cancer. PAX-2 is a transcription factor that promotes renal tubular differentiation during embryogenesis. PAX-2 expression in Met RCC is studied and compared with that of the RCC Marker antigen (RCCM).

Design: From an archive of 94 unequivocal cases of Met RCC, 64 cases were immunostained for PAX- 2 and 58 cases for RCCM. Among them, both PAX-2 and RCCM stain were performed on consecutive tissue sections for 28 cases. Features selected for evaluation included histologic type of the Met in relation to that of the primary tumor, staining intensity (graded 0-3), staining extent (% of positive tumor cells), and the correlation of staining of PAX-2 and RCCM relative to tumor type.

Results: For **PAX-2**, 47/64 (73%) cases showed positive stain (mean score 2.06) in 5-100% tumor cells (mean 55) with a nuclear pattern, and a weak nonspecific cytoplasmic pattern in 39 cases. The positive stain was not correlated with the tumor type or grade. For **RCCM**, 33/58 (57%) cases showed positive stain (mean score 2.5) in 2-100% tumor cells (mean 62) with a cytoplasmic pattern only. The positive stain was seen predominantly in Met with low nuclear grade, and clear cell or papillary features. The results of the 28 cases submitted to staining for both **PAX-2 and RCCM** were shown in Table 1.

Conclusions: 1) Immunostain for PAX-2 is successful in routinely processed human tissue, with a nuclear pattern, expected for a transcription factor; 2) PAX-2 expression, noted in a majority of Met RCC, should substantially facilitate a correct diagnosis. 3) PAX-2 is a significantly more sensitive marker than the RCCM, but there are rare cases with the – PAX-2/+ RCCM phenotype, justifying the use of both as a panel.

Table 1: 28 Cases Submitted for Both RCCM and PAX-2 Staining

	PAX-2 Positive (n=20)	**PAX-2 Negative (n=8)**
RCCM Positive (n=12)	10	2
RCCM Negative (n=16)	10	6

846 Expression of SmgDGS in Prostatic Adenocarcinoma and Its Function in Cancer Cell Migration

HY Zhi, XJ Yang, J Kuhnmuench, R Thill, CL Williams, CG Becker, R Li. Medical College of Wisconsin, Milwaukee, WI; Northwestern Memorial Hospital, Chicago, IL.

Background: SmgDGS, a guanine nucleotide exchange factor, actives small GTPases which promote malignancy of different carcinoma cells. Surprisingly, the expression and function of SmgDGS in human cancers have not been reported. In this study, we evaluated the expression of SmgGDS in benign prostatic tissue, prostatic adenocarcinoma and prostatic intraepithelial neoplasia (PIN) using immunohistochemistry. In addition, we performed functional studies of SmgGDS using siRNA in different prostatic cancer cell lines.

Design: Large sections of 18 radical prostatectomy specimens contained invasive carcinoma (Gleason score G3-G5) and benign prostatic tissue, with or without PIN were selected. In addition, a section of tissue microarray containing 90 cases including 30 high grade PIN, 30 G3 and 30 G4/5 carcinoma, with or without benign prostatic tissues was also used. Totally, there were 108 cases including 44 PINs and 78 adenocarcinomas with 32 cases containing benign tissues. Immunohistochemistry was performed using a monoclonal anti-SmgGDS antibody (BD Transduction laboratories). In the functional studies, the LnCap and PC-3 cell lines were transfected with SmgGDS siRNA to silence SmgGDS expression. The MTT assay was used to measure cell proliferation and the Wound-Healing and Colloidal Gold Phagokinesis assays were used to measure cell migration.

Results: SmgGDS was either weakly expressed or undetectable in benign prostatic glands. Seventy of 78 (89.7%) invasive adenocarcinomas and 38/44 (86.4%) PINs showed a strong and increased staining when compared with adjacent benign glands. There was no difference of expression between different Gleason patterns with 30/33 (90.9%) in G3 versus 40/45 (88.9%) in G4/5 adenocarcinomas. Silencing SmgGDS expression slightly inhibited cell proliferation in both LnCap and PC-3 cells. However, silencing SmgGDS significantly diminished cell migration in PC-3 cell line.

Conclusions: 1) SmgGDS is overexpressed in prostatic adenocarcinoma and PIN, indicating a potential role as a marker in the diagnosis of prostatic cancer. 2) The increased SmgGDS expression in both adenocarcinoma and PIN suggest its role in early prostatic carcinogenesis. 3) SmgGDS enhances the migration of the androgen-independent PC-3 cells. This finding indicates that SmgGDS may be used as a potential target in the treatment of advanced prostatic cancers, because androgen-independence is correlated with advanced tumor stage after hormonal therapy.

847 Cancer Risk Associated with High Grade Prostatic Intraepithelial Neoplasia (HGPIN) in a Contemporary Single-Institution Cohort

M Zhou, E Klein, C Magi-Galluzzi. Cleveland Clinic, Cleveland, OH.

Background: HGPIN has been shown to denote a 25-30% risk of finding cancer in subsequent biopsies. Its significance, however, has been questioned recently as several studies have shown that the cancer risk associated with HGPIN is comparable to that associated with a benign initial diagnosis. This study examined the cancer risk associated with HGPIN in a contemporary cohort of patients evaluated at a single institution.

Design: Patients met the following criteria: (1) initial and subsequent prostate biopsies were performed after January 2003; (2) initial biopsy contained ≥ 6 cores; (3) time interval between the initial and 1st repeat biopsy ≤24 months; (4) initial biopsy had a diagnosis of benign prostatic tissue (BPT), HGPIN, atypical glands suspicious for cancer (ATYP). All the biopsies were diagnosed by the GU pathology service at the authors' institution.

Results: 262 patients were included in this study. The initial diagnosis was BPT in 28.2%, HGPIN in 47.3%, and ATYP in 24.4%. The mean number of re-biopsy was 1.2 (range 1-3), and the time interval between the initial and subsequent biopsy (ies) was 10.6 (range 0.5-39.5) months. Of the patients with an initial HGPIN diagnosis, 33.1% had PCa in subsequent biopsies, compared to 14.9% cancer risk associated with an initial BPT diagnosis ($p<0.01$, Table 1). If the initial biopsy had ≥ 10 cores, the cancer risk associated with HGPIN was 33.7%, significantly higher than that associated with the initial BPT biopsy (12.5%, $p<0.01$). However, the cancer risk associated with HGPIN was not significantly different from that associated with the initial BPT biopsy (30.4% vs 19.2%, $p>0.05$) when the initial biopsy had 6-9 cores.

Table 1: The Cancer Risk Assessment with BPT, HGPIN and ATYP in Initial Diagnosis

		PCa in Subsequent Biopsy		
		Biopsy core #6-9	Biopsy core # ≥10	All Cases
Initial Diagnosis	BPT	5/26 (19.2%)*	6/48 (12.5%)**	11/74 (14.9%)^
	HGPIN	7/23 (30.4%)*	34/101 (33.7%)**	41/124 (33.1%)^
	ATYP	5/9 (55.6%)	22/25 (40%)	27/64 (42.2%)

*p>0.05, **p<0.01, ^p<0.01

Conclusions: HGPIN is associated with a significantly increased cancer risk in our contemporary cohort. However such increased cancer risk is only significant when the initial biopsy has ≥ 10 cores. Our study suggests that HGPIN in needle biopsy should still be considered as a risk factor for PCa. In addition, a biopsy scheme with ≥ 10 core is recommended in order to detect such an increased cancer risk associated with HGPIN.

848 Expression of Carbonic Anhydrase IX (CA9) in Renal Neoplasms: Implications for Use as Diagnostic Marker

M Zhou, C Magi-Galluzzi. Cleveland Clinic, Cleveland, OH.

Background: CA9 is a tumor associated antigen found on the cell surface of a number of human cancers. Recently, CA9 has been shown to be a useful diagnostic and prognostic biomarker for clear cell renal cell carcinoma. Its expression in other renal neoplasms, and its utility as a differential diagnostic marker, however, is not well documented.

Design: A tissue microarrays (TMA) was constructed from 60 normal kidneys, 23 clear cell renal cell carcinoma (CCRCC), 20 papillary renal cell carcinomas (PRCC), 16 chromophobe renal cell carcinomas (ChRCC), and 19 oncocytomas (ONC), 14 pelvic urothelial carcinoma (TCC) and 20 angiomyolipoma (AML). The TMA was immunostained for CA9. Membranous CA9 expression was scored as negative, weak and strong. The percentage of positive cells was also recorded.

Results: CA9 expression was absent in normal renal tissues. Strong and weak positive staining was present in 83% and 4% of CCRCC, respectively, with an average of 47.6% of tumor cells positive for CA9 expression (Table 1). Thirteen % of CCRCC was negative. Ten % of PRCC was also positive for CA9. One such case had papillary structures lined with clear cells. All pelvic TCCs were positive for CA9, with strong expression in 86% and weak expression in 14% of cases. However, only an average of 11.3% of tumor cells were positive for CA9. All ChRCC, ONC and AML were negative for CA9.

Table 1: Expression of CA9 in Renal Neoplasms

CA9 Expression	CCRCC	PRCC	ChRCC	ONC	TCC	AML
Strong Positive	19/23 (83%)	2/10 (10%)	0	0	12/14 (86%)	0
Weak Positive	1/23 (4%)	0	0	0	2/14 (14%)	0
Negative	3/23 (13%)	18/20 (90%)	16/16 (100%)	19/19 (100%)	0	20/20 (100%)
% postivie cells [mean (range)]	47.6% (3-100)	21.5% (20-23)	N/A	N/A	11.3% (5-50%)	N/A

Conclusions: Although expressed by the majority of CCRCC, CA9 is also found in all the renal pelvic TCC and in a minority of PRCC, but is absent in ChRCC and oncocytoma. CA 9 may potentially be useful in the differential diagnosis of selected renal tumors, such as CCRCC vs ChRCC, and TCC vs ChRCC.

Gynecologic

849 p16 Is Expressed in Ovarian Clear Cell Carcinoma

AJ Adeniran, ED Euscher, MT Deavers, A Malpica. M.D. Anderson Cancer Center, Houston, TX.

Background: p16 is a cyclin-dependent kinase-4 inhibitor that is expressed in tumors of the female genital tract. Of the gynecologic tumors, p16 expression in cervical carcinoma (squamous, glandular, and small cell) is best studied and characteristically overexpressed. p16 expression in low grade and benign ovarian tumors is reported to be low while ovarian serous carcinomas are reported to have overexpression. The expression of p16 in ovarian clear cell carcinoma has not been studied. This study evaluates the expression of p16 in a series of fifteen cases of ovarian clear cell carcinoma.

Design: Fifteen cases of pure clear cell carcinoma of the ovary were retrieved from the pathology files of M.D. Anderson Cancer Center. H&E slides were reviewed in all cases. Immunohistochemical analysis for p16 (clone 16P07, dilution 1:25, Labvision/Neomarkers, Fremont, CA) was performed on formalin-fixed, paraffin embedded archival tissue utilizing standard avidin-biotin technique. Nuclear and cytoplasmic expression in tumor cells was considered positive with results scored semiquantitatively (0, no staining; 1+, 1-25%; 2+, 26-50%; 3+, 51-75%; 4+, 76-100%) by two independent observers.

Results: Fourteen of the 15 (93%) cases over-expressed p16 immunohistochemically. Eleven of 15 (73%) cases had a score of 3+ or 4+, and 3 of 15 (20%) cases had a score of 1+ or 2+. One of fifteen cases (7%) had no p16 expression.

Conclusions: The majority of ovarian clear cell carcinomas overexpress p16, although the mechanism for this is unknown. p16 may be less useful as a marker to distinguish metastatic cervical carcinoma to the ovary from a primary ovarian carcinoma. However, p16 may be a useful marker in an immunopanel to distinguish a primary ovarian clear cell carcinoma from a metastatic carcinoma with clear cell morphology to the ovary.

850 Inverse Relationship between Epithelial and Stromal Expression of CD44 and Clinical outcome in Recurrent Endometrial Carcinoma: A Gynecologic Oncology Group (GOG) Study

A Akalin, K Darcy, B Brady, KC Torkko, K Leslie, CW Whitney, M Singh. University of Colorado at Denver and Health Sciences Center, Aurora, CO; Gynecologic Oncology Group, Buffalo, NY; University of New Mexico, Albuqurque, NM; Christiana Gynecologic Oncology, LLC, Newark, DE.

Background: Although CD44 is recognized for its role in cell-cell and cell-matrix interaction, very little is know about the relationship between CD44 expression in recurrent endometrial carcinoma and measures of clinical outcome including progression-free survival (PFS), overall survival (OS) and tumor response.

Design: A retrospective study was undertaken to examine whether CD44 expression in epithelium or stroma was associated with clinico-pathologic characteristics and measures of clinical outcome. Expression was evaluated using an optimized immunohistochemistry assay (Dako anti-CD44 antibody, clone DF1485) on sections from a tissue microarray (TMA) created using residual blocks from women who participated in GOG protocol 119. GOG-119 was a prospective phase II trial of tamoxifen combined with intermittent progestin in women with recurrent endometrial carcinoma.

Results: CD44 expression was positive in the epithelium in 26 (70.3%) and in the stroma in 25 (67.6%) of 37 evaluable cases. Epithelial, but not stromal, expression was associated with younger age (p=0.048), worse tumor grade (p=0.023) and no prior radiotherapy (p=0.036). After adjusting for patient age, tumor grade and performance status, proportional hazards regression models suggested that women with positive epithelial expression had a reduced risk of disease progression (hazard ratio [HR]= 0.40; 95% confidence interval [CI]=0.16-0.96; p=0.040) and death (HR=0.33; 95% CI=0.13-0.84; p=0.019). In contrast, adjusted models suggested that women with positive stromal expression of CD44 had an increased risk of disease progression (HR= 3.13; 95% CI=1.25-7.81; p=0.014) and death (HR=2.65; 95% CI=1.09-6.47; p=0.032). Neither epithelial nor stromal expression was associated with tumor response (p=0.715 and p=0.157, respectively).

Conclusions: Expression of epithelial CD44 was directly related to PFS and OS, while stromal CD44 was inversely related to PFS and OS. A larger study is needed to further evaluate an interaction between epithelial and stromal expression and clinical outcome in this disease. This work was supported by the Receptor Core Laboratory of the GOG and the TMA was prepared by the GOG Tissue Bank.

851 Endometrial Carcinoma (EC) in Women 35 Years of Age or Younger

R Ali-Fehmi, M Cote, MH Arabi, CS Bryant, JP Shah, L Mughrabi, VL Schimp, K Sauder, F Abdul-Karim, AR Munkarah. Karmanos Cancer Center, Wayne State Universtiy, Detroit, MI; Case Western University Hospital, Cleveland, OH.

Background: Although the majority of women with EC are postmenopausal, 5% to 30% are under the age of 50 years of age at the time of diagnosis. The aim of this study is to describe the demographic and survival data for women presenting with EC at an age of 35 years or younger (YOY).

Design: Patients with a diagnosis of uterine cancer were identified from the Surveillance, Epidemiology, and End Results (SEER) cancer database from 1973 to 2003. Demographic and pathologic data were collected. Statistical analysis and survival data were calculated using the Kaplan Meier method and Cox regression.

Results: Of the 109,192 women diagnosed with EC in the study period, 924 patients (less than 1%) were 35 YOY. The study population included 57% Caucasian (C), 10% African-American (AA), and 33% other/missing. The mean age was 32 years (range 14 to 35). Age at diagnosis was greater for the C population compared to the AA (33 years vs. 31 years, p=0.004). The overall distribution by stage was 43% Stage I, 4% Stage II, 2% Stage III, and 3% Stage IV, (48% unstaged). Histopathologic classification by tumor type and grade was as follows: 90% endometrioid, 5.8% mucinous, 1.8% serous, and 2.2% clear-cell. Fifty-two percent, 23%, and 8% of grade 1, 2, and 3 tumors were reported, respectively. Type II tumors comprised 4% (N=36) of the study population. There was no significant difference in stage, histology, or grade distribution by ethnicity. Median survival of the entire population was 133 months. There was a significant difference in survival by race (median survival in C patients was 149 months vs. 100 months in AA patients, p=0.002).

Conclusions: Early stage, well-differentiated EC represents the most common type of EC in this cohort of younger patients. Overall survival is relatively very good. Unlike other reports that include patients of all ages, our study did not reveal a higher proportion of aggressive histologic types in the young African American population. However, AA patients suffered from a worse prognosis.

852 Should Intraoperative Frozen Section Diagnosis Be Used To Determine Surgical Staging for Patients with Endometrial Carcinoma?

R Ali-Fehmi, MH Arabi, RT Morris, VL Schimp, CS Bryant, JP Shah, AR Munkarah, W Sakr. Karmanos Cancer Center, Wayne State University, Detroit, MI.

Background: For patients with endometrial cancer (EC), intraoperative frozen section assessment (IFSA) at the time of hysterectomy is often used to determine which patients should undergo lymph node sampling (LNS). At our institution, LSN is not usually performed for patients with low grade carcinomas and superficial or no myometrial invasion (MI). The objectives of this study were to evaluate the accuracy of frozen section diagnosis in patients with early stage endometrial carcinoma and accordingly, to investigate the use of the IFSA results to determine the need for surgical staging.

Design: All patients with EC treated by radical hysterectomy at our institution from 1995 to 2004 were considered for the study. The subset that had intraoperative pathologic evaluation of EC stage and grade was identified. The correlation between the permanent and IFSA diagnoses was evaluated by a gynecologic pathologist who also reviewed the complete surgical pathology material on the study cases to identify patients who underwent LNS.

Results: Four hundred fifty-seven cases of EC were identified during the study period of whom 253 (55%) had IFSA performed. Fifty-eight patients were reported by IFSA to have superficial (<3mm) or no MI. Thirty two of the 58 patients (55%) had LNS performed while 26 patients (45%) did not undergo LNS. Upon evaluating the entire specimens on permanent sections, 10 of the LNS patients (31%) were upstaged and 12, (34%) were upgraded. The corresponding figures for the 26 patients who did not have LNS were 12 (48%) upstages and 12 (48%) upgraded patients (p=NS).

Conclusions: Our data indicate a low correlation between the frozen section assessment of an early stage, low grade endometrial carcinoma and the final pathologic findings. More than 40% of early disease patients in our study were upstaged and/or upgraded on final pathology. These results question the usefulness of relying on intraoperative frozen section evaluation as a decision making tool to proceed with surgical staging for patients with endometrial carcinoma.

853 Sampling of the Grossly Negative Omentum in Ovarian Neoplasms

C Allen, SM Lele. University of Kentucky College of Medicine, Lexington, KY; University of Nebraska Medical Center, Omaha, NE.

Background: Optimal pathologic staging of borderline or malignant ovarian neoplasia includes omentectomy. Histopathologic evaluation of the grossly negative omental specimen in a case with a malignant or borderline ovarian epithelial tumor is important in detecting stage IIIA disease. The Borderline Ovarian Tumor Workshop (*Human Pathology* 2004;35:910-917) suggests sampling one section per two centimeters of largest dimension of the grossly negative omentum. However, the authors acknowledge that no prospective studies with published data exist to support this extent of sampling. A prospective study was designed to determine the optimal number of sections needed to adequately assess such specimens.

Design: Omental specimens received in the surgical pathology laboratory from cases with borderline or primary malignant ovarian epithelial tumors were carefully inspected for evidence of gross disease. Those that were macroscopically positive were excluded. Grossly negative omental specimens were sampled by submitting one section or one block per cm of largest dimension. Foci with even slight firmness or thickening if present were submitted in the first three blocks. Hematoxylin and eosin stained sections of the ovarian tumors and omental specimens were reviewed.

Results: Thirty omental specimens were examined from cases with a borderline or primary malignant ovarian epithelial tumor. In 21 cases the omentum was grossly negative. 11 were borderline tumors (6 serous and 5 mucinous) and 10 were carcinomas (5 endometrioid, 2 serous, 2 clear cell and 1 carcinosarcoma). In 18 cases (86%) the omentum was negative on microscopic examination. In each of the remaining 3 cases (14%) the omentum revealed microscopic tumor: non-invasive implants in two serous borderline tumors and metastatic carcinoma in a case of primary carcinosarcoma of the ovary. In all three cases microscopic tumor was detected in the omentum in the first three sections/blocks corresponding to areas of slight firmness/thickening on gross inspection.

Conclusions: A thorough gross evaluation of the grossly negative omentum with sampling of areas of even slight firmness/thickening is important in identifying microscopic tumor. When such foci are sampled, as was done in this study in the first 3 blocks, additional sections do not yield any information that will change the pathologic stage.

854 Diagnosing Endometrial Hyperplasia: Why Is It so Difficult To Agree?

KH Allison, SD Reed, L Voigt, RL Garcia. University of Washington Medical Center, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

Background: The current WHO classification of endometrial hyperplasia (EH) is problematic because of its poor diagnostic reproducibility. We sought to determine the factors that caused diagnostic disagreement in a large review of endometrial specimens.

Design: Endometrial specimens were reviewed by two pathologists, and by three in cases with disagreement. All cases diagnosed as EH or endometrial carcinoma (EC) were scored for degree of glandular crowding, architectural complexity and cytologic atypia. In addition, the sample adequacy, neoplasia volume, presence of metaplasia or endometrial polyp were noted.

Results: 2,147 endometrial samples were reviewed by two pathologists, with disagreements resulting in a third review in 568 cases (26.4%). The primary pathologists agreed on a diagnosis of no hyperplasia 82% of the time, simple hyperplasia 20%, complex hyperplasia 36.1%, atypical hyperplasia 31.6% and adenocarcinoma 41.1%. The overall unweighted kappa value was 0.55, with a lower kappa of 0.35 when only EH or EC were included. Cases with adequacy categorized by either pathologist as "scant" had significantly more diagnostic disagreement than those without (67% versus 54%, $p < 0.00001$). Cases categorized as "low volume neoplasia" had significantly more diagnostic disagreement than cases categorized as "high volume neoplasia" (50% versus 37%, $p = 0.003$). Cases with features of a polyp had 69% diagnostic disagreement compared to 55% of cases without ($p= 0.037$). Metaplasia was not a statistically significant factor for disagreement. The most problematic diagnostic criteria category was cytologic atypia (50% of cases with diagnostic disagreement also had disagreement on the degree of cytologic atypia verses 16% of cases with diagnostic agreement, $p <0.00001$). Less significant were disagreements on architectural crowding ($p = 0.007$) and architectural complexity ($p = 0.028$).

Conclusions: The high frequency of diagnostic disagreement in EH appears to be related to both "confounding factors" (e.g. scant sample, polyp) as well as lack of objectivity in diagnostic criteria (e.g. cytologic atypia). While increasing adequacy of endometrial samples may ensure greater diagnostic reproducibility, differences in evaluating subjective criteria like cytologic atypia remain a major factor associated with diagnostic disagreement.

855 Type and Degree of T Cell Immune Response to Ovarian Carcinoma Matters: Correlation of Cytotoxic T Cells and Foxp3+ Immunosuppressive Tregs with Survival

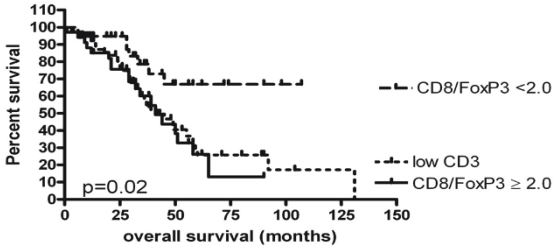
KH Allison, CA Shah, RL Garcia, HJ Gray, C DelloRusso, EM Swisher. University of Washington, Seattle, WA.

Background: Immunosuppressive regulatory T cells (Tregs) have been associated with immune tolerance to neoplasia. We hypothesized that the balance between cytotoxic and immunosuppressive T cell responses in the tumor micro-environment influences survival in ovarian carcinoma.

Design: 119 cases of ovarian carcinoma were immunostained with CD3 antibodies. 57 of these cases had significant CD3+ intra-tumor infiltrates and were subsequently stained with CD8 and Foxp3 antibodies. Cases were scored by two investigators independently, averaged for a final score and correlated with available survival data.

Results: Patient ages ranged from 28-88 years (median = 59). 89.9% had advanced stage disease. Survival ranged from 0-131 months (median = 32 months). The CD8:FoxP3 ratio was strongly associated with survival (p = 0.009). There was also a significant association between CD8+ T cells and survival (p = 0.03). FoxP3+ T cells or CD3+ T cells alone were not associated with survival. Interestingly, an intermediate CD8: FoxP3 ratio as well as intermediate number of CD8+ T cells had the best survival (median survival not yet reached, p = 0.02) (See below Figure). Median survival in cases with low levels of CD3+ infiltrates was 41 months. Similarly, cases with the highest CD8:FoxP3 ratios (≥ 2) and CD8+ T cells (≥ 30) had median survivals of 41 and 34 months respectively.

Conclusions: The type and degree of intra-tumor T cell response is a statistically significant predictor of survival. Our results suggest that there is an "ideal" balance between cytotoxic and immunosuppressive T cell responses, with both low and high cytotoxic responses having worse survival. These findings have implications for tailoring tumor vaccines and immunotherapies.



856 Mast Cells in Sex Cord-Stromal Tumors of the Ovary

L Andino, A Malpica, ED Euscher, MT Deavers, EG Silva. The University of Texas Medical School at Houston, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: It is known that mast cells can be present in the ovary. Some authors have proposed that mast cells are present in the medulla and absent in the cortex while others propose that mast cells are found throughout the ovary. Mast cells are the primary source of histamine in tissue, and histamine can stimulate ovarian steroidogenesis by stimulating or increasing progesterone secretion. In this study, we investigate their presence in sex cord-stromal tumors of the ovary.

Design: We compared 15 sex cord-stromal tumors classically associated with clinical manifestations of steroid hormone production (8 thecomas, 5 Sertoli-Leydig cell tumors and 2 adult granulosa cell tumors) with 10 ovarian fibromas, tumors which typically do not have clinical evidence of steroid hormone production, serving as a control group. These tumors were stained for tryptase (1:60, Dako, Carpinteria, CA), c-Kit (1:200, Dako, Carpinteria, CA), estrogen receptor (1:100, Novocastra, Norwell, MA) and androgen receptor (1:30, Dako, Carpinteria, CA) utilizing standard avidin-biotin technique. Staining intensity was assessed quantitatively by counting the number of positive cells in the single most active 10X field of the tumor.

Results: The immunoperoxidase results are summarized in Table 1.

Conclusions: 1. Mast cells, as identified by tryptase and c-Kit, are rare in fibromas and frequent in thecomas, Sertoli-Leydig cell tumors and adult granulosa cell tumors. The presence of mast cells may correlate with steroidogenesis. 2. Tryptase and c-Kit may be helpful in the differential diagnosis between fibromas and thecomas.

Number of Positive Cells per Most Active 10X Field in Sex Cord Stromal Tumors

	Tryptase (median)	c-Kit (median)	Estrogen receptor (median)	Androgen receptor (median)
Fibromas	0-44 (1)	0-7 (0)	0-400 (0)	0 (0)
Thecomas	60-400 (75)	2-65 (15)	0-500 (20)	0-400 (95)
Granulosa cell tumors	60-280 (170)	15-90 (52.5)	0-10 (5)	0-100 (0)
Sertoli-Leydig tumors	45-400 (90)	3-40 (21)	0-600 (0)	0-150 (0)

857 Immunohistochemical Characterization of the Stromal Components in Endocervical Adenocarcinoma

C Aoyama, P Liu, N Ostrzega. Olive View-UCLA Medical Center, Sylmar, CA.

Background: Endocervical stroma is composed of multiple layers; superficial layer containing CD34(+) cells, deeper layer containing Actin(+) cells, and vascular-rich layer in the middle. We investigated if immunohistochemical distinction of the stromal components could be useful for objective histologic evaluation of endocervical adenocarcinoma.

Design: Immunohistochemistry with CD 34 and Actin was performed on a total of 44 cases of adenocarcinoma of endocervix. Clear cell carcinoma and adenosquamous cell carcinoma were excluded. There were 32 tumors whose stroma contained only CD34(+) cells, 4 tumors contained both CD34(+) and Actin(+) cells, and 8 cases contained only Actin(+) cells. Those stromal immunophenotypes were analyzed with histologic parameters by using Chi-square test.

Results: As shown in the table 1, all in situ adenocarcinoma had Actin(-) stroma. In contrast, 86% of invasive adenocarcinoma had Actin(+) stroma (p<0.0001). Depth of tumor from the basement membrane was also correlated with different stromal compositions: deeply seated tumors (≥ 5 mm) often had Actin(+) stroma, and superficial tumors (<5mm) frequently had Actin(-) stroma (p<0.0001). Coexistent squamous dysplasia was almost exclusively seen in tumor with Actin(-) stroma (p<0.003). As shown in the table 2, presence of CD34(+) cells in the stroma indicated a significantly lower risk of lymphovascular invasion (p<0.0001).

Conclusions: Stromal characterization with Actin and CD34 immunostains is useful for objective assessment of endocervical adenocarcinoma.

Table 1

	Final Dx= in situ	Final Dx= invasive	tumor depth <5mm	tumor depth >/=5mm	squamous dysplasia(+)	squamous dysplasia (-)
Actin (-)	30	2	29	2	19	13
Actin (+)	0	12	3	10	1	11

Table 2

	LVI (+)	LVI (-)
CD34 (-)	5	3
CD34 (+)	2	34

858 Impact of Microsatellite Instability (MSI) on Survival in High Grade Endometrial Carcinoma

MH Arabi, M Cote, CS Bryant, JP Shah, AR Munkarah, R Ali-Fehmi. Wayne State University, Detroit, MI.

Background: MSI is a hallmark of defective DNA- mismatch repair during replication. Genetic instability resulting from the inactivation of mismatch repair system genes (MLH1, MSH2, and MSH6) is known to be one of the molecular pathways involved in oncogenesis. The objective of this study was to identify by immunohistochemically (IHC), the correlation between MSI and survival in high grade (HG), Type I (T1) and Type II (T2) endometrial carcinomas (EC).

Design: Between 1995 and 2002, we identified 460 patients with EC who underwent hysterectomy. 91 HG EC cases (T1=44 and T2=47) were available. Two paraffin blocks from each case were immunostained using antibodies against MLH1, MSH2, and MSH6. Semi-quantitative scoring of immunoreactivity was based on percentage of tumor staining and staining intensity. The distribution of demographic variables and tumor characteristics were available using SEER and institutional databases. Statistical analysis and survival data were calculated using the Kaplan-Meier method & Cox regression.

Results: The mean age was 60.7 and 62.6 years for T1 and T2 EC, respectively. The study population included 44 white and 47 black patients. The overall distribution by stage was 34 Stage I and 57 Stage II/III/IV. 58 patients (T1=31, T2=27) were alive at the time of the study. 33 (T1=13, T2=20) were deceased from cancer-related causes. The median survival was 45.5 months for T1 and 27.0 months for T2. IHC status is described in table 1.

Table 1

MSI Status	Type I	Type II
0 markers positive	4 (9.1%)	1 (2.1%)
1 marker positive	10 (22.7%)	10 (21.3%)
2 markers positive	9 (20.5%)	12 (25.5%)
3 markers positive	21 (47.7%)	24 (51.1%)

Tumors were considered microsatellite unstable when immunostaining was negative for all three markers. MSI was present in 5 cases (T1=4, T2=1). After adjusting for race, stage and tumor type, the risk of death was 13.2 times greater among women with MSI tumors compared to women with non-MSI tumors (OR=13.20 95% CI 3.50-49.76). A significant difference in survival was noted for MSI tumors (3.3 months) compared to non-MSI tumors (71.6 months) in this cohort of patients (p=0.004).

Conclusions: Although this study has its limitation due to the small sample size, it confirms the prognostic significant of MSI in high grade endometrial carcinoma, the risk of death was significantly higher in patients whose tumors tested negative for the three mismatch repair genes MLH1, MSH2, and MSH6. Evaluating these genes might be valuable prognostic indicator for survival in HG EC patients.

859 Expression of COX-2, VEGF, Ki-67, and p53 in Advanced Stage, High-Grade Serous Ovarian Carcinoma with Prolonged Survival

MH Arabi, JP Shah, CS Bryant, RT Morris, V Schimp, M Mughrabi, AR Munkarah, R Ali-Fehmi. Wayne State University, Detroit, MI.

Background: Tumor grade and histologic type are important prognostic factors in epithelial ovarian cancer. In addition, tumor biology is also influenced by molecular changes that are not reflected phenotypically. The goal of our study is to compare the expression of a number of molecular markers in histologically similar tumors obtained from patients with significantly different survivorship.

Design: Patients with stage III-IV, high-grade ovarian serous carcinoma (SOC) were identified from our pathology database. A retrospective chart review collected clinicopathologic information. 22 SOC patients with a survival of >5 years (prolonged survival-PS) and 40 SOC patients with a survival of < 2 years (short survival-SS) were identified and included in this study. Paraffin sections were stained by immunohistochemistry for COX-2, VEGF, Ki-67, and p53. COX-2 and VEGF expression was scored high or low based on intensity and percentage of positive cells. Ki-67 and p53 was scored positive if staining present in >5% of cells. Pearson Chi-squared and T test were used to determine differences in age, COX-2, VEGF, Ki-67, p53 between SOC patients with prolonged survival and SOC patients with < 2 years survival.

Results: There was no difference in mean age between the two groups. The median survival in the SS and PS groups were 27 month and 106 months respectively. 51 Stage III and 11 Stage IV SOC patients were included. The only statistically significant

difference between the groups was COX-2 expression. In fact, 63% (7/11) of patients with low COX-2 expression were alive at 5 years compared to 30% (14/47) of patients with high COX-2 expression (p=0.042). No difference in expression of VEGF, Ki-67, and p53 was noted between the two groups.

Survival of SOC Patients	Mean Age (Years)	COX-2 n=58	VEGF n=45	Ki-67 n=62	p53 n=60
		Low / High	Low / High	Negative / Positive	Negative / Positive
≥ 5 years (n=22)*	60.9	7(33%)/14(67%)	3(19%)/13(81%)	14(64%)/8(36%)	8(36%)/14(64%)
≤ 2 years (n=40)*	62.0	4(11%)/33(89%)	4(14%)/25(86%)	21(52%)/19(48%)	14(37%)/24(63%)
P Value		0.042	0.0484	0.283	0.597

Conclusions: Our study confirms the prognostic significance of COX-2 in high grade serous ovarian carcinoma. Even though 67% of patients who survived more than five years had COX-2 overexpressing tumors, patients with low COX-2 expression were significantly more likely to be alive beyond five years than those with high expression.

860 Institutional Gynecologic Pathology Review Has an Impact on the Practice of Gynecologic Oncology

H Asad, R Soslow. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Our hospital mandates examination of histopathologic slides before treating patients previously diagnosed at a referring institution. This constitutes a useful quality assurance implement and allows study of diagnostically problematic lesions that often require a second opinion diagnosis.

Design: We retrieved pathology reports from patients referred to the gynecologic oncology service between mid-2003 and mid-2006, the period of time during which only gynecologic pathologists reviewed relevant consult material. The referral diagnoses were compared with the diagnoses rendered at our institution. Major disagreements were defined as altered diagnoses leading to a significant change in therapy or prognosis.

Results: Of the approximately 4300 cases examined, only 27 (0.63 %) had a major disagreement. The most frequent discrepancy, seen in 8 cases (30%), involved a change from a malignant to a benign/borderline diagnosis. Other discrepancies included a change from benign/borderline to malignant 6 (22%), a change in tumor classification 6 (22%) , a change from non-invasive to invasive disease 2 (7%), a change from invasive to non-invasive disease 2 (7%), a change in primary to secondary tumor 1(4%), a change in secondary to primary tumor 1(4%) and a change in benign classification 1 (4%). Most of the errors occurred while examining endometrial pathology (29%), followed by ovarian (26%), cervical (22%), myometrial (11%), vulvar (4%), serosal (4%) and gestational pathology (4%). Most errors could be traced to problems well known to gynecologic pathologists that have been addressed in detail in the gynecologic pathology literature, including invasion assessment, the appearance of rare tumors, nuances in diagnostic criteria, and uncommon morphologic manifestations of common gynecologic tumors.

Conclusions: Aside from being a valuable educational exercise, institutional pathology review remains an important quality assurance tool and can result in a change of diagnosis with major therapeutic implications.Though, these major disagreements taken numerically appear uncommon, their impact in terms of the individual patient concerned cannot be understated.

861 The Bethesda System 2001 Recommendation of Reporting Endometrial Cells Seen in Pap Tests of Women Age 40 Years and Older Is Overly Conservative

DL Aslan, JP Crapanzano, M Harshan, M Erroll, B Vakil, EC Pirog. New York Presbyterian Hospital Weill-Cornell Medical Center, New York, NY.

Background: Benign appearing spontaneously exfoliated endometrial cells seen in Pap tests obtained from postmenopausal women have been associated with endometrial adenocarcinoma and its precursors. The 2001 update of the Bethesda System (TBS2001) recommends reporting of endometrial cells seen in Pap tests of all women age 40 and older. The purpose of this study is to determine the incidence of reporting and significance of endometrial cells over the age of 40 after implementation of TBS2001.

Design: Pap tests of women ≥ 40-years-old and containing cytologically benign appearing endometrial cells outside of the first half of menstrual cycle (EM40) reported over 2 years before and 4.5 years after TBS2001 implementation were included. Significant endometrial pathology (SEP) was defined as simple hyperplasia or a higher diagnosis.

Results: 41 of 24157 and 361 of 73478 women before and after TBS2001, respectively, (0.17% vs. 0.49%, p=0.0001) were included. The increase in EM40 reporting rate was exclusively due to reporting in women with premenauposal out-of-cycle status and unknown menopausal status. 159 of 402 (39.5%) women with EM40 had endometrial biopsies (EMB). 22 (13.84%) of these EMB were performed solely because of the EM40 diagnosis. 2/22 (9.1%) showed SEP (1 endometrial carcinoma [EMCA]; 1 simple endometrial hyperplasia [SHP]); both patients were postmenopausal. 137 (86.16%) of the EMB were performed for reasons other than EM40 (postmenopausal or irregular bleeding etc). Of these patients, 12 of 137 (8.7%) were diagnosed with SEP within 18 days prior to 18 months subsequent to the index Pap test; all were postmenopausal (5 EMCA, 3 complex atypical hyperplasia, 3 complex hyperplasia without atypia, 1 SHP). Only 1 of overall 14 (7.14%) SEP was detected in a woman under the age of 45 (SHP). No change in biopsy rates for EM40 were observed in postmenopausal women after TBS2001. Biopsies performed for EM40 before TBS2001 involved postmenopausal women exclusively, whereas after TBS2001 68.4% (13/19) of such patients were premenopausal.

Conclusions: TBS2001 led to increased reporting of EM40. EM40 can lead to detection of SEP in asymptomatic women; however, this finding is non-specific and should lead to an endometrial biopsy only in the presence of other risk factors such as postmenopausal status. The age limit for reporting cytologically benign appearing endometrial cells can be safely raised to women age 45 and older.

862 Amniotic Fluid Material Is Not Identified in the Uterine Veins of Non-Amniotic Fluid Embolus Patients

CZ August, L Escuadro. Advocate Illinois Masonic Medical Center, Chicago, IL; University of Illinois at Chicago, Chicago, IL.

Background: Amniotic fluid embolus is a rare but frequently fatal complication of pregnancy. Many studies have focused on the identification of amniotic fluid elements in the maternal blood and pulmonary vasculature, a phenomenon generally limited to amniotic fluid embolus. While amniotic fluid elements appear to enter the circulation via the uterine and cervical vasculature, factors predisposing to this event are not clearly defined; placenta accreta, uterine rupture, tears in endocervical and lower uterine segment veins, and iatrogenic causes have been suggested. If any of these factors alone predisposed to amniotic fluid elements entering the uterine vasculature, such elements would be expected to be present in the uterine vasculature of non-amniotic fluid embolus patients with these conditions.

Design: Prompted by the autopsy finding of amniotic fluid material in the uterine veins of a 36 year old female with acute chorioamnionitis who died of amniotic fluid embolus, we reviewed uterine sections of 16 non-amniotic fluid embolus patients undergoing hysterectomy at or around the time of delivery to identify factors associated with the presence of amniotic fluid material in uterine or cervical veins. The indications for hysterectomy were as follows: placenta accreta, 9 cases; placenta previa, 1 case; uterine perforation/rupture, 3 cases; placental abruption, 1 case; ovarian carcinoma, 1 case; leiomyomata uteri, 1 case. The average number of H&E sections of uterus and cervix examined for each case was 10 (3-26). For the amniotic fluid embolus patient, 8 such sections were reviewed.

Results: Although amniotic fluid material (degenerating squamous cells, leukocytes, and bacteria) was identified in the uterine veins of the patient who died of amniotic fluid embolus, such material was not present in any of the sections of the other 16 uteri examined in this study.

Conclusions: Amniotic fluid material is not commonly found in the uterine and cervical vasculature of patients without amniotic fluid embolus, even if the uterus is removed for conditions associated with amniotic fluid embolus, such as placenta accreta and uterine rupture. This suggests that the etiology of amniotic fluid entering the uterine vasculature may be multifactorial or related to as yet unidentified factors. Further investigation into this event early in the development of amniotic fluid embolus may elucidate early avoidable risk factors for this potentially lethal complication of pregnancy.

863 ProEx C as a Marker of HPV-Associated Squamous Lesions of the Cervix

RE Badr, AE Walts, S Bose. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Based upon DNA microarray analysis, TriPath Imaging (Burlington, NC) recently developed ProEx C, an antibody that targets minichromosome maintenance protein 2 and topoisomerase II, two novel biomarkers associated with cervical neoplasia. Initial studies suggesting high specificity and sensitivity for detection of high grade squamous intraepithelial lesions (HSIL) in cytology liquid preparations have been reported. Our study was designed to assess ProEx C staining as an adjunct in the diagnosis and grading of cervical biopsies for HPV-associated SILs.

Design: Slides of 67 cervical biopsies were retrieved from our file. Patients ranged in age from 19 to 67 years (mean: 35 yrs). Based on H&E stains, the diagnoses were: negative (28), condyloma without overt dysplasia (8), CIN I (8), CIN II/III (23). Each biopsy and appropriate controls were immunostained for ProEx C in accordance with standard protocols and manufacturer's recommendations. Fifty-six of the cases were also immunostained for p16 (Biocare Medical) and Ki67 (Ventana) and subjected to in-situ hybridization (ISH) utilizing the Inform HPV Family 6 and 16 probes (Ventana). ProEx C was recorded as positive when >50% of lesional nuclei stained. P16 was recorded as positive (spotty >10% or band-like) and Ki67 as positive when >50% of lesional nuclei stained as previously described by us in a study of anal biopsies. ProEx C staining was correlated with H&E diagnoses, p16/Ki67 stains, and HPV ISH.

Results: ProEx C positivity was present in 50% condylomas, 25% CIN I, and 100% CIN II/III. None of the negative cases was positive for ProEx C. ProEx C positivity was strongly correlated with presence of HPV-associated lesions, high risk HPV DNA, Ki67 positivity, and p16 positivity ($p<0.001$ for each). As defined in our study, ProEx C positivity was associated with 100% sensitivity and 62.5% specificity for HSIL.

Conclusions: Our findings indicate that ProEx C positivity (a) is a reliable indicator of HPV-associated SIL, (b) is strongly associated with HR HPV and CIN II/III, (c) correlates significantly with p16 and Ki67 staining, and (d) is associated with 100% sensitivity but only 62.5% specificity for HSIL. Additional studies are warranted to determine if ProEx C positivity is predictive of disease progression.

864 Use of p57KIP2 and Ki-67 Immunohistochemistry and Interphase FISH in the Diagnosis of Hydatidiform Mole: A Unique Algorithmic Approach

TS Barry, PM Kim, DO Treaba, SE Tirrell, SJ Kussick, CH Tse, PL Kandalaft, AM Gown. PhenoPath Laboratories, PLLC and IMPRIS, Seattle, WA.

Background: There is significant interobserver and intraobserver variability in the diagnosis of hydatidiform mole, particularly those from the 1st trimester. Since distinguishing between partial/complete hydatidiform moles (PHM, CHM) and hydropic spontaneous abortions (SA) can impact patient treatment, the use of ancillary studies, including p57KIP2 and Ki-67 immunohistochemistry and interphase FISH, may be helpful in supplementing morphology in the diagnosis of hydatidiform moles.

Design: We retrospectively examined 191 cases (including 53 PHMs, 60 CHMs, and 78 SAs) that were submitted for routine evaluation to exclude hydatidiform mole. Histologic evaluation was performed using criteria established by Szulman and Surti (Am J Obstet Gynecol 132:20, 1978). P57KIP2 (57P06/KP10) and Ki-67 (MIB-1) immunohistochemistry and interphase FISH using a centromeric chromosome 17 probe (CEP-17, Vysis) were performed. Expression of p57KIP2 and Ki-67 was scored based

on the percentage of cytotrophoblasts positive as follows: 1-(0-25%), 2-(26-50%), 3-(51-75%), 4-(76-100%) with loss of p57KIP2 defined as <25% of cytotrophoblasts positive. Non-overlapping nuclei of villous stromal cells and cytotrophoblasts containing three hybridization signals were counted (average of over 400 nuclei per case). Cases displaying a mean of >5.4% of cells with 3 signals (threshold represents three standard deviations above the mean of a negative control group of SA cases) were considered positive for triploidy.

Results: Loss of p57KIP2 and high Ki-67-defined proliferative index were noted in 59 of 60 CHMs, whereas PHMs and SAs displayed no loss of p57KIP2 and had a significantly lower Ki-67-defined proliferative rate (2.33+/-1.26; p<0.05). As expected, an increased percentage of cells containing 3 chromosome 17 signals, indicative of triploidy, was noted in 29 of 29 PHMs (23.0 +/- 6.1%; n= 53), compared to CHMs (1.2 +/- 0.8%; n= 56) and SAs (2.0 +/- 1.0%; n= 77) which were diploid.

Conclusions: P57KIP2 and MIB-1 immunohistochemistry and interphase FISH are useful in the diagnosis of hydatidiform moles, and are particularly helpful in cases in which morphology alone may not be definitive.

865 Distribution of High-Risk HPV Subtypes in HGSIL Cervical Lesions from a Midwest, Affluent-Suburban, Screened Population

SK Bhagavathi, E Odish, S Forbes, NS Goldstein. William Beaumont Hospital, Royal Oak, MI.

Background: HPV 16 and 18 are considered to be the dominant high-risk (HR) subtypes in HGSIL lesions. There is substantial variation in the distribution of oncogenic HPV strains between populations. We assessed the distribution of type specific HPVs in HGSILs from a low-risk, regularly-screened, affluent-suburban population.

Design: 103 consecutively-accessioned HGSIL (CIN 2/3) formalin-fixed cervical biopsies/ LEEP cone specimens from 103 patients with recent prior cervical smears, and positive HPV PCR assay results were retrieved from our files. Distribution and type-specific prevalence and extent of multiple infections were assessed using the GP-E6/E7 primer set followed by four type-specific nested multiplex PCR reactions.

Results: The mean and median patient ages were 28.9 and 28 years, respectively (range, 17 – 62). 83 (80.5%) had single genotype infections. Only two cases (1.9%) had high-risk HPV (31, 33) coinfection with a low-risk HPV (6/11). The genotypes in order of frequency (%) were 16 (41.7%), 31 (12.6%), 18 (11.6%), 33 (6.8%), 35 (5.8%), 52 (4.9%), 58 (4.9%), 56 (2.9%), 42 (1.9%), 43 (1.9%), 39 (1%), 51 (1%), 59 (1%), 66 (1%), other (1%). Among the 10 HPV16+ multiple subtype cases, 3 were 16/43, 2 were 16/18, and one each had 16/31, 16/33, 16/51, and 16/52. Among the 48 (46.6%) non-16/18 cases, 40 (38.8%) were single genotype and 8 were multiple genotypes (7.8%). The mean patient age was similar in single and multiple genotype infection patients. Although the mean patient age of patients with HPV 16 was significantly younger than those with HPV 18 subtypes (25.8 vs 33.8 years, p=0.032), non-HPV16 subtypes were significantly more common in young patients (<23 years) than older patients (>32 years) (50% vs 71%, Fisher's exact, p=0.045).

Conclusions: Non-HPV16/18 subtypes constituted almost half of the strains found in HGSIL lesions in this midwest, suburban, affluent patient population. Additionally, younger patients significantly more often had non-HPV 16/18 subtype infections. Almost all multiple subtype infections were by multiple HR strains, coinfection of a HR HPV with low risk strain was rare. These results differ from other patient populations in which HPV-16/18 subtypes strongly dominate over other genotypes and are the predominant HR strains in young patients. The extent to which results of HPV subtype epidemiologic studies can be extrapolated from one population onto another may be limited. Similarly, the efficacy of HR-HPV vaccination may vary between populations.

866 Expression of Maspin in Ovarian Carcinoma, and Its Correlation with Vascular Endothelial Growth Factors A, C and D

F Bolat, F Kayaselcuk, D Gumurdulu, S Erkanli, H Zeren. Baskent University, Adana, Turkey; Cukurova University, Adana, Turkey.

Background: Maspin (mammary-specific serpin) is a 42 kD tumor suppressor protein which belongs to the serine proteinase inhibitor (serpin) family. Maspin is expressed in normal human mammary and prostate epithelial cells but is down-regulated during cancer progression. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a glycoprotein of 32-42 kDa. VEGFA, VEGFC, and VEGFD are the members of the VEGF family. VEGFA, plays essential role in vasculogenesis and angiogenesis. Both VEGFC and VEGFD have functions in angiogenesis as well as lymphangiogenesis. There is limited information on the role of Maspin,VEGFA, VEGFC, and VEGFD expression in ovarian carcinoma patients. This study was designed to investigate the correlation of maspin, vascular endothelial growth factor A,C,and D expression with clinicopathologic variables.

Design: We examined maspin, VEGFA, VEGFC, and VEGFD expression in 60 ovarian carcinomas (35 serous papillary carcinomas, 18 endometriod carcinomas and 7 primary ovarian mucinous carcinomas) tissues by immunohistochemistry. The degree of tumor cytoplasmic immunoreactivity was semiquantitatively by scoring the intensity of staining (0-3+) and percantage of positive cells.

Results: Maspin, VEGFC, and VEGFD overexpression was associated with a high tumor grade (p<0.001, p=0.004, p<0.001 respectively), clinical stage (p=0.002, p=0.01, p=0.001 respectively), presence of ascites (p<0.001, p=0.03, p=0.001 respectively), presence of metastatic lymph node (p=0.002, p<0.001, p<0.001 respectively).The expression of Maspin was correlated with expression of VEGFA (p=0.01), VEGFC (p<0.001), VEGFD (p<0.001) in ovarian carcinoma. VEGFA score was positively correlated with high tumor grade (p=0.04), clinical stage (p=0.009), maspin, VEGFC (p=0.003), and VEGFD (p=0.003), but was not correlated with presence of ascites and metastatic lymph nodes.

Conclusions: Maspin, VEGFC and VEGFD were expressed in ovarian tumors with poor prognostic parameters, and seem to play role in ovarian cancer angiogenesis, progression and lymph node metastasis. These results indicate that, in contrast to other carcinomas, maspin expression is directly associated with the biological aggressiveness of ovarian carcinoma. These results may offer new insights regarding the role of maspin in ovarian cancer that may also impact diagnosis and treatment strategies.

867 Differential Expression of Matrix Metalloproteinases in Ovarian Carcinoma

BD Bolton, S Bishu, J Symowicz, K Gleason, MS Stack, BP Adley. NU Feinberg School of Medicine, Chicago, IL.

Background: Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes whose function is to degrade collagen and other extra cellular matrix proteins in various biological processes. They not only play a crucial role in the physiologic remodeling of connective tissue that occurs in wound repair, but also have been shown to be involved in invasion and metastases by malignant tumors. Sakata et al (Int J Oncol. 17(4):673-81) demonstrated that MMP-2, MMP-9, and MMP-14 were more highly expressed in ovarian carcinomas than in borderline or benign ovarian tumors. The purpose of our study was to further characterize expression of MMP-2, MMP-9, and MMP-14 in specific histological subtypes (serous, endometrioid, clear cell, and mucinous) of ovarian carcinoma using immunohistochemistry and tissue microarrays.

Design: Tissue microarray blocks containing a total of 71 serous, 45 endometrioid, 18 clear cell, and 9 mucinous carcinomas were constructed with 1.5mm cores of formalin-fixed, paraffin-embedded archival tissue. Immunohistochemistry was performed by standard avidin-biotin technique using monoclonal antibodies for MMP-2, MMP-9, and MMP-14. Staining intensity was scored from 0 (negative) to 3+ (strong).

Results: Ninety-four percent of clear cell carcinomas (16/17) showed at least moderate (2+) expression of MMP-2 compared with 55% (39/71) of serous carcinomas, 49% (22/45) of endometrioid carcinomas, and 33% (3/9) of mucinous carcinomas. Similarly, 94% of clear cell carcinomas (17/18) showed at least moderate expression of MMP-14 compared to 46% (32/70) of serous carcinomas, 52% (23/44) of endometrioid carcinomas, and 55% (5/9) of mucinous carcinomas. Most carcinomas were negative to weakly (1+) positive for MMP-9 including 89% (16/18) of clear cell carcinomas, 72% (51/71) of serous carcinomas, 78% (35/45) of endometrioid carcinomas, and 77% (7/9) of mucinous carcinomas.

Conclusions: Expression of MMP-2 and MMP-14 appears significantly higher in clear cell carcinomas compared to non-clear cell ovarian carcinomas. There appears to be no differential expression of MMP-9 in any of the carcinoma subtypes. MMP-2 and MMP-14 may be especially important in clear cell carcinoma invasion and metastasis and at least partially explain the worse prognosis associated with ovarian clear cell carcinoma compared with other carcinoma subtypes.

868 Does the Knowledge That a Reflex HPV DNA Test for a Diagnosis of Atypical Squamous Cells Is Ordered Lower the Diagnostic Threshold of the Pathologist?

SEH Cameron, K Kjeldahl, C Curran, S Miller, B Thyagarajan, HE Gulbahce, SE Pambuccian. University of Minnesota, Minneapolis, MN; University of Minnesota Medical Center-Fairview, Minneapolis, MN.

Background: Since the introduction of reflex HPV DNA testing to triage women with Papanicolaou test (PT) diagnoses of ASC-US, there has been concern that reflex HPV testing would lower the threshold for diagnosing ASC-US. Such a lower threshold for ASC-US diagnosis would translate into lower HPV rates for women with ASC-US (and ASC-H) and a lower biopsy yield of significant lesions (CIN2/3). Our institution serves both clinics that prefer reflex HPV testing of all ASC-US (and some also ASC-H) diagnoses and others that prefer to order HPV testing on an individual basis. Since the fact that a reflex HPV test will follow is known to the cytotechnologist and the pathologist, it could result in a lower threshold for a diagnosis of ASC-US (and ASC-H) in PT that had a reflex HPV test ordered (the REF group) as compared to those that had and HPV test requested subsequently (the REQ group).

Design: All ASC-US and ASC-H PT with HPV tests performed between 12/01/2002 and 9/30/2005 were retrieved from our institution's databases. HPV DNA genotyping test result and whether the HPV test was a reflex or subsequently requested test were extracted, as were the results of subsequent cervical biopsies. During this interval HPV testing was performed with a PCR-based method on the residual liquid-based PT sample (Surepath) and the resulting genotypes were classified according to Munoz, et al, 2003. In each category (ASC-US and ASC-H) we compared the overall HPV+, hr-HPV+ rates as well as the CIN2/3+ rates.

Results: 8789 (6975 REF and 1814 REQ) ASC-US PT cases and 504 (377 REF and 127 REQ) ASC-H cases with HPV results were identified.

	ASC-US			ASC-H		
	REF	REQ	p-value	REF	REQ	p-value
HPV+	2342/6919 33.9%)	616/1790 (34.4%)	p=0.65	166/372 (44.6%)	65/127 (51.2%)	p=0.20
hr-HPV+	1568/6919 (22.7%)	427/1790 (23.9%)	p=0.29	135/372 (36.3%)	51/127 (40.2%)	p=0.44
biopsy rate	1611/6975 (23.1%)	543/1814 (29.9%)	p<0.001	223/377 (59.2%)	78/127 (61.4%)	p=0.65
CIN2/3+	200/1610 (12.4%)	62/541 (11.5%)	p=0.55	66/223 (28.3%)	30/78 (38.5%)	p=0.15

Conclusions: Our results show that the knowledge that a reflex HPV test will follow an ASC-US (and ASC-H) diagnosis did not significantly change the threshold for these diagnoses.

869 Expression of PAX2 in Endometrial Hyperplasia and Carcinomas: Immunohistochemical Analysis of 136 Cases

D Cao, AM Gown, RS Vang, RJ Kurman, BM Ronnett. The Johns Hopkins Hospital, Baltimore, MD; Seattle, WA.

Background: A paired box gene, *PAX2*, has been reported to be activated by estrogen and tamoxifen in endometrial carcinomas but not in normal endometrium, with activation associated with cancer-linked hypomethylation of the *PAX2* promoter (Wu et al, *Nature* 2005;438:981-7). However, PAX2 expression in normal and hyperplastic endometrium and endometrial carcinomas has not been systematically studied.

Design: Immunohistochemical analysis of PAX2 expression (nuclear staining) using a rabbit polyclonal anti-PAX2 (1:50 dilution, Zymed Laboratories Inc., San Francisco, CA) was performed on 136 endometrial specimens: non-atypical hyperplasia (simple or complex [SH/CH]; n=22), complex atypical hyperplasia (CAH, n=31), FIGO grade 1 endometrioid carcinoma (EC-G1; n=24), FIGO grade 2 endometrioid carcinoma (EC-G2; n=28), FIGO grade 3 endometrioid carcinoma (EC-G3; n=19), and serous carcinoma (invasive or endometrial intraepithelial carcinoma [SC/EIC]; n=12).

Results: Staining of lesional cells was semi-quantitatively scored: 0 = <5% , 1+ = 5-25%, 2+ = 26-50%, 3+ = 51-75%, 4+ = >75% (Table 1). Normal endometrial glands, when present, were uniformly positive (usually 4+, rarely 3+) and served as internal positive control.

Conclusions: PAX2 was uniformly expressed in normal endometrium and demonstrated progressive loss along the spectrum of endometrial proliferations considered pathogenetically related to unopposed estrogenic stimulation (hyperplasias and endometrioid carcinomas). In contrast, loss of expression in serous carcinomas was less frequent compared with endometrioid carcinomas. These results appear contrary to the concept of *PAX2* gene activation in carcinomas related to estrogenic stimulation, suggesting a need for further analysis, including correlation of PAX2 and ER expression with *PAX2* promoter methylation status, and comparison of results with this polyclonal anti-PAX2 with a monoclonal anti-PAX2.

Table 1: PAX2 expression in endometrial hyperplasia and carcinomas

Endometrial lesion	0	1+	2+	3+	4+
SH/CH (n=22)	4 (18%)	5 (23%)	3 (13%)	5 (23%)	5 (23%)
CAH (n=31)	17 (55%)	7 (23%)	2 (6%)	2 (6%)	3 (10%)
EC-G1 (n=24)	19 (79%)	1 (4%)	1 (4%)	0 (-)	3 (13%)
EC-G2 (n=28)	22 (79%)	1 (3%)	2 (7%)	0 (-)	3 (11%)
EC-G3 (n=19)	18 (95%)	0 (-)	0 (-)	1 (5%)	0 (-)
SC/EIC (n=12)	6 (50%)	4 (33%)	0 (-)	2 (17%)	0 (-)

870 High Grade Cervical Intraepithelial Neoplasia (CIN 2 & 3) Excised with Negative Margins by Loop Electrosurgical Excision Procedure: The Significance of Grade 1 CIN at the Margins of Excision

G Cardoza-Favarato, O Fadare. Wilford Hall Medical Center, Lackland AFB, TX.

Background: The prognostic significance the status of margins in loop electrosurgical excision procedure (LEEP) specimens is well-established when the variable in question is cervical intraepithelial neoplasia, grades 2 and 3 (CIN2/3). However, the significance of CIN 1 at the surgical margins of LEEP specimens has not been rigorously studied. Herein, we evaluate the significance of finding CIN 1 at one or more margins of a LEEP specimen when these margins are negative for CIN 2/3. Any potential relationship to glandular involvement and extent of CIN 2/3 was also investigated.

Design: Consecutive LEEP specimens with a CIN 2/3 diagnosis (and whose margins were negative for the latter) were retrieved and their slides were reviewed. The cases were classified as to whether CIN 1 was present at the margins, the extent of disease (using a >50% versus ≤50% threshold), and glandular involvement by CIN 2/3. Follow-up cytologic and histologic data were compared between patients with and without CIN 1 at the margin. For patients with multiple follow-up samples, only the most severe abnormality (1 sample) was counted, using the following scales: HSIL>LSIL>ASC-H>ASC-US (for cytologic samples) and CIN 3>CIN2>CIN1(for histologic samples).

Results: 73 cases were evaluated, with CIN 1 present at the margin in 59 (81%, Group 1) and absent in 14 (19%, Group 2). Follow-up information was available in 60 patients, with a median follow-up of 19 months. The cytologic follow-up of Groups 1 and 2 patients were not significantly different regarding the diagnostic frequency of any one of the Bethesda 2001 diagnostic categories ($p > 0.05$ for all, Fisher's Exact and X^2 tests). There were no statistically significant differences even after adjusting for glandular involvement and extent of disease. Of the 33 patients who received a biopsy and/or endocervical curettage during the follow-up period, 29 belonged to Group 1 and 4 to Group 2. The distribution of "Negative", "CIN 1" and "CIN 2/3" diagnoses for Group 1 were 16/29 (55%), 7/29 (24%) and 6/29 (21%) respectively. Parallel figures for Group 2 were 2/4 (50%), 2/4 (50%) and 0/4 (0%) respectively. Although CIN2/3 was more frequent in Group 1, none of the comparisons attained statistical significance.

Conclusions: Although this is a small study, our preliminary analysis suggests that the isolated presence of CIN 1 at the margin of a LEEP specimen whose margins are otherwise negative, lacks adverse prognostic significance.

871 Evidence That Many Primary Peritoneal Serous Carcinomas (PPSC) Originate in the Distal Fallopian Tube

JW Carlson, D Kindelberger, M Parast, MS Hirsch, A Miron, Y Lee, CP Crum. Brigham and Women's Hospital, Boston, MA.

Background: Primary peritoneal serous carcinoma (PPSC) presumably originates on the peritoneal surface, but in practice the diagnosis of this entity is made following exclusion of an ovarian, endometrial or tubal primary. We recently identified tubal intraepithelial carcinoma (TIC) as 1) the most common early serous carcinoma in BRCA+ women and 2) a common feature in women with ovarian serous carcinoma. This study examined the association of TIC with PPSC and their genetic relationship.

Design: Consecutive cases were selected which met the 2001 WHO criteria for PPSC. Fallopian tube involvement was assessed in two groups: 1) 26 consecutive archived cases with non-uniform sampling of the fallopian tubes; 2) 9 consecutive prospectively accrued samples with complete tubal exam, using a protocol for sectioning and extensively examining the fimbriated end (SEE-FIM). TIC was identified using published criteria and the frequency recorded for each group. In two cases, laser-capture-microdissected material from the TIC and peritoneal tumor was analyzed for p53 mutations by PCR and direct sequencing of exons 1-11.

Results: Thirteen of 26 (50%) cases in Group 1 involved the endosalpinx and 9 (35%) had TIC. Five of 8 (63%) cases in Group 2 with both tube available for analysis contained endosalpingeal involvement; 4 (50%) had TIC. TIC was typically fimbrial, confined to one region and demonstrated minimal tubal invasion. In the two cases analyzed, reproducible sequence-specific p53 mutations were shared by both TIC and peritoneal tumor.

Conclusions: TIC is frequently present in PPSC and is genetically linked to the latter. Therefor the fimbria is a candidate site of origin for many of these tumors. Thorough examination of the distal fallopian tube is recommended in all cases of presumed PPSC to more precisely determine the frequency of TIC and delineate this subset of pelvic serous carcinomas for further study. A model of tumor evolution permitting disparity in tumor size between the tube and peritoneum is presented.

872 Diagnostic Utility of ProEx™C in Evaluating Cervical Biopsy Specimens for High-Grade Dysplasia

B Carmichael, F Fan, M Danley, OW Tawfik, PA Thomas. KU Medical Center, Kansas City, KS.

Background: High-risk human papilloma virus (HR-HPV) is recognized as a cause of cervical carcinoma and its precursors. The Pap smear and HPV testing detect HPV infection by identifying HPV associated morphologic abnormalities and the presence of HR-HPV DNA respectively. However, the majority of HPV infections is associated with low-grade cervical intraepithelial neoplasia (CIN) and will spontaneously be cleared by the immune system. Only the high-grade CIN represents the true precursor for invasive cervical carcinoma. Many efforts have been made in identifying specific markers associated only with high-grade CIN lesions. ProEx™C (TriPath Imaging, Inc., Burlington, NC) containing antibodies against minichromosome maintenance and topoisomerase proteins has emerged as a promising new marker. It is used in evaluating aberrant S-phase induction, an important process occurring during transformation from low-grade to high-grade CIN. The aim of this study was to test the diagnostic utility of ProEx™C in routine cervical biopsy specimens.

Design: Immunohistochemical staining with ProEx™C antibody was performed on 34 cervical biopsy specimens retrieved from the surgical pathology file at KU Medical Center. The immunostaining results were interpreted by two pathologists independently without the knowledge of the biopsy diagnoses. A positive staining was identified as strong brown nuclear staining in squamous cells.

Results: Positive ProEx™C staining was confined to the basal layer of squamous epithelium in 24 cases, among them, 2 were normal and 22 were CIN I on histology. ProEx™C positivity was identified in the middle and upper third of squamous epithelium in 8 cases, all of which were diagnosed as CINII-III on histology. Positive ProEx™C was also strongly expressed in squamous cells present in the stroma representing 2 cases of invasive squamous cell carcinoma. Metaplastic squamous cells and HPV-associated koilocytes were negative for expression of ProEx™C.

Conclusions: This study proves a specific association between ProEx™C expression and high-grade CIN lesions. It can be used diagnostically in differentiating reactive/metaplastic squamous epithelium and low-grade CIN from high-grade CIN. More studies are needed in further exploring the clinical utilities of this promising new marker for cervical neoplasia.

873 Microsatellite Instability Is Associated with K-RAS Transition Mutations in Endometrioid Carcinomas

LL Catasus, A Ribe, A Gallardo, M Cuatrecasas, J Prat. Hospital de la Santa Creu i Sant Pau. Autonomous University of Barcelona, Barcelona, Spain.

Background: *K-RAS* mutations occurring in 10-30% of endometrioid carcinomas are point mutations mainly at codons 12 and 13 that may influence tumor development. PTEN loss and microsatellite instability (MI) are common alterations in uterine endometrioid carcinomas. The relationship between *K-RAS*, MI, and PTEN is controversial.

Design: Genomic DNA was obtained from 78 frozen (72 endometrioid and 6 mixed) endometrial carcinomas. Mutation analysis for *K-RAS* (codons 12 and 13) and *PTEN*, LOH at chromosome 10q23, and microsatellite instability (MI) for five different loci (BAT25, BAT26, D2S123, D5S346, and D17S250) were done. Tissue-arrays were made for immunohistochemical analysis of hMLH1 and hMSH2. Clinicopathologic data was obtained.

Results: *K-RAS* mutations were detected in 21% (16/78) of cases; 15 were pure endometrioid carcinomas (EC) and one was a mixed EC and clear cell carcinoma. PTEN alterations were detected in 54% (42/78), and *PTEN* mutations in 44% (34/78). MI was found in 38% (30/78). Of the sixteen *K-RAS* mutations, 11 were in codon 12, and 5 in codon 13. 69% (11/16) of cases with *K-RAS* mutations also exhibited MI (p=0.009). Five were transversions and eleven were transitions. Ten of the 11 transitions coexisted with MI, whereas only one transversion was associated with MI (p=0.013). PTEN alterations and *K-RAS* mutations coexisted in 69% (11/16) of cases (p=0.180), 8 with MI, and 3 without MI. The three alterations coexisted In 50% of cases (8/16 p=0.026).

Conclusions: Our results confirm a relationship between MI and *K-RAS* mutations, particularly transitions. On the other hand, association between *K-RAS* mutations and PTEN alterations may be due to their coexistence with MI.

874 Microsatellite Instability and PTEN Expression in Mucinous Endometrioid Adenocarcinomas

HP Cathro, MH Stoler. University of Virginia, Charlottesville, VA.

Background: Endometrioid adenocarcinoma (EAC) may occur as often as colon carcinoma (CC) in women with hereditary nonpolyposis colon cancer (HNPCC). These patients have germline mutations of DNA mismatch repair genes causing microsatellite instability (MSI). ~30% of sporadic EAC are associated with MSI due to somatic hypermethylation. HNPCC are typically right-sided and mucinous with better survival than usual CC; however, the data on MSI EAC are conflicting. This study of mucinous endometrioid carcinomas (MEC) examines MSI rates, loss of PTEN expression and survival.

Design: Mucinous EAC had intracytoplasmic mucin in >50% of sampled tumor. 20 mucinous EAC (4 Stage ≥2) were matched by grade with 21 conventional EAC (2 Stage ≥2). Immunohistochemistry for MLH1, MSH2 and PTEN was performed on the 2 EAC groups and on 3 groups of EIN/complex hyperplasias; 10 "atypical mucinous proliferations", 9 mucinous hyperplasias without atypia and 11 atypical complex hyperplasias.

Results: MEC had higher rates of MSI than conventional (48% vs 30% respectively) and higher associated rates of loss of PTEN expression (80% vs 67%) in the mucinous and non-mucinous MSI tumors respectively. Additionally two MEC had weak MLH1 expression, possibly correlating with MSI. 3 EAC (1 conventional and 2 mucinous) demonstrated clonal loss of MLH1 and/or PTEN expression. 10% of the atypical mucinous proliferation biopsies lacked MLH1 expression, but none did in the other two biopsy groups. Rates of loss of PTEN expression were similar in the 3 groups (50% in the atypical mucinous proliferations, 56% in the mucinous hyperplasias and 64% in the complex hyperplasia group). Overall average survival was 43 vs 33 mn after MEC and conventional EAC respectively ($p = 0.098$), with average survival after Stage 1 tumors 49 mn vs 33 mn in MEC and conventional EAC respectively ($p = 0.06$).

Conclusions: MSI is commoner in MEC than usual EAC and MSI-MEC have higher rates of loss of PTEN expression than MSI-EAC. One of 2 MEC with clonal PTEN loss demonstrated 2 different subclones; a dual-negative clone and a dual-positive clone in a background of MLH1–/PTEN+ carcinoma, suggesting that in this case the MSI preceded the PTEN abnormality (Fig 1). Survival is better in Stage 1 MEC than in Stage 1 conventional EAC.

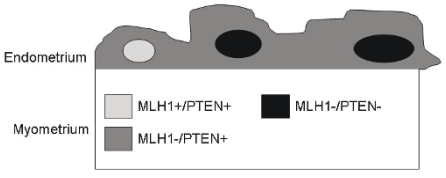


Figure 1
Subclones within Mucinous Endometrioid Adenocarcinoma

875 Immunohistochemical Comparison of Endometrioid Carcinomas of Ovary Versus Endometrium

I Chebib, DN Ionescu, A Alkushi, AM Gown, CB Gilks. University of Calgary and Calgary Laboratory Services, Calgary, AB, Canada; BC Cancer Agency, Vancouver, BC, Canada; King Fahad National Guard Hospital, Riyadh, Saudi Arabia; PhenoPath Laboratories and IMPRIS, Seattle, WA; Vancouver General Hospital, Vancouver, BC, Canada.

Background: Endometrioid adenocarcinoma arising in the ovary and endometrium are morphologically indistinguishable. The objective of this study was to identify differences in the immunostaining profile of endometrial and ovarian endometrioid carcinoma.

Design: A cohort of 131 sequential cases of ovarian endometrioid adenocarcinoma (with median follow-up of 12 years) and 156 endometrial endometrioid carcinoma (with median follow-up of 8.0 years) were reviewed for this study. Tissue microarrays (TMA) of endometrial and ovarian endometrioid carcinomas were constructed from formalin-fixed paraffin-embedded tissue. Immunostains for CK5/6, CK7, CK20, p16, p53, estrogen and progesterone receptor (ER, PR), WT1, Mesothelin, HBME, podoplanin, beta-catenin, e-cadherin, p-cadherin, and HER-2 were performed.

Results: Six of the immunohistochemical markers showed statistically significant differences in staining of ovarian and endometrial endometrioid carcinomas (Table 1).

Table 1. Immunostaining of Endometrial versus Ovarian Endometrioid carcinoma.

	Endometrium		Ovary		
Immunostain	# Positive	# Negative	# Positive	# Negative	p-value
p16	63	83	49	80	NS
ER	133	9	99	29	< 0.001
p53	16	120	39	90	< 0.001
CK 5/6	84	82	25	104	< 0.001
CK 7	131	11	117	10	NS
CK 20	0	149	3	124	NS
PR	131	19	88	14	NS
WT1	1	144	6	122	< 0.05
Mesothelin	50	91	35	95	NS
HBME	130	12	57	71	< 0.001
Podoplanin	20	122	9	120	NS
β-Catenin membrane	132	3	123	7	NS
β-Catenin nuclear	17	119	40	89	< 0.001
E-Cadherin	27	112	31	97	NS
P-Cadherin	117	17	117	12	NS
Her2Neu	3	128	7	121	NS

Conclusions: There are significant differences in the immunoprofiles of endometrioid carcinoma of ovarian and endometrial origin, however no single immunohistochemical stain or combination of stains has the ability to differentiate ovarian from endometrial primary site with certainty. Thus it is not possible to identify primary site in patients with endometrioid carcinomas of uncertain primary site, based on immunohistochemistry with the panel of antibodies used in this study.

876 14-3-3 sigma Protein Expression in Ovarian Sex-Cord Stromal Neoplasms: An Immunohistochemical Study of 60 Cases

L Chen, W Liu, B Yang. Cleveland Clinic, Cleveland, OH; Case Western Reserve University, Cleveland, OH.

Background: The 14-3-3 sigma protein is a p53-regulated G2/M inhibitor, which regulates numerous signaling pathways involved in cell cycle control and DNA repair. Additionally, 14-3-3 sigma also inhibits apoptosis through interaction with pro-apoptotic proteins such as Bax and BAD. Recent studies indicate that 14-3-3 sigma plays an essential anti-apoptotic role in maintaining the viability of immortalized granulosa cells in vitro. The expression of 14-3-3 sigma in non-epithelial ovarian neoplasms is largely unknown, and as such, we studied the expression of this protein in 60 cases of non-epithelial ovarian tumors.

Design: Using an anti-14-3-3 sigma antibody, we evaluated the immunohistochemical expression of this protein in 25 adult granulosa cell tumors, 2 metastatic adult granulosa cell tumors, 2 juvenile granulosa cell tumors, 6 Leydig/hilar cell tumors, 3 Sertoli cell tumors, 7 thecomas, 10 fibromas, 2 cellular fibromas, 2 primary ovarian endometrial stromal sarcomas, and 1 unclassified sex cord-stromal tumor. Eight ovaries with cystic follicles were included as controls. Perinuclear cytoplasmic stain was considered as positive. Percentage of positive cells and staining intensity (+ to +++) were recorded.

Results: Normal granulosa cells from the cystic follicles express 14-3-3 sigma protein with weak to moderate intensity. Overexpression (diffuse and strong staining) of 14-3-3 sigma protein was seen in 100% (29/29) of granulosa cell tumors and 100% (9/9) of Sertoli-Leydig cell tumors. There was no significant difference in staining intensity and percentage of positive cells between adult and juvenile and between primary and metastatic granulosa cell tumors. In contrast, expression of 14-3-3 sigma was not seen in fibromas (0/12), thecomas (0/7), ovarian endometrial stromal sarcomas (0/2), and unclassified sex cord-stromal tumor (0/1).

Conclusions: This study provides the first evidence for overexpression of 14-3-3 sigma protein in neoplastic granulosa, Sertoli and Leydig cells. Given the absence of this protein in ovarian fibroma-thecoma and other sex cord-stromal tumors, this may be a useful marker in facilitating the differential diagnoses of granulosa cell and Sertoli-Leydig cell tumors from other ovarian sex-cord stromal neoplasms.

877 Ovarian Mucinous Borderline Tumor of Intestinal Type: Can We Eliminate the Borderline Category?

AG Chiesa, E Veras, EG Silva, MT Deavers, L Posligua, A Malpica. The University of Texas M.D. Anderson Cancer Center, Houston, TX; The University of Texas Medical School, Houston, TX.

Background: Pathologists participating at the Borderline Ovarian Tumor Workshop sponsored by the National Cancer Institute in 2003 were willing to consider a more benign term than borderline for ovarian intestinal-type mucinous tumors (tus) currently designated as such if additional data could confirm their benign behavior. The purpose of this study is to present our experience with cases of ovarian mucinous borderline tus, intestinal type (OvMBTI) in which an adequate sampling and at least 5-year follow-up (f/u) (unless recurrent) were guaranteed.

Design: Thirty-four cases of OvMBTI, in which the above-mentioned criteria had been met, were retrieved from our department files. H&E slides were reviewed in all cases and the diagnosis of OvMBTI confirmed according to the WHO classification. Cases with intraepithelial carcinoma (Ca), expansile Ca or microinvasion were excluded. Clinical information was obtained from the patients' (pts') charts. Tu size, laterality, and the presence of pseudomyxoma ovarii (PO) were recorded. According to the ratio of number of sections of tu to the largest dimension of the tu, cases were considered either optimally sampled (one or more sections per cm) or adequately sampled (1 section per 1 to 2 cm).

Results: Pts' age ranged from 16 to 89 years (yrs), (mean 50 yrs). Tu size ranged from 8 to 39 cm (mean 20 cm). In 33 cases the tu was unilateral and in 1 case it was bilateral. PO was detected in 14 cases (focal, 10; multifocal, 4). Twenty-nine tus were optimally sampled and 5 were adequately sampled. Thirty-two pts had oophorectomies (O) and 2 pts had cystectomies (Cystx). FIGO staging was available in 27 cases, all of them with stage I disease. F/u ranging from 5 to 18 yrs (mean 10 yrs) was available in 33 cases: 31 pts had no recurrences and the 2 pts that had Cystxs recurred, were treated, and were free of disease after a f/u of 7 yrs. In a single case, the tu recurred 4 yrs after the initial diagnosis. This pt, who had had FIGO stage I disease and a tu optimally sampled without PO, developed numerous omental tus and pleural effusion. Cytologic examination of the latter showed a high-grade mucinous adenoCa. Imaging studies showed no evidence of a secondary primary.

Conclusions: In our opinion, the borderline category for OvMBTI cannot be eliminated since in a rare case malignant recurrences can occur (1/34 cases, 3%, in this series).

878 Epidermal Growth Factor Receptor (EGFR) Protein Overexpression Is Significant in Endometrial Carcinosarcoma (EMCS) in Contrast to Other Endometrial Carcinomas and Is Not Associated with EGFR Gene Amplification

DA Chitale, RA Soslow. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: EMCS are aggressive biphasic neoplasms characterized by a poor prognosis. EGFR tyrosine kinase has been implicated in the development and progression of many human cancers. Few studies exist in the literature studying EGFR expression in EMCS, all demonstrating high frequency of overexpression suggesting a potential therapeutic target, however, data is limited. The aim of the study was to determine the frequency of EGFR expression and its correlation with gene amplification in EMCS in contrast to other endometrial carcinomas (endometrioid adeonocarcinoma [EMC], clear cell carcinoma [CCS], papillary serous carcinoma [PSC]).

Design: Formalin-fixed paraffin-embedded tissues of 118 endometrial carcinomas were obtained and tissue micro-array blocks were constructed. The tumors were assessed for EGFR using immunohistochemistry (Zymed). EGFR immunopositivity was scored

as 0 to 3+, according to the manufacturer's guidelines. EGFR gene amplification was evaluated by chromogenic in-situ hybridization using commercially available kit (Zymed). Tumors were interpreted as positive for gene amplification when the average number of gene copies was > 5 per nucleus.

Results:

IHC for EGFR

Tumor type	0	1+	2+	3+	Total=118
EMCS	6 (16.2%)	6(16.2%)	17 (45.8%)	8 (21.8%)	37
EMC-FIGO grade 1	23 (85.2%)	2 (7.4%)	2 (7.4%)	0	27
EMC-FIGO grade 2	6 (60%)	3 (30%)	1 (10%)	0	10
EMC-FIGO grade 3	21 (77.7%)	4 (14.8%)	2 (7.5%)	0	27
CCS	3 (42.8%)	0	2 (28.6%)	2 (28.6%)	7
PSC	8 (80%)	2 (20%)	0	0	10

EGFR overexpression was present in 84% of cases of EMCS and was seen in both carcinomatous and sarcomatous components. Most EMC [76%], irrespective of the FIGO grade, EMCS with an adjacent EMC component [89%] and serous carcinomas [80%] did not express EGFR, but more than half of CCS were positive [57%]. None of the cases showed EGFR gene amplification.

Conclusions: Presence of EGFR over-expression in both components of EMCS supports the hypothesis of a common carcinogenic mechanism for both components. High frequency of over-expression and dismal prognosis make EMCS patients good candidates for trials of tyrosine kinase inhibitors. Gene amplification does not appear to be the mechanism for protein over-expression in EMCS and alternative molecular mechanisms seem to be involved in EGFR up-regulation. EMC and PSC appear to follow pathways other than EGFR for promoting tumor growth.

879 Microsatellite Instability at a Tetranucleotide Repeat in Invasive Squamous Cell Carcinoma of Uterine Cervix

YD Choi, JH Kim, C Choi, CS Park, SW Juhng, HS Choi, JH Nam. Chonnam National University Medical School, Gwangju, Republic of Korea.

Background: Although the role of human papillomavirus(HPV) in the development of cervical neoplasia is strong and independent of other risk factors, the pathogenesis is not completely explained. Defects in the DNA mismatch repair (MMR) system result in a mutator phenotype, which is manifested as microsatellite instability (MSI). A distinct type of MSI where microsatellite alterations are present at selected tetranucleotide repeat regions (elevated microsatellite alterations at selected tetranucleotide repeat, EMAST) has been recently described. But, the underlying genetic mechanism of EMAST is unclear. The *p53* gene plays a role in maintaining genome integrity by repairing damaged DNA. Therefore, we desired to investigate the prevalence of EMAST in invasive squamous cell carcinoma of the uterine cervix and to determine the correlation between EMAST and clinicopathologic parameters, HPV infection state or *p53* mutation.

Design: We examined the 3 mono-, 3 di-, and 5 tetranucleotide repeat markers in 47 cases of invasive squamous cell carcinoma (SCC) and also performed immunohistochemical staining for *p53*. HPV detection and genotyping was performed by using a commercially available HPV DNA chip (provided by Mygene Co., Seoul, Republic of Korea).

Results: Thirteen out of 47 cases (27.7%) were EMAST(+) with at least one of five tetranucleotide repeat markers. But, MSI at mono- and dinucleotide markers was noted in only one case (2.1%). EMAST was not related with stage, size, lymph node invasion, vascular/lymphatic invasion, or depth of invasion. Positive immunostaining for *p53* was significantly more common in EMAST(+) tumors than EMAST(-) tumors (P=0.04). HPV-infection was positive in 32 cases with a single genotype (25 cases (78.1%) of HPV-16, 4 (12.5%) of HPV-18, 1 of HPV-33, 1 of HPV-35, and 1 of HPV-58) and 2 cases with two genotypes (2 of HPV-16/18). However, EMAST was not correlated with HPV infection state or HPV genotype.

Conclusions: Our results showed that 27.7% of invasive SCC of the uterine cervix exhibited EMAST, and EMAST in invasive SCC of uterine cervix was significantly associated with *p53* mutation.

880 Morphologic and Immunohistochemical Patterns Induced by Neoadjuvant Chemotherapy in Epithelial Ovarian Cancer

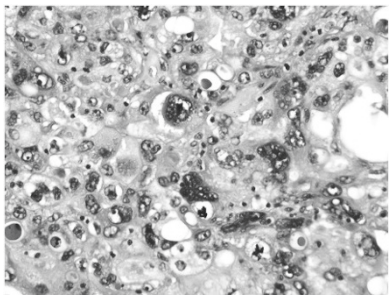
S Chopra, A Mahadavi, K Tewari, I Ramzy. UCI Medical Center, Orange, CA.

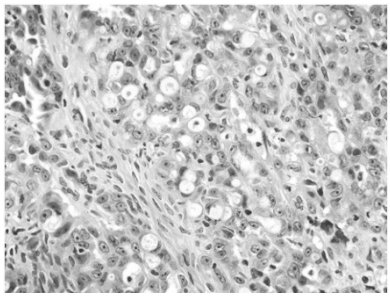
Background: Management of ovarian carcinoma involves cytoreductive surgery and adjuvant chemotherapy. Factors impacting survival include age, stage, debulking and speed of CA-125 declination. Neoadjuvant chemotherapy is used if debulking is not surgically or medically feasible. We examined tumor tissue exposed to neoadjuvant chemotherapy in an attempt to characterize histopathologic or immunohistochemical features, and identify parameters that correlate with clinical reponse.

Design: Histological sections were examined, in a double blinded study, from 21 patients treated with neoadjuvant chemotherapy. Based on clinical and imaging studies, responses to neoadjuvant therapy were classified as complete, partial and poor response. Parameters tabulated were nuclear grade, mitosis, cytoplasmic vacuolization, necrosis, fibrosis, histiocytes, apoptosis, multinucleation, chromatin, percentage of cells positive for CA-125, P53, Ki-67 and HER-2-neu. Results were analysed using Fisher exact test.

Results: 14/21 patients had papillary serous tumors. The remainder included clear cell, endometroid, mucinous, mixed and poorly diffrentiated carcinomas. Only nuclear grade (Fig 1)and degenerative cytoplasmic vacuoles (Fig 2) were significant as predictors of response: low nuclear grade and presence of vacuoles were associated with good response (p value = 0.0476). Other variables showed no significant difference between reponders and nonresponders.

Conclusions: Preoperative chemotherapy results in several morphologic changes in ovarian tumors. Nuclear grade predicts response to therapy, with high grade tumors showing poor response. Cytoplasmic vacuoles may predict better response. CA-125 serum levels, used as clinical indicators, do not correlate with % reactivity as depicted by immunohistochemical stains.





881 P16, a Sensitive Biomarker in HPV-Negative High Grade Cervical Intraepithelial Neoplasia

DW Cohen, NL Prescott, TS Scheidemantel, JA Brainard, RR Tubbs, B Yang. Cleveland Clinic, Cleveland, OH.

Background: Reflex testing for high risk (HR) HPV, triggered by ASCUS, has improved the sensitivity and specificity of cervical dysplasia detection. However, approximately 5% of high grade CIN lesions lack detectable HPV. In these cases, cytology-triggered histologic evaluation plays an important role in the pathologic detection of CIN lesions. Although p16 immunohistochemical expression has been used as a surrogate biomarker for high grade CIN associated with HR-HPV infection, its role in the detection of CIN2-3 in patients with negative HR-HPV has not been well examined. We present our findings of p16 expression in 30 HR-HPV-negative cases and correlate p16 expression with cytologic and histopathologic features.

Design: Between 2000 and 2006, surgical cases with a diagnosis of CIN 2-3 were reviewed and those cases negative for HR-HPV by Digene HC II were identified. Status of HPV in all cases was re-evaluated by in situ hybridization (ISH) with the new Ventana HPV detection system. A p16 immunostain was performed in all cases with available tissue. Slides were reviewed and diagnoses and p16 staining patterns recorded.

Results: A total of 516 cases of CIN2-3 had available either reflex or synchronous testing for HR-HPV. Of these, 484 (94 %) cases were HPV positive, and 32 (6%) cases were HPV negative. The Pap test showed an abnormality in all but one of the 32 HPV-negative cases with available cytology: 4 HSIL (13%), 6 ASC-H (20%), 4 LSIL (13%) and 15 ASCUS (50%). Follow up biopsies were performed in 29 cases and LEEP procedures were done on 24 patients. A p16 immunostain was performed on all 24 LEEP specimens and 22 biopsies with available tissue. Review of histopathology yielded the following: 22 cases of CIN2-3 or SCC, 5 cases of CIN1. Five borderline cases were interpreted as atypical immature squamous metaplasia (AIM). P16 immunostaining demonstrated 20 cases with a high grade CIN pattern, including 16 of the 17 cases of histologically diagnosed CIN2-3 and 3 cases of AIM. Three AIM cases were negative for p16 expression. Overall, based on histopathologic features and p16 staining patterns, final diagnoses were: 21 (65%) CIN2-3/SCC, 5 (16%) CIN1, and 6 (19%) negative.

Conclusions: Although cytologic abnormalities are the mainstay in directing clinical follow-up in HPV-negative cases, the specificity is relatively low. As a sensitive biomarker, the p16 immunostain will be useful in enhancing specificity in the diagnosis of CIN2-3 lesions in patients with undetectable HPV status.

882 Analysis of EGFR Status in Endometrial Stromal Sarcoma

P Cossu-Rocca, F Pili, A Festa, M Contini, A Mura, L Bosincu, M Brunelli, G Martignoni, G Massarelli. University of Sassari, Sassari, Italy; University of Verona, Verona, Italy.

Background: Endometrial stromal sarcomas (ESS) are rare neoplasms, which are currently classified in low grade ESS, with indolent growth, tendency to local recurrences and, more rarely, to metastasize, and undifferentiated endometrial sarcomas (UES), with a very aggressive behavior. Recently, Epidermal Growth Factor Receptor (EGFR) expression has been described in a large series of ESS, and a potential role of EGFR-targeted adjuvant therapies has been proposed. Aim of our study was to analyze EGFR immunohistochemical expression and EGFR gene amplification by FISH in a series of ESS.

Design: EGFR status was investigated in 10 ESS, including 7 low grade ESS and 3 UES. EGFR expression levels were assessed by immunohistochemistry with Dako EGFR pharmDX kit (DAKO, Glostrup, Denmark). Staining intensity and percentage of positive cells were scored for each case. Gene amplification analysis was performed with Dual-Color Fluorescence *in situ* hybridization (FISH) (Vysis, Downers Grove, IL, USA) with specific probes for EGFR gene (LSI Spectrum Orange) and Chromosome 7 centromeric region (CEP7 Spectrum Green). At least 50 neoplastic nuclei for each case were scored for both green and orange signals, and the ratio EGFR/CEP7 was evaluated. Only cases with ratios of 2 or higher were considered amplified.

Results: Nine out of ten (90%) ESS showed positive immunostaining. Six out of seven low-grade variants were positive, showing both cytoplasmic and membranous (5 cases) or only membranous staining (1 case). The staining intensity was interpreted as 3+ (3 cases), or 2+ (3 cases), with percentages of positive cells ranging from 60 to 80%. All three cases of UES were positive for EGFR, with membranous and cytoplasmic (2 cases) or only membranous (1 case) staining. The staining intensity was evaluated as 1+, 2+ and 3+, with percentages of positive tumor cells ranging from 50% to 80%. FISH analysis showed EGFR/CEP7 ratios constantly below the cut-off value, ranging from 0.9 to 1.3.
Conclusions: Our study confirms that EGFR is frequently over-expressed in ESS. FISH analysis did not show EGFR amplification in any of the tumors, thus EGFR over-expression in ESS should be related to different genetic mechanisms. Further studies are needed to identify specific EGFR genetic abnormalities, potentially useful to select patients who might benefit from current EGFR-targeted therapeutic options.

883 Low-Grade Endometrial Stromal Sarcoma (LG-ESS): Is There an Immunophenotype Predictive of Clinical Behavior?

S Dahiya, A Felix, P Branton, S Campbell, DC Sgroi, E Oliva. Massachusetts General Hospital, Boston, MA; Portuguese Oncologic Institute, Lisbon, Portugal; Inova Fairfax Hospital, Falls Church, VI.
Background: LG-ESS, derived from endometrial stromal cells, is associated with frequent recurrences and metastases. Estrogen and progesterone are important regulators of endometrial stromal function and aromatase (ARO) is a key enzyme in estrogen biosynthesis. It has been hypothesized that ARO expression correlates with aggressive behavior in LG-ESSs. Recently, PAX-2 has been identified as an effector of estrogen mediated endometrial carcinogenesis. In addition, cDNA microarray data have shown deregulation of genes involved in Wnt-signalling pathway, including beta-catenin (B-Cat) mainly in undifferentiated endometrial sarcomas. The goal of this study was to look for possible correlations between expression of estrogen (ER), progesterone (PR), androgen (AR) receptors, ARO, PAX-2, B-Cat and Wilm's tumor gene (WT-1) and tumor progression in LG-ESSs and its potential impact on prognosis and treatment modalities.
Design: Twenty two LG-ESSs were identified in patients ranging in age from 29-72 years. Ten were limited to the uterine corpus at initial diagnosis and remained disease-free at last follow-up (f/u) and 12 had either advanced disease at the onset (3), local recurrence (2), or metastatic disease (7) on f/u. Immunohistochemistry to quantify ER, PR, AR, ARO, PAX-2, B-Cat and WT-1 expression was performed and score-results from the product of staining intensity (1-mild, 2-moderate and 3-marked) and percentage of positive cells (0-10%-1, 10-50%-2 and >50%-3) were analyzed.
Results: ER and PR nuclear expression was seen in 19/21 and 21/21 tumors (score ≥6 in 16 and 20 respectively)(ER score 0-2 in 5 aggressive LG-ESS) and AR nuclear expression in 7/22 (score ≥6 in 4 LG-ESS, all with aggressive disease). B-Cat showed only membranous/cytoplasmic staining in 17/19 cases, and WT-1 showed nuclear staining in 10/21 cases (score ≥6 in 8, four of them either with metastasis or locally advanced disease). PAX-2 was seen in 2/22 (score ≤ 3), and ARO was negative in all 22 cases.
Conclusions: All LG-ESSs with high AR or low ER expression significantly correlated with an aggressive behavior. However, no significant difference in PR, ARO, B-Cat, WT-1 or PAX-2 expression was noted between the two groups. In contrast to published data, absence of ARO expression in this series may indicate that ARO inhibitors may only be beneficial in isolated cases.

884 Estrogen Receptor (ER) Co-Activators and Co-Repressors in Endometrial Carcinoma Associated with Tamoxifen Therapy

S Dahiya, E Carney, E Lopez-Varela, E Oliva, X-J Ma, DC Sgroi. Massachusetts General Hospital, Boston, MA; AviaraDx Inc, Carlsbad, CA.
Background: Tamoxifen (TX), a selective estrogen receptor modulator (SERM), demonstrates partial estrogenic activity in the uterus and has been associated with an increased incidence of endometrial cancer (EC) in breast cancer patients. Cell-type and promoter-specific differences in co-regulator recruitment have been shown to play a critical role in determining SERM function in breast and uterine tissues. Recently, microarray gene expression analysis has demonstrated that TX and estrogen have distinct but overlapping gene expression profiles. One overlapping gene, PAX-2, a paired-box transcription factor, mediates cell proliferation and tumorigenesis in the endometrium. The goal of this study was to compare estrogen-receptor (ER) co-activator, ER co-repressor and PAX-2 expression levels in sporadic ECs with TX-associated ECs.
Design: Formalin fixed paraffin embedded tissue blocks from 12 ECs {9 endometrioid (7 G1), 2 serous, and 1 clear-cell} arising in the setting of TX therapy (ECTX cohort) were matched (for subtype and grade) with a control cohort of ECs (ECCON cohort) arising sporadically (in the absence of TX therapy). RNA was extracted, followed by cDNA synthesis and quantitative real-time RT-PCR. Using the relative standard curve method, quantitative gene expression levels of PAX-2, ER, PR, ER co-repressors (HDAC-2, HDAC-3, HDAC-4, NCOR-1, NCOR-2) and ER co-activators (AIB-1, SRC-1, pCAF-1 and GRIP-1) were determined and a comparison performed between the ECTX and ECCON cohorts.
Results: The ER co-activators GRIP-1 and SRC-1, and the ER co-repressor HDAC-3 demonstrate a higher level of expression in sporadic ECs as compared with TX-associated ECs. No difference in PAX-2 expression levels were observed between the ECTX and ECCON cohorts.
Conclusions: Differential gene expression of a subset ER-coactivators and co-repressors is observed between sporadic and TX-associated endometrial carcinomas, but not in PAX2. These data suggest that differential ER coregulator recruitment may play a critical role in TX-induced endometrial carcinoma.

885 The Development of High-Grade Serous Carcinoma from Atypical Proliferative (Borderline) Serous Tumors and Low-Grade Micropapillary Serous Carcinoma – A Morphologic and Molecular Genetic Analysis

R Dehari, RJ Kurman, S Logani, I Shih. Johns Hopkins Medical Institutions, Baltimore, MD; Emory University School of Medicine, Atlanta, GA.
Background: We have proposed a model for the development of ovarian surface epithelial tumors. In this, all histologic types of surface epithelial tumors are divided into two categories designated Type I and Type II which correspond to two pathways of tumorigenesis. Type I tumors include low-grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, malignant Brenner tumor and clear cell carcinoma which develop slowly in a stepwise fashion from well-recognized precursors, namely "borderline" tumors. Type II tumors are high-grade rapidly growing tumors that typically have spread beyond the ovaries at presentation. They include high-grade serous carcinoma, malignant mixed mesodermal tumors and undifferentiated carcinoma. These tumors are rarely associated with morphologically recognizable precursor lesions and it has been proposed that they develop "de novo" from ovarian inclusion cysts. This model implies that the pathogenesis of Type I and Type II tumors are separate and independent but it is not clear whether some Type II tumors develop from Type I tumors.
Design: We attempted to address this issue by determining the clonality of six cases of high-grade serous carcinomas that were closely associated with serous borderline tumors and invasive low-grade micropapillary serous carcinomas.We reviewed 210 ovarian serous tumors from the surgical pathology files of the Johns Hopkins Hospital and identified two high-grade serous carcinoma that were directly associated with SBTs and four that were associated with invasive low-grade micropapillary serous carcinomas. Mutational analyses for KRAS, BRAF and p53 genes were performed on micro-dissected samples from the high-grade and low-grade tumor areas for each case.
Results: All six tumors demonstrated wild-type BRAF and p53 genes. Only two of the six cases were informative from a molecular genetic standpoint. In those two cases we found the same mutations of KRAS in both the SBT and the high-grade serous carcinoma component of the tumor, indicating a clonal relationship.
Conclusions: The majority of high-grade and low-grade carcinomas develop independently but in rare cases, a high-grade serous carcinoma may arise from a serous borderline tumor and an invasive low-grade serous carcinoma.

886 Peritumoral Lymphatic Vessel Density as a Prognostic Marker in Endometrial Carcinoma

KL Denning, JF Silverman, YL Liu, M Tung, RS Saad. Allegheny General Hospital, Pittsburgh, PA.
Background: Lymphatic invasion and nodal metastasis plays a major role in the spread of endometrial adenocarcinoma (EC) and serves as a major prognostic indicator for disease progression and a guide for therapeutic strategies. There is limited data evaluating the significance of lymphatic microvessel density (LMD) as a prognostic marker in patients with EC. In this study, we investigated tumor lymphangiogenesis, detected by D2-40, as a predictive marker for the risk of lymph node (LN) metastasis and its relation to other prognostic parameters in EC.
Design: Fifty-five cases of EC treated with total abdominal hysterectomy and pelvic LN dissection were reviewed. All cases were immunostained for D2-40 and CD31. Positively stained microvessels (MV) were counted in densely vascular/lymphatic foci (hot spots) at x400 field in each specimen (=0.17 mm^2) by 2 pathologists. Results were expressed as the highest number of MV count identified within any single field. Spearman correlation was used to compare MV count with FIGO grade, depth of myometrial invasion, presence of angiolymphatic invasion, LN metastases, pelvic washing cytology and clinical stage.
Results: Peritumoral LMD was significantly higher than intratumoral LMD ($P = 0.009$). D2-40 LMD count showed significant correlation with CD31 MV (mean 13 ± 7 and $17\pm7/0.17$ mm^2). There was a positive correlation of both D2-40 and CD31 MV counts with FIGO grade ($r=0.3$ and 0.36, $P<0.05$), and depth of invasion ($r=0.35$ and 0.33, $P< 0.01$). However, only D2-40 counts correlated significantly with the presence of angiolymphatic invasion, LN metastases and disease stage ($r= 0.36$, 0.31, 0.44, respectively). Angiolymphatic invasion was detected in 23/55 patients by D2-40, 20/55 by CD31 and 13/55 by routine H&E. Angiolymphatic invasion detected by D2-40 showed significant correlation with FIGO grade, LN metastases and stage of the tumor ($r=0.36$, 0.31, 0.43, respectively).
Conclusions: Our study showed that both angiogenesis and lymphangiogenesis play an important role in the progression of EC. Peritumoral LMD detected by D2-40 showed prognostic significance with positive correlation with FIGO grade, depth of invasion, the presence of angiolymphatic invasion, LN metastases and tumor stage. In addition, D2-40 increases the frequency of detection of lymphatic invasion relative to conventional H&E staining and the commonly used pan-endothelial marker, CD31. Therefore, D2-40 as an indicator for tumor lymphangiogenesis may serve as a prognostic marker in EC.

887 A Wide Spectrum of Serous Carcinomas Arise in Ovarian Serous Borderline Tumors: A Report of 25 Cases

A Dionigi, E Oliva, AJ Iafrate, DA Bell. Massachusetts General Hospital, Boston, MA.
Background: It has been suggested that low- and high-grade serous carcinomas (SC) arise via different genetic pathways. Low-grade SC probably arise via an "adenoma-borderline tumor-carcinoma" progression from typical (T) to micropapillary (MP) serous borderline tumor (SBT) to low-grade SC via alteration of the RAS-RAF signaling pathway secondary to mutations in *KRAS* and *BRAF*. High-grade SC probably arise from surface epithelial inclusion glands with *TP53* mutations. In this study, we report the clinicopathologic features of 25 cases of SC arising in SBT, which are being studied for *TP53*, *KRAS, BRAF* mutations.

Design: The clinicopathologic features of 33 serous carcinomas arising from SBT from 25 women were reviewed. The H and E stained slides were evaluated for the type of underlying SBT (MP, T or both) as well as the following features of SC: number of foci, maximum size of focus, presence of stromal invasion, different architectural stromal-epithelial patterns and cytologic atypia. Representative paraffin embedded blocks were obtained for molecular and immunohistochemical studies.
Results: The patients' mean age was 45 yrs (range 14-73 yrs). 48% of the patients were in FIGO Stage I, 12% in II, and 40% in III. There was peritoneal involvement in 14 cases (56%: 11 SC; 3 non-invasive). 17 (68%) SC were unilateral and 8 (32%) bilateral: the tumor mean size was 11 cm (range 2-23 cm). 7 morphologically different patterns of SC were observed. 4 types showed obvious stromal involvement: 7 SC(21%) had typical features of "stromal microinvasion", but the size of the foci exceeded 5 mm; 7 (21%) contained infiltrating micropapillae (mp) and nests with grade 1 nuclear features; 2 (6%) showed mp but had grade 2 nuclear atypia; and 3 (9%) contained inverted macropapillae. 3 patterns of SC had no obvious stromal invasion: 12 (37%) had confluent masses of epithelium with slit-like spaces and low nuclear grade; 1 had MP architecture with a higher nuclear grade; and 1 resembled conventional invasive grade 2-3/3 SC.
Conclusions: These data demonstrate that the histologic spectrum of SC that arise within SBT is wider than previously documented. Although many of the SC are well-differentiated and micropapillary, consistent with the presently accepted pathogenesis of low-grade SC, SC with high nuclear grade or extensive florid epithelial proliferation occur. Immunohistochemistry for p53 and molecular studies for *TP53*, *KRAS*, *BRAF* mutations of these cases are planned to better investigate the molecular pathogenesis of SC.

888 Immunohistochemical Comparison of Endocervical Adenocarcinomas and Endometrial Endometrioid Carcinomas

B Djordjevic, D El Demellawy, W Chapman. University of Toronto, Toronto, ON, Canada.
Background: Distinguishing endometrial and endocervical adenocarcinomas on biopsy specimens may be difficult. However, substantial differences in pathogenesis exist between these two tumor types. Retinoblastoma (Rb) and cyclin D1 proteins are both important factors in the regulation of the cell cycle. The majority of cervical adenocarcinomas are causally related to human papilloma virus (HPV), whose oncogenic form exerts some of its effects by interfering with the normal cell cycle control. It is therefore hypothesized that the expression of Rb and Cyclin D1 may differ in cervical carcinomas when compared to endometrial carcinomas; as such these markers may be potentially useful in discriminating between the two types of cancers.
Design: Specimens from patients with invasive or in-situ cervical adenocarcinomas of usual types (endometrioid, mucinous) and from patients with endometrioid endometrial adenocarcinomas from 2001 to 2006 were reviewed. Only cases where the primary site was clear were selected. Immunohistochemistry was performed on blocks containing tumor from 48 cases of cervical and 48 cases of endometrial adenocarcinoma. The markers assessed were Cyclin D1 and Rb, as well as more traditional markers used for the distinction of cervical and endometrial primaries: CEA, vimentin, ER and PR. The cutoff for positive cases was > 5% for CEA and vimentin (membranous), >10% for ER and PR (nuclear), >10% for Cyclin D1 (nuclear) and >75% for Rb (nuclear).
Results: 17% of the cases of cervical carcinoma stained positive (>75%) for Rb, while the majority of the negative cases showed reduced Rb expression (<50%). Diffuse Rb staining was noted in 98% of endometrial carcinomas. Cyclin D1 positivity was present in 29% of cervical carcinoma cases. The remainder of the cases demonstrated a complete absence of Cyclin D1 signal. In contrast, Cyclin D1 was diffusely expressed in 90% of endometrial carcinomas.
Conclusions: Cyclin D1 and Rb are expressed sufficiently differently in endocervical vs endometrial carcinomas to be useful in distinguishing between these two types of cancers in difficult cases. Rb is downregulated vs diffuse, while Cyclin D1 is negative vs diffuse. The patterns observed with these proteins are substantially more distinct than with traditional markers such as ER, PR, vimentin and CEA.

Comparison of staining profiles of endocervical adenocarcinomas and endometrioid endometrial carcinomas

	CEA % +	Vim % +	ER % +	PR % +	Cyclin D1 % +	Rb % +
cervix	82	13	52	23	29	17
endometrium	6	69	90	73	90	98

889 Primary Leiomyosarcoma of Vagina: A Clinicopathologic Study

X Duan, Z Qu, CT Abarracin, A Mapilca, M Deavers. University of Texas, Houston, TX; MD Anderson Cancer Center, Houston, TX.
Background: Primary vaginal tumors are unusual and leiomyosarcomas of the vagina are even more uncommon. Because of the rarity of this tumor, few studies of pathologic features and clinical behavior are available. The objectives of this study were to evaluate and correlate pathologic characteristics to the clinical course.
Design: The pathology database of MD Anderson Cancer Center, Memorial Hermann Hospital and Ben Taub General hospital were retrospectively reviewed in search of cases of vaginal leiomyosarcomas. We identified 31 patients with vaginal involvement of leiomyosarcoma and confirmed 10 cases of primary leiomyosarcoma of the vagina. Two pathologists independently reviewed archived slides of these selected ten cases to evaluate tumor margin, mitotic activity, nuclear atypia, and necrosis. Follow-up information was retrieved from patient records and ancestry registration.
Results: The age of patients at presentation ranged from 39 to 72 years with a mean age of 54. Most common symptoms at presentation were vaginal bleeding or mass. Six patients had complete excision of their tumor, one patient had total hysterectomy and tumor resection, one patient had radical resection with lymph node dissection and two patients had unknown surgery. Half of ten cases had infiltrating tumor margins and the remaining half had well circumscribed tumor margins. Tumor size ranged from 1 to 8 cm (mean of 4.9 cm). Two tumors had rare mitoses while the remaining tumors all had high mitotic counts ranging from 11 to 30/10HPFs. Eight cases had moderate to severe cytologic atypia and two had only mild atypia. Mitotic counts of these two cases with only mild atypia were high (13 and 30/10 HPFs). Follow-up information was available for five patients at this time of whom three died 5y to 6y after diagnosis, and one died 16 y after diagnosis. The last mentioned patient was young at diagnosis (47 years old) and had a tumor with very low mitotic activity. The only alive and well patient is a newly diagnosed young patient (44 years old) who had a small high grade tumor resected this year.
Conclusions: Pathologic features of primary leiomyosarcoma of the vagina are varied and the clinical behavior of most vaginal leiomyosarcomas is aggressive.

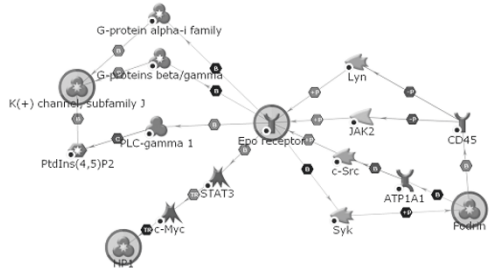
890 Dermatofibrosarcoma Protuberans of the Vulva: A Clinicopathological Study of 11 Cases

M Edelweiss, A Malpica. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: Dermatofibrosarcoma protuberans (DFSP) is a low-grade sarcoma with a high local recurrence rate. Rare cases have been described in the vulva and typically have been managed by wide local surgical excision. In this study, we present the clinicopathologic features of eleven cases of vulvar DFSP seen in our institution in a period of 28 years (yrs).
Design: Eleven cases of vulvar DFSP were retrieved from the files of the department of pathology at The University of Texas MD Anderson Cancer Center. Institutional Review Board approval was obtained prior to initiation of this study. Clinical information was retrieved from the patients' (pts') charts or from treating physicians. H&E stained slides were reviewed in all cases ranging from 1 to 40 per case (mean, 9 slides per case). Clinical presentation, tumor size, location, presence or absence of fibrosarcomatous transformation, and margin status were recorded. Immunohistochemical studies were performed as follows: CD34 (8 cases), and estrogen receptors and progesterone receptors (3 cases). Follow-up was obtained in all 11 cases.
Results: Pts' age ranged from 23 to 76 yrs (mean, 46.8 yrs). Clinically, 10 pts presented with a vulvar tumor and 1 with a pigmented skin lesion. The most common location was the labium majus (left or right). Tumor size ranged from 1.2 cm to 15 cm. Histologically, all the lesions had the typical features of DFSP. Fibrosarcomatous transformation was noted in three cases, all noted in recurrent tumors. The associated pigmented lesion seen in one case was represented by a melanoma in situ. CD34 was positive in the 8 cases tested and estrogen and progesterone receptors were negative in the 3 cases tested. After a follow-up, ranging from 2 months (mos) to 14 yrs, six pts had recurrences. Of these 6 pts, only one had had negative margins after the initial surgical procedure. One pt died of disease, one pt died of other causes, two pts are alive with disease, and seven pts are with no evidence of disease.
Conclusions: Vulvar DFSP is an uncommon tumor that occurs in a wide age range. Fibrosarcomatous transformation can be seen in tumors in this location. As described at other sites, recurrences appear to be related to the margin status of the initial surgical specimen.

891 Identifying Candidate Signalling Pathways That Confer Resistance to Therapy from Gene Expression Array Data for Ovarian Cancer

ME Edgerton, DH Fisher, Z Chen, JM Lancaster. MD Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; H. Lee Moffitt Cancer Center, Tampa, FL.
Background: Ovarian cancer is the eighth most common cancer in women but ranks fifth as the cause of cancer death in women. The high mortality rate is attributed to both delayed diagnosis and resistance to therapy. Identification of gene networks that confer resistance to therapy would allow for the introduction of novel molecular based therapies for this disease.
Design: Gene expression profiles were generated for tissue from 117 ovarian cancer patients using Affymetrix arrays. All patients were diagnosed with serous carcinoma, Stage III or higher. Eight-four (84) patients had a complete response and thirty- three (33) patients were non-responders (NR), as measured by the decrease in ca-125 following de-bulking surgery. Data were analyzed using Backward Chaining Rule Induction (BCRI) to build hypothetical gene networks relevant to classification as NR vs. CR. This was compared to published results comparing the same gene expression profiles to expression patterns generated from cell lines that over-express *myc, src, beta-catenin*, and *ras*.
Results: Using BCRI identify *src, syk, jak2*,and *lyn* kinases, two *G-protein* coupled pathways, and the *myc* oncogene as candidates for conferring resistance to therapy in serous ovarian carcinoma. In a separate study, gene expression profiles for model systems with dysregulation of *src, myc, ras* and *beta-catenin* were compared against the same data, and demonstrated that overexpression of *src, beta-catenin*, and *myc* were associated with a poor outcome.

Conclusions: These results support the utility of BCRI in identifying candidate mechanisms from gene expression array data. BCRI as a data mining strategy can be used to guide subsequent research of oncogenic pathway signatures to guide the use of targeted therapeutics. In addition, BCRI can identifying markers for the oncogenic pathways, thus suggesting immunohistochemical tests for identifying patients that would benefit from targeted therapy.



The potassium channel, subfamily J, the Epo receptor, HP1, and Fodrin (Spectrin) are all members of rules that are linked back to the non-responders of the ovarian cancer data. These genes appear to be markers for c-src, syk, jak2, lyn, myc and some of the g-protein signaling pathways, leading to the hypothesis that dysregulation of these signals may be involved in resistance to therapy.

892 Expression of the Urothelial Differentiation Markers GATA3 and Placental S100 (S100P) in Female Genital Tract Transitional Cell Proliferations

GE Esheba, TA Longacre, JP Higgins. Stanford University, Stanford, CA.

Background: The degree of urothelial differentiation in putative transitional (urothelial) proliferations in the female genital tract is still controversial. To further investigate the similarities (or dissimilarities) of female genital tract transitional proliferations and bladder urothelium, we evaluated the expression of S100P and GATA3, two proteins that are strongly expressed in bladder urothelial tumors.

Design: 25 Walthard cell nests (24 tubal and 1 ovarian hilus), 28 benign ovarian Brenner tumors, 1 mature teratoma with a benign urothelial proliferation, 2 proliferating ovarian Brenner tumors, and 2 ovarian transitional cell carcinomas (TCC) were identified through a review of pathology records from Stanford University Division of Surgical Pathology. Representative paraffin tissue blocks from each case were assessed using immunohistochemical markers for S100P and GATA3.

Results: Most Brenner tumors, including the proliferating Brenner tumor were positive for S100P (86.7%), whereas Walthard cell nests were negative or showed only scattered positive cells. Most Brenner tumors (78.6%) and Walthard nests also showed immunoreactivity for GATA 3. The teratoma-associated urothelial proliferation was negative for S100P but positive for GATA 3, similar to the staining pattern for Walthard nests. Both TCC were negative for S100P and one of two was weakly positive for GATA 3.

Transitional Cell Proliferation	S100P	GATA 3
	N/W/S/E	N/W/S/E
Walthard nest, fallopian tube (n=24)	18/0/0/6	2/10/6/6
Walthard nest, ovarian hilus (n=1)	0/1/0/0	0/1/0/0
Mature teratoma with urothelial differentiation (n=1)	1/0/0/0	0/0/1/0
Brenner tumor (n=28)	1/13/11/3	2/12/10/3
Proliferating Brenner tumor (n=2)	0/2/0/0	1/0/0/0
TCC (n=2)	2/0/0/0	1/1/0/0

N- negative, W-weak, S-strong, E- equivocal

Conclusions: Expression of S100P and GATA3 by a majority of ovarian Brenner tumors underscores the similarity between these neoplasms and urothelial proliferations of bladder origin. However, Walthard nests and ovarian TCC show an indeterminate phenotype.

893 Oncofetal Protein Glypican-3 Distinguishes Yolk Sac Tumor from Clear Cell Carcinoma of the Ovary

GE Esheba, LL Pate, M van de Rijn, TA Longacre. Stanford University, Stanford, CA.

Background: Clear cell carcinoma (CCC) of the ovary is the surface epithelial neoplasm most often confused with primitive germ cell tumors (GCT), particularly yolk sac tumor (YST) and dysgerminoma. OCT3/4 has proven to be a sensitive and relatively specific marker for the latter entity, but existing markers for the former differential diagnosis are limited. Recent studies suggest that glypican-3 (GPC3), an oncofetal protein expressed in fetal liver and malignant tumors of hepatocytic lineage, is also expressed in GCT, especially YST. To investigate whether GPC3 is useful in distinguishing YST from ovarian CCC, we studied the expression pattern of GPC3 in a large series of ovarian neoplasms and compared it with GCT.

Design: Tissue microarrays containing over 400 benign and malignant ovarian neoplasms, including 34 CCC were stained with monoclonal GPC3 (clone 1G12, Biomosaics, Burlington, VT). These arrays contained a wide assortment of ovarian surface epithelial neoplasms and sex cord stromal neoplasms, as well as GCT. A separate array containing only testicular GCT was studied in parallel. Full paraffin tissue sections from selected cases were also assessed.

Results: All YST, including those associated with mixed GCT were positive for GPC3, as were syncytiotrophoblastic giant cells, whereas all teratomas and embryonal carcinomas were negative. Both cytoplasmic and membrane staining were present in the positive cases, with no background staining. The syncytiotrophoblast of placental villi included in the arrays was also positive for GPC3. Almost all CCC (88%) were negative for GPC3, as were serous, endometrioid and mucinous tumors. All other tissues, including normal ovary were negative for GPC3.

Tumor Type	GPC3
	N/W/S/E
Clear cell carcinoma (n=34)	30/2/2/0
Endometrioid carcinoma (n=32)	30/1/1/0
Serous carcinoma, ovary (n=157)	156/0/1/0
Serous carcinoma, peritoneal (n=38)	38/0/0/0
Serous tumor of low malignant potential (n=59)	59/0/0/0
Mucinous carcinoma (n=10)	10/0/0/0
Sex cord stromal tumors (n=32)	32/0/0/0
Carcinosarcoma (n=4)	4/0/0/0
Undifferentiated carcinoma (n=2)	2/0/0/0

N-negative, W-weak, S-strong, E-equivocal

Conclusions: GPC3 appears to be a promising diagnostic marker for differentiating YST from ovarian CCC ($p<0.0001$). Since GPC3 may be associated with AFP expression, further studies are required to determine the utility of GPC3 in differentiating YST from clear cell tumors with hepatoid differentiation.

894 Sentinel Lymph Node Evaluation in Cervical Cancer

ED Euscher, A Malpica, MT Deavers. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Background: Pelvic lymph node (LN) status is an important prognosticator in cervical cancer (cx ca). Sentinel lymph node (SLN) biopsy is less morbid than radical pelvic LN dissection and may predict the regional LN status in cx ca. We present a series of patients (pts) with stage I-IIA squamous cell cx ca who underwent SLN biopsy.

Design: The institutional database was searched from 1998-2005 for all LN dissections with SLNs reported, and a total of 48 cases were identified. Clinicopathologic features recorded included: pts age, follow up, tumor size, presence/absence of vascular invasion, number and status of SLNs and nonSLNs, location of SLNs, and size of metastases in SLNs. Ultrastaging [5 HE at 40µm intervals, if negative→ pankeratin immunostain] was performed when all SLNs from a site were negative by standard processing.

Results: Table 1 contains the clinicopathologic features of the 48 identified pts. Table 2 contains the pathologic features of the 15 pts with positive SLNs. Ultrastaging detected metastasis in 3 SLNs, which were otherwise negative by routine processing. One patient had a false negative SLN biopsy. No pts with positive SLNs have died of disease.

Clinicopathologic Features of Pts with SLN

Feature	Positive SLN (n=15)	Negative SLN (n=33)
Age (years)	37.5 ± 6	41 ± 9
Follow up (median, months)	48, no documented deaths	52, one documented death
Tumor size (cm)	2.3 ± 1.3	2.3 ± 1.3
Vascular invasion	13/15, present	13/33, present
Total SLNs detected	76	127
Most common site	External iliac	External iliac
SLN laterality	13/15, bilateral	19/33, bilateral

Features of Positive SLN Cases

Case # (total SLN)	#pos SLNs	Met. Size (mm)	Ultrastaging	# pos/total non SLN
1 (11)	5	3.0-5.0	neg	0/15
2 (6)	5	unknown	N/A	0/4
3 (5)	1	3.0	neg	0/10
4 (2)*	1	Single cells	Single cells by immuno only	0/5
5 (5)*	1	<0.1	detected level 3	0/12
6 (2)	2	<0.5, 27	N/A	0/5
7 (8)*	1	1.0	detected levels 2-5	0/7
8 (5)	1	<0.1	neg	0/9
9 (2)	1	unknown	neg	0/11
10 (3)	1	unknown	neg	0/6
11 (1)	1	<1.0	N/A	0/9
12 (5)	1	4.5	neg	1/16
13 (7)	2	1.5, 1.6	neg	4/24
14 (4)	1	11.0	neg	1/12
15 (5)	2	unknown	neg	0/11

*metastasis detected only with US

Conclusions: The only feature possibly predictive of a positive SLN is vascular invasion. Ultrastaging improves detection of microscopic metastases with wide HE intervals most additive. Only 3/15 pts with positive SLN had positive regional LNs. In these patients, total tumor exceeded 3.0 mm, suggesting that metastasis size may predict regional LN status.

895 Cervical Biopsy p16^{INK4a} Immunostaining Patterns and the Relationship to Follow-Up LEEP Diagnoses

MF Evans, AN Kalof, E Brooks, L Simmons-Arnold, K Cooper. University of Vermont College of Medicine, Burlington, VT.

Background: p16^{INK4a} is a putative surrogate marker of high-risk (HR) human papillomavirus (HPV) infection and cervical lesion grade. Accordingly, p16^{INK4a} staining may aid the diagnosis of lesions of equivocal grade and/or identify lesions at increased risk for progression. The aim of this study was to examine the correlation of p16^{INK4a} staining patterns and HPV type among consecutively collected primary cervical biopsy samples with follow-up loop electrosurgical excision procedure (LEEP) data.

Design: One hundred and ninety-four unselected cervical biopsy samples were stained for p16^{INK4a} using the CINTech™ p16^{INK4a} Histology kit (DakoCytomation). Nuclear and cytoplasmic staining patterns of the squamous epithelium were graded as 'focal/sporadic' (FS), 'diffuse lower third' (LT), 'diffuse lower two-thirds' (LTT), or, 'diffuse full-thickness' (FT). HPV typing was performed by GP5+/6+ PCR and dot blot hybridization.

Results: A diagnosis of reactive/metaplastic changes was recorded for 107 samples: 50 (47%) tested HPV positive (49 HR); p16^{INK4a} staining was obtained for 30 (28%) samples (19 FS, 8 LT, 2 LTT, 1 FT). Ten samples were diagnosed with 'atypical metaplasia': 5 (50%) tested HPV positive (4 HR); p16^{INK4a} staining was obtained for 5 (50%) samples (1 LT, 3 LTT, 1 FT). CIN1 was identified among 38 samples: 23 (60%) were HPV positive (all HR); p16^{INK4a} staining was obtained for 29 (74%) samples (6 FS, 20 LT,

1 LTT, 2 FT). CIN2-3 was recorded in 39 samples: 35 (90%) tested HPV positive (all HR); $p16^{INK4a}$ staining was obtained for 39 (100%) samples (3 LT, 25 LTT, 11 FT). Of 31 reactive/metaplastic/atypical/CIN1 cases referred for LEEP, 13 were subsequently diagnosed with CIN2-3; all five patients with LTT or FT biopsy $p16^{INK4a}$ staining had a subsequent LEEP CIN2-3 diagnosis (P<0.008, Specificity 100%, Sensitivity 38.5%). Among the 31 cases, HR HPV was detected in 17 samples, and in 9 of the 13 with a subsequent LEEP diagnosis of CIN2-3 (P=0.275, Specificity 56%, Sensitivity 69%).
Conclusions: These data suggest that profuse $p16^{INK4a}$ staining in low-grade cervical lesions may be a highly specific but insensitive marker for high-grade lesion identification on a subsequent LEEP. HR HPV types are relatively common among all grades of cervical diagnoses restricting their positive predictive value. The findings support the possible use of $p16^{INK4a}$ staining in the diagnosis of equivocal/low-grade lesions that may be associated with CIN2-3 at subsequent LEEP, potentially aiding clinical management decisions.

896 Cervical Involvement by Non-Myoinvasive [Otherwise FIGO Stage 1A] Uterine Endometrioid Carcinoma: Incidence and Prognostic Significance

O Fadare, L Desrosiers, F Xiao, SA Wang. Wilford Hall Medical Center, Lackland AFB, TX; University of Massachusetts, Worcester, MA.
Background: In the current FIGO and AJCC staging systems, cervical involvement in an endometrial endometrioid adenocarcinoma [EEC] upstages a patient to stage II. However, cervical involvement is tightly associated with high tumor grade and deep myometrial invasion, which limits assessment of the impact of cervical involvement alone. Since cervical involvement in non-myoinvasive (otherwise stage 1A) EEC represents a potential decision point (at which some oncologists apply adjuvant vaginal cuff brachytherapy in patients that otherwise would only be observed postoperatively), we investigated the incidence and prognostic significance of this finding.
Design: Clinicopathologic data for all patients diagnosed with FIGO stage 1 EEC during a 9-yr period (1997-2005) were reviewed. Patients with stage1A EEC were then compared with patients who had non-myoinvasive EEC and who were upstaged solely due cervical involvement. Fisher's exact test was used for statistical comparisons at a p<0.05 threshold for significance.
Results: Of 345 stage 1 EEC diagnosed during this period, 109 were staged 1A [Group 2] and sufficiently documented. Only 4 patients were upstaged solely due to cervical involvement [Group 1, IIa n=2, IIb n=2] for an overall incidence of 4 (3.5%) of 113 non-myoinvasive cancers.One of 4 and 65 of 113 patients in Group 1 and Group 2 respectively were completely staged. Neither group had any case with positive lymph nodes. Lymphovascular invasion was present in 1/4 patients in Group 1 and 0/109 patients in Group 2. 3 of 4 and 2 of 109 Groups 1 and 2 tumors were, respectively, of architectural grade 3. Parallel figures for grades 1 and 2 were 1/4 and 0/4 (Group 1) and 90/109 and 17/109 (Group 2). At a mean follow-up of 3.7 and 5.3 years, there were 0 and 2 vaginal relapses in Groups 1 and 2 respectively. No significant differences were noted between these groups with respect to mean body mass index (35.9 vs 35.3, p=0.9) or patient age (55.2 vs 57.9, p=0.8).
Conclusions: Cervical involvement in non-myoinvasive EEC is rare (4/113, 3.5%). In this study, patients upstaged solely due to cervical involvement did not have higher relapse rates than patients with FIGO stage 1A disease. Furthermore, no distinctive differences were noted between these 2 groups. Our findings suggests a possible need to re-evaluate any adjuvant therapeutic measures being undertaken solely due to cervical involvement of a non-myoinvasive, otherwise stage 1A EEC.

897 MicroRNA Gene Expression Profiling in Human Ovarian and Primary Peritoneal Serous Carcinomas

RJ Flavin, S O'Toole, P Smyth, A Laois, S Cahill, J Li, K Denning, C Martin, S Finn, B Shepherd, K Lao, O Sheils, JJ O'Leary. Trinity College, Dublin, Ireland; Applied Biosystems, Foster City, CA.
Background: MicroRNAs (miRNAs) are a group of small non-coding RNA's approximately 22 nt in length. Over 320 miRNAs have been discovered in humans thus far. These molecules have been found to control cell growth, differentiation and apoptosis and have a role in cancer tumorigenesis acting as both oncogenes and tumour suppressor genes. Unique miRNA expression profiles having been able to classify various cancers, however ovarian and primary peritoneal serous carcinomas have not been specifically looked at.
Design: Total RNA was extracted from 6 fresh frozen serous carcinomas (3 ovarian and 3 primary peritoneal carcinoma) using the mirVANA™ miRNA isolation kit. All 6 tumours were of advanced stage and of high grade.A novel Applied Biosystems 330 multiplex stem –loop RT/PCR kit was used for miRNA gene expression profiling. Analysis of relative miRNA expression data was performed using the ΔΔCt method.
Results: A unique miRNA expression signature differentiated between ovarian and primary peritoneal serous carcinoma. Differentially upregulated miRNA's included mir-152 and mir-184. mir-187 and mir-205 were differentially upregulated in tumour with distant (pleural) metastases versus tumour with locally advanced (peritoneal) disease.mir-1 was differentially downregulated.
Conclusions: Several of the up/down regulated miRNAs may have a role in ovarian and primary peritoneal serous carcinoma pathogenesis.

898 A Candidate Precursor to Pelvic Serous Cancer (p53 Signature) and Its Prevalence in Prophylactically Removed Ovaries and Tubes from BRCA+ Women

AK Folkins, EA Jarboe, Y Lee, TA Ince, R Drapkin, CP Crum. Brigham and Women's Hospital, Boston, MA.
Background: Early serous carcinomas predominate in the fimbria of BRCA+ women and are associated with p53 mutations. p53-immunopositive epithelial foci in normal-appearing secretory tubal cells (p53 signatures) have been recently described in both benign and neoplastic fallopian tubes, may contain p53 mutations, and have been proposed as candidate precursors to serous cancer. This study compares the prevalence of p53 signatures in fully sectioned fallopian tubes and ovaries from BRCA+ women.
Design: Forty-five BRCA+ cases were studied; all were analyzed by a protocol to completely examine the ovaries and tubes, including sectioning and extensively examining the fimbriated end (SEE-FIM). A total of 332 blocks of ovarian and 297 blocks of tubal tissue were analyzed by immunostaining for p53. p53 signatures were defined as 12 or more adjacent nuclei with moderate to intense p53 staining (obscuring nuclear detail).
Results: p53 signatures were found in 12 of 45 cases (27%). Eleven of the signatures were associated with the fallopian tube, 9 of which were in the fimbria. Only 1 of 332 blocks of ovarian tissue was positive, and this consisted of a single cortical focus of p53-positive epithelium. Four carcinomas were present, and all of them were associated with the fimbria. In these four cases, no p53 signatures were seen in the ovarian cortex, although an implant of serous carcinoma was identified in one.
Conclusions: p53 signatures are rare on the ovarian surface and in cortical inclusions in BRCA+ women. The disparity in frequency between ovarian cortical and fimbrial p53 signatures validates the tubal origin of many BRCA+ serous carcinomas and supports the concept that the secretory epithelial cells (which are abundant in the tube and less common in the ovarian cortex) are more likely to be targeted. In this model, the site of tumor (ovary vs. tube) is determined by the location of the cells vulnerable to developing p53 signatures.

899 Reproducibility of the Diagnosis of Atypical Endometrial Hyperplasia (AEH) and Endometrioid Adenocarcinoma (EA) with Histologic Effects of Hormonal Therarpy of Medroxyprogesterone Acetate (MPA)

M Fukunaga, H Tsuda, A Sakamoto, T Kaku, K Ushijima, T Kamura, H Yoshikawa. Jikei University Daisan Hospital, Komae, Tokyo, Japan; National Defense Medical University, Tokorozawa, Saitama, Japan; Kyorin University, Mitaka, Tokyo, Japan; Kyushu University, Fukuoka, Japan; Kurume Medical University, Kurume, Fukuoka, Japan; Tsukuba University, Tsukuba, Ibaraki, Japan.
Background: A multi-instituitional Japanese group initiated a protocol to assess the efficacy of hormonal therapy of MPA with AEH and EA, grade 1(EAG1). The therapy is based on its ability to inhibit DNA synthesis and induce regression of abnormal proliferation. This study prospectively investigated the reproducibility of the diagnosis of lesions with hormanal effects by a panel of 3 gynecologic pathologists.
Design: 53 women under 40 years of age with referral hospital diagnosis of AEH or EAG1 (stage Ia) were entered into the protocol. After available slides were interpreted independently by each panel pathologist using the ISGP/WHO criteria, 22 patients with EAG1 and 17 with AEH were given MPA 600mg orally daily. Treatment continued for 26 weeks. Endometrial tissue was histologically assessed by the panel every 8 weeks. A total of 112 specimens were examined.
Results: For 68 specimens, unanimous agreement for any diagnosis was reached among the panel. Pairwise kappa values ranged from 0.54 to 0.77 with an overall kappa value of 0.51. Diagnostic problems identified included those related to application of diagnostic criteria including the presence or absence of architectural or cellular atypia, quantity of atypical area, complicated metaplasia, and a small quantity of tissue obtained after therapy. Complete response, defined as the absence of EA or hyperplasia, was observed in 10 patients with EAG1(45%) and 14 with AEH(82%) at 16 weeks and in one with EAG1 at 26 weeks. Two patients with EAG1 and 2 with AEH developed recurrence between 9 to 16 months after the therapy.
Conclusions: The efficacy of the treatment was proven and most responders showed complete response at 16 weeks. Reproducibility of the diagnosis was not sufficent. The presence or absence of architectural or cellular atypia, a small quanity of tissue, and complicated metaplasia caused diagnostic problems. Reproducibility must be improved and new diagnostic criteria applicable to horomonally affected EA and AEH are needed for precise evaluation of the fertility-sparing treatment.

900 Reproducibility of the Diagnosis of Atypical Endometrial Hyperplasia (AEH) and Endometrioid Adenocarcinoma (EA): A Multi-Institutional Study for Conservative Therapy

M Fukunaga, H Tsuda, A Sakamoto, T Kaku, K Ushijima, T Kamura, H Yoshikawa. Jikei University Daisan Hospital, Komae, Tokyo, Japan; National Defense Medical University, Tokorozawa, Saitama, Japan; Kyorin University, Mitaka, Tokyo, Japan; Kyushu University, Fukuoka, Japan; Kurume Medical University, Kurume, Fukuoka, Japan; Tsukuba University, Tsukuba, Ibaraki, Japan.
Background: Gynecologists determine therapy based on the WHO classification of endometrial hyperplasia and carcinoma, but the reproducibility of histologic diagnosis has been questioned. A multi-institutional Jananese group initiated a protocol among 16 institutions to assess the efficacy of hormonal therapy of AEH and EA, grade 1(EAG1). This study prospectively investigated the reproducibility of diagnosis by a referral hospital pathologist and by a panel of 3 gynecologic pathologists.
Design: Fifty-three women with a referral hospital diagnosis of AEH or EA based on biopsy or curettage were entered on this protocol. Available slides were assessed independently and interpreted by each of a panel of 3 gynecologic pathologists using the ISGP/WHO criteria. The diagnosis was then based on diagnostic concordance by at least 2 of the 3 panelists.

Results: The hospital diagnosis of AEH or EA was supported by the majority of the panel in 15/18 and 28/35, respectively. In 2 cases, hospital diagnosis of AEH was reclassified as EAG1, one was reclassifed as atypical polypoid adenomyoma (APA). 4 EAG1 were reclassified as AEH, 2 as EA, grade G2, (EAG2) and 1 as APA. Unanimous agreement for all diagnoses was reached among all 3 of the panel in 35/52 and 16 cases were agreed by two panelists. For the panel, pairwise kappa values for any diagnosis ranged from 0.59 to 0.76 with an overall kappa value of 0.59. Based on the diagnosis of the panel, 2 each of EAG2 and APA were excluded from the planned hormonal therapy. Diagnostic problems identified by the panel included those related to the application of diagnostic criteria including the presence or absence of stromal invasion and those related to a small quantity of tissue.
Conclusions: The reproducibility of hospital diagnosis is not satisfactory. Both under- and over diagnosis are very common. However, the histologic selection of patients (with AEH or EAG1) eligible for the hormonal therapy was properly done based on the panelists' histologic diagnosis. Central pathological review is essential for multi-institutional studies.

901 Can HPV 16 Typing Adjudicate the Accuracy of Cervical Biopsy Interpretation?

MT Galgano, PE Castle, MH Stoler. University of Virginia, Charlottesville, VA; National Cancer Institute, NIH, DHHS, Bethesda, MD.
Background: ASC to SIL ratios and HPV typing can be used to quality control cervical cytology; however, clinical management is most often based on the subsequent colposcopic biopsy. Yet, interobserver variability of histologic interpretation is no better than that of cytologic interpretation. This is especially relevant for CIN2, a diagnosis which crosses a critical threshold for therapeutic decisions while having the least reproducibility. Since high-risk HPV is prevalent across all CIN, yet HPV 16 prevalence varies with grade, we propose using the fraction related to HPV 16 as a variable for assessing diagnostic accuracy.
Design: From women enrolled in ALTS, we compared HPV 16 prevalence, as detected by PCR from cervical sampling, to the subsequent biopsy diagnosis, as determined by the consensus interpretation. The HPV 16 prevalence was similarly compared to the diagnostic interpretations of each individual pathologist, and averaged within the two groups of pathologists (clinical center (CC), and quality control (QC)).
Results: Stratified by the consensus diagnosis of 4854 biopsies, HPV 16 prevalence is 9.76% in negative, 18.39% in CIN1, 37.3% in CIN2, and 60.94% in CIN3. While the averages for the CC group were not that different from the QC group, or the consensus standard, the ranges for each group were quite broad, with CC having significantly more variability than QC. The range of HPV 16 prevalence rates across the CC group is 4.48-11.49% in normal, 9.26-22.78% in CIN1, 29.27-66.67% in CIN2, and 47.73-79.17% in CIN3. The range for the QC group is 5.47-10.65% in normal, 14.05-17.94% in CIN1, 38.93-47.71% in CIN2, and 56.94-68.12% in CIN3.
Conclusions: There is substantial variation in biopsy interpretation even among experts. HPV 16 prevalence is correlated with biopsy severity; with CIN2 having rates much more like CIN3 than CIN1. Monitoring HPV 16 fractions on populations of biopsy interpretation can help adjudicate diagnostic variation and allow benchmarking of individuals or groups of individuals relative to important treatment or management thresholds.

902 *PIK3CA* Mutations Are Associated with *PTEN* Alterations and Correlate with Microsatellite Instability in Endometrial Carcinomas

A Gallardo, LL Catasus, A Ribe, A Gonzalez, J Munoz, M Cuatrecasas, J Prat. Hospital de la Santa Creu i Sant Pau. Autonomous University of Barcelona, Barcelona, Spain.
Background: The p110a catalytic subunit of phosphatidylinositol 3'-kinase (PI3K), encoded by *PIK3CA*, acts as an oncogene that activates several downstream targets including Akt. PTEN counteracts the activity of PI3K and is frequently mutated in endometrial carcinoma (EC). Simultaneous mutations of *PIK3CA* and *PTEN* have been reported. We investigated the coexistence of *PIK3CA*, *PTEN*, *K-RAS,* and B-catenin gene mutations in a series with known microsatellite instability (MI) status and *PTEN* LOH.
Design: Genomic DNA was obtained from 64 frozen (59 endometrioid and 5 mixed) EC. Mutational analyses for *PIK3CA* (exons 9 and 20), *PTEN*, *K-RAS* (exon 1), and B-catenin gene (exon 3) were performed. Also, *PTEN* LOH at chromosome 10q23 and microsatellite instability (MI) for five different loci (BAT25, BAT26, D2S123, D5S346, and D17S250) were done. Tissue-arrays were done for immunohistochemical analysis of pAkt, PTEN, mTOR, ER, PR, neu, B-Catenin, bcl2, p53 p27, MMP7, E-cadherin, cyclin-D1, hMLH1, and hMSH2. Clinicopathologic data was obtained.
Results: *PIK3CA* mutations were detected in 25% (16/64) of cases. Nine were in exon 20 and 9 in exon 9. Two cases carried double mutations. All mutated cases were pure endometrioid carcinomas and 50% were grade 3. PTEN alterations (either mutation or LOH) were detected in 45% (29/64), MI in 39% (25/64), *K-RAS* mutations in 20% (13/64), and B-catenin gene mutations in 19% (12/64) of cases. *PIK3CA* mutations coexisted with PTEN alterations in 56% (9/16), MI in 37% (6/16), *K-RAS* in 12% (2/16), and B-catenin in 6% (1/16). *PIK3CA* mutations were associated with strong pAKT immunoexpression in 70% (7/10) of cases.
Conclusions: Our results show that *PIK3CA* mutations are frequent in high-grade endometrioid carcinoma, coexist with PTEN alterations in 56%, and pAKT overexpression in 70% of cases. We also found moderate correlation of *PIK3CA* mutations with MI (37%) and occasional coexistence with *K-RAS* and B-catenin gene mutations.

903 Assessment of TTF-1 Immunoreactivity in Ovarian and Uterine Carcinomas and Its Potential Impact on the Differential Diagnosis

HG Gao, VA LiVolsi, T Pasher, LA Litzky, PJ Zhang. Hospital of the University of Pennsylvania, Philadelphia, PA.
Background: Thyroid transcription factor-1 (TTF-1), a 38kDa protein, belongs to the NKx2 family of DNA-binding proteins, highly expressed in normal and neoplastic tissues of thyroid and lung origins. TTF-1 has been widely regarded as a reliable marker for differentiating adenocarcinoma of the lung from those of other origins. The notion that TTF-1+ adenocarcinoma is lung primary has been well accepted in the current practice. However, TTF-1expression has been reported in rare cases of ovarian serous carcinoma (OSC) in a small series using TMA tissue. TTF-1 expression has not been thoroughly investigated in adenocarcinomas of gynecological (GYN) tract in large series using routine surgical specimens (RSS).
Design: Paraffin sections of 194 primary endometrial and ovarian carcinomas, 94 RSS and 100 TMA samples, were immunostained with a monoclonal antibody against TTF-1 with heat-antigen retrieval on DAKO autostainer. For RSS, there were 54 OSC, 2 ovarian endometrioid (OEC), 3 ovarian mucinous (OMC) and 6 ovarian clear (OCC) and 43 uterine endometrioid (UEC) carcinomas. For TMA, there were 20 cases for each OSC, OEC, OMC, OCC and ovarian poorly differentiated carcinoma (OPDC). Immunostain for surfactant was also performed in cases tested positive for TTF-1.
Results: Of total 194 cases, TTF-1 nuclear reactivity was detected in 6; 3 OSC (4%), 1 UEC (3%), 1 OEC (4%), and 1 OCC (4%). Five of these 6 cases were RSS and the one was TMA specimen. Immunoreactivity was strong and diffuses in all cases. No TTF-1 expression was detected in any non-neoplastic ovarian or endometrial tissues. Surfactant was negative in all TTF-1 positive cases. The histological features and clinical presentations and follow-ups of all TTF-1+ cases were reviewed and support an ovarian or endometrial primary in all cases.
Conclusions: OSC, OCC and endometrioid carcinomas of both ovary and uterus can express TTF-1. The incidence of TTF-1 expression in ovarian and endometrial tumors is no more than 5%, but when present, it is strong and diffuse as seen in thyroid and lung tumors which is unusual for aberrant expression. Although it is not common, if unaware of, this strong TTF-1 reactivity can potentially mislead a pathologist to conclude that the tumor is of lung primary, particularly when lung is considered as possible primary site. The biologic significance of the strong TTF-1 expression in GYN tumors is unclear and warranted for further investigation.

904 Utility of Trichrome and Reticulum Stains in Characterizing Necroses in Uterine Smooth Muscle Tumors

HG Gao, VA LiVolsi, PJ Zhang. Hospital of the University of Pennsylvania, Philadelphia, PA.
Background: Coagulation necrosis (CN) has been considered as one of the main diagnostic features of uterine leiomyosarcoma (ULES). On routine H&E section however, it can be difficult to differentiate CN from the hyaline necrosis (HN) commonly seen in uterine leiomyoma (ULEA). To differentiating these two types of necrosis, much of the focus is placed on morphologic evaluation of the cellular elements with little attention to the changes in the extracellular fibrous network (EFN) of the areas which could be hard to appreciate on H&E section. We evaluated EFN changes in CN and HN in uterine smooth muscle tumors (USMT) on Masson-trichrome (MT) and reticulum (RC) stains.
Design: MT and RC stains were performed on 25 USMT including 12 ULEA, 1 smooth muscle tumor of uncertain malignant potential (STUMP), and 12 ULES. HN or CN was found in all ULEA or ULES respectively. The necrotic areas of the STUMP were hard to classify at the time of the diagnosis. EFN changes were evaluated in the necrotic areas in comparison to the viable tumor and normal myometrium.
Results: MT and RC staining differentiated the acellular fibrous matrix from necrotic tissue and degenerated red cells. RC highlighted the delicate/intact EFN wrapping individual cells in the myometrium, viable tumor and HN in USMT. The EFN was broken down and lost in CN of all but one ULES in which CN area was no longer present on section. In addition, because of the better separation of extracellular matrix and cells, the integrity of individual cells was better appreciated in necrotic areas on MT and RC stains. In HN of ULEA, the ghost cells were "intact" with little difference from cells in the viable area while cells in all CN were fragmented and degraded. With the aids of MT and RC stains, HN was also detected coexisted with CN in 5 (40%) ULES and focal EFN breakdown but with intact ghost cells in 1 (8%) ULEA, and areas of CN and HN in the STUMP case.
Conclusions: MT and RC are better staining to evaluate EFN and cellular change in necroses than H&E. It is easier to differentiate acellular fibrous matrix from necrotic cellular elements and degenerated red cells, all of which otherwise appear to be amorphous hypereosinophilic ground material on H&E stain. The EFN and cells are intact in HN and broken-down and lost in CN. HN and CN can be assessed and differentiated from each other more objectively and reliably on MT and RC stains which can be very useful in the diagnosis of USMT when difficulty in differentiating HN vs. CN arises.

905 Potential Use of p53, ER and CA125 Immunohistochemical Panel in Distinction between Endosalpingiosis and Metastatic Carcinomas

MT Garcia, P Ganjei-Azar. University of Miami, Miami, FL.
Background: Benign Mullerian inclusions or endosalpingiosis is encountered in approximately 5-25% women in pelvic, inguinal and periaortic lymph nodes as well as in peritoneal tissues overlying the uterus, salpinx, ovary, colon, appendix, and omentum.These inclusions consist of small glands lined by a simple, often ciliated cuboidal or columnar mullerian-type cell with bland nuclei. In patients with diagnosis of adenocarcinoma, these inclusions may be mistaken for metastatic adenocarcinoma. The aim of this study is to evaluate the use of ER, P53 and CA125 immunohistochemistry, as helpful markers for endosalpingiosis.

Design: We evaluated fifteen cases of endosalpingiosis encountered in lymph nodes (3) and in different peritoneal surfaces (12) from women diagnosed with benign gynecological pathology (8), borderline serous neoplasm (4), papillary serous carcinoma (PSC)(2), and cervical adenocarcinoma (CAC)(1). As control group, ten cases of gynecological and gastrointestinal malignancy (3 CAC, 2 colonic adenocarcinomas, 2 PSC and 3 endometrial endometrioid adenocarcinomas (EEA)) were also stained. Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded 4-micron sections using the following primary antibodies: ER (DAKO, 1:25, mouse), CA125 (DAKO, 1:50, mouse) and P53 (EMD Biosciences Inc., 1:250, mouse).
Results: Of the fifteen endosalpingiosis cases, thirteen (86.6%) cases stained negative for P53 and positive for ER and CA125, one (6.6%) stained negative for P53 and ER and positive for CA125, and one (6.6%) stained negative for P53 and CA125 and positive for ER. All the fifteen cases were negative for P53. All the CAC were negative for ER, positive for CA125 and one was focally positive for P53. All the colonic adenocarcinomas were negative for ER and CA125 and one was positive for p53. All the EEA stained positive for ER and CA125; two were negative for P53 while one high-grade tumor was positive for this antibody. All the PSC were positive for CA125, negative for P53 and one was ER negative.
Conclusions: The P53 negative, ER positive and CA125 positive immunophenotypic profile supports the diagnosis of endosalpingiosis. It also excludes malignancies that are usually associated with P53 positivity and/or ER negative such as colonic adenocarcinoma, high-grade endometrioid adenocarcinoma and PSC as well as endocervical adenocarcinomas. Low-grade endometrial and ovarian tumors could potentially represent a diagnostic difficulty.

906 Endometrial Endometrioid Adenocarcinoma (EEA): A Pathological Analysis of 827 Consecutive Cases

Z Ghorab, S Nofech-Mozes, N Ismiil, I Ackerman, G Thomas, A Covens, MA Khalifa. University of Toronto, Toronto, ON, Canada.
Background: Histopathologic features of EEA have not been comprehensively reviewed in the past two decades. The aim of this study is to update the prevalence of the tumor's pathologic features with special emphasis on the association between its grade and other prognosticators.
Design: In a population-based study we assessed the various clinical and pathological features of 827 patients with pure EEA accessioned in our department (July 1999 – June 2004). All patients were treated with hysterectomy. Tumor grade was reported in a 2-tiered system, with a "high grade" defined as having > 50% solid areas. Grade, pathological stage, depth of myometrial invasion, presence of cervical involvement, lymphovascular invasion (LVI) and lymph node status were evaluated.
Results: The median age at diagnosis was 62 years (range 30-94). Forty-three patients were premenopausal. In 85 patients, lymph nodes were sampled. The tumor was low grade in 733 patients (88.6%) and high grade in 94 (11.4%). Tumor pathologic stage distribution was as follows: IA,149 (18%); IB, 343 (41.5%); IC, 151 (18.2%); IIA, 55 (6.6); IIB, 82 (9.9%); III, 42 (50.8%); and stage IV, 5 (0.6%) cases. The tumor was confined to the endometrium in 154 (18.6%) cases, invaded into the inner myometrial half in 424 (51.3%) cases and the outer half in 249 (30.1%) cases. Cervical involvement was observed in 171 (20.7%) patients and LVI was noted in 182 (22%) cases. The associations between tumor grade and depth of myometrial invasion, cervical involvement and LVI are shown in Table 1. Lymph nodes were sampled in 85 patients. Lymph node metastases were observed in 13 (1.6%) cases and ovarian metastases in 15 cases (1.8%). Chi-square test showed that high tumor grade was significantly associated with deep myometrial invasion ($p<0.0001$), cervical involvement ($p=0.0065$) and LVI ($p<0.0001$).
Conclusions: Endometrial endometrioid adenocarcinoma presents most commonly with a low histologic grade. In approximately 70% of the patients, the tumor does not invade into the outer half of the myometrium. High tumor grade is significantly associated with deep myometrial invasion, cervical involvement and LVI.

Histologic characteristic per tumor grade

Variable	Low Grade (n=733)	High Grade (n=94)
Myometrial Invasion		
Absent	152	2
Inner half	387	37
Outer half	194	55
Cervical involvement		
Glandular	60	9
Stromal	81	21
Lymphovascular involvement	131	51

907 EGFR and HER2/neu Expression in Stage Matched Type 1 and Type 2 Endometrial Carcinoma

V Gowda, S Logani. Emory University Hospital, Atlanta, GA.
Background: Expression of Epidermal growth factor receptor (EGFR) and Human epidermal growth factor type II receptor (HER2/neu) have been shown to be of prognostic and therapeutic significance in many carcinomas. A high level of expression of these markers correlates with advanced stage, poor prognosis and high metastatic potential. Newly developed drugs that target these receptors may have a role in the treatment of endometrial carcinoma. In this study we evaluated the expression of EGFR and HER2/neu in stage matched, type 1 and type 2 endometrial cancer.
Design: The pathology database at Emory University Hospital was searched from 2001 to 2005 for patients with endometrial carcinoma. Tumor slides were retrieved, reviewed and selected tumor blocks were immunohistochemically stained with antibodies to EGFR (Zymed, clone 31G7) and HER2/neu (Dako, polyclonal) using established methodology. Tumors were sub-divided into type 1 and 2 and into 2 stage groups (stage1; stage ≥2) for statistical analysis (chi-square test). Over- expression of HER2/neu was defined as greater than 10% of cells with moderate membranous staining (≥2+ staining). EGFR expression was interpreted as positive (any membranous staining) or negative (absence of any membranous staining).
Results: 27 cases of type 1 (23 grade 1-2, 4 grade 3) were compared to 32 cases of type 2 (25 serous, 7 clear cell) endometrial carcinoma. HER2/neu was over expressed in 11% of type 1 and 44% of type 2 endometrial carcinomas ($p<0.05$). EGFR expression was demonstrated in 37% and 16% of type 1 and type 2 tumors, respectively ($p<0.10$).

	HER2/neu over expression		EGFR positivity	
Endometrial Cancer	Stage 1	Stage ≥2	Stage 1	Stage ≥2
Type I (n=27)	2 (12%)	1 (10%)	9 (53%)	1 (10%)
Type II (n=32)	4 (33%)	10 (50%)	3 (25%)	2 (10%)

HER2/Neu was over-expressed in high stage type 2 tumors in comparison to low stage tumors of similar type (50% vs 33%; $p<0.05$). Low stage type 1 tumors more frequently expressed EFGR compared to high stage type 1 tumors but this result was not statistically significant ($p<0.10$).
Conclusions: HER2/neu is preferentially expressed in type 2 endometrial carcinoma. Moreover, HER2/neu expression increases with stage in this tumor subtype. Type 1 endometrial carcinoma express EGFR especially when low stage. Distinct patterns of immunohistochemical expression of these markers in subtypes of endometrial carcinoma may potentially have therapeutic implications using newly developed drugs that act by blocking their receptors.

908 Distribution and Viral Load of Eight Oncogenic Types of Human Papillomavirus (HPV) and HPV 16 Integration Status in Cervical Intraepithelial Neoplasia and Carcinoma

M Guo, N Sneige, W Zhang, E Silva, D Cogdell, YJ Jan, E Lin, R Luthra. The University of Texas M.D. Anderson Cancer Center, Houston, TX; Taichung Veterans General Hospital, Taichung, Taiwan.
Background: Current human papillomavirus (HPV) DNA testing using pooled probes, although sensitive, lacks specificity in predicting risk of cervical intraepithelial neoplasia (CIN) progression. To evaluate selected HPV genotyping, viral load and viral integration status as potential predictive markers for CIN progression, we performed HPV genotyping in formalin-fixed paraffin-embedded cervical tissue.
Design: Cervical tissue specimens included cervical carcinoma (29 cases) and CINs (CIN 1, 27 cases; CIN 2, 28 cases; CIN 3, 33 cases). General HPVs were screened using consensus primers (GP5+/GP6+ and PGMY09/11). HPV genotyping and viral load measurement were performed using quantitative real-time PCR (qRT-PCR) for eight oncogenic HPV types (16, 18, 31, 33, 35, 45, 52, and 58). HPV 16 viral integration status was evaluated by measuring HPV 16 E2/E6 ratio.
Results: We observed that the HPV DNA positivity increased in parallel with the severity of CINs and carcinoma, with 59% positivity in CIN 1, 68% in CIN 2, 76% in CIN 3, and 97% in carcinoma (P trend = 0.004). The eight oncogenic HPV types were significantly associated with CIN 2/3 (81%) and carcinoma (93%) (Odds ratio [OR], 15.0; 95% confidence interval [CI], 5.67-39.76; $P < 0.0001$) compared with the unknown HPV types (OR, 2.87; 95% CI, 0.89-9.22; $P = 0.08$). HPV 16 was the predominant oncogenic HPV type in CIN 2/3 (51%) and carcinoma (71%) and integrated significantly more frequently in carcinoma than in CIN 2/3 ($P = 0.004$). No significant differences in viral load were observed across the disease categories.
Conclusions: Our findings suggest that selected genotyping for the eight oncogenic HPV types might be useful in separating women with a higher risk of CIN progression from those with a minimal risk. HPV 16 integration status has potential to be a marker for risk assessment of CIN progression.

909 Comparison of INFORM HPV *In Situ* Hybridization with PCR Assay for Detection of Human Papillomavirus (HPV) in Cervical Carcinoma and Cervical Intraepithelial Neoplasia

M Guo, N Sneige, Y Gong, M Deavers, E Silva, YJ Jan, W Zhang, D Cogdell, E Lin, R Luthra. The University of Texas M.D. Anderson Cancer Center, Houston, TX; Taichung Veterans General Hospital, Taichung, Taiwan.
Background: In situ hybridization (ISH) assay for HPV DNA testing in cervical tissue, although is preferred by pathologists, has a relatively low sensitivity. In this study, we compared a new ISH probe, INFORM HPV3 (Ventana Medical Systems) with PCR assays to detect HPV DNA in formalin-fixed, paraffin-embedded cervical tissue.
Design: The cervical specimens included normal cervix (20), cervical intraepithelial lesions (CIN1,27; CIN2,28; CIN3,33) and cervical carcinoma (29). General HPVs were tested using consensus primers (GP5+/GP6+, PGMY09/11). Genotyping for eight oncogenic HPV types (HPV 16,18,31,33,35,45,52 and 58), viral load and HPV 16 integration status were performed using quantitative real-time PCR.
Results: The positivities of HPV DNA by the combined HPV testing assays were 74% in CIN1, 82% in CIN2, 85% in CIN3 and 97% in carcinoma. The ISH and PCR assay were not significantly different in detecting HPV DNA across all CIN categories with moderate to good agreements (Kappa coefficient: 0.53-0.64). In carcinoma, ISH detected significantly fewer HPV positive cases compared with PCR (Kappa coefficient, 0.2; P value = 0.03). The diffuse distribution pattern of HPV DNA by ISH was significantly associated with higher viral load ($P <0.0001$). However, no statistical significant difference was observed between staining distribution and grades of the diseases. The punctate staining pattern of ISH significantly increased with the severity of the diseases (P trend = 0.01). Although punctate pattern was more frequently observed with higher HPV 16 integration status (E2/E6 ratio, 0.29) compared with mixed punctate/diffuse pattern (E2/E6 ratio, 0.42), no significant difference of the E2/E6 ratio was observed between the two groups ($P = 0.4$). Five carcinoma cases with ISH -/PCR + testing results showed a significantly high HPV 16 integration status (E2/E6, 0.05; $P = 0.008$).
Conclusions: INFORM HPV3 ISH shows comparable and complementary testing results to detect HPV DNA compared with PCR assay in cervical tissue. The punctate staining pattern can be a useful marker for high-grade CINs. The high false negative results of HPV 16 in carcinoma by ISH may be cuased by high level of viral integration.

910 Interobserver Variation in the Diagnosis of Ovarian Clear Cell Carcinoma and Mixed Ovarian Epithelial Carcinomas with a Clear Cell Component

G Han, CB Gilks, S Leung, CA Ewanowich, JA Irving, TA Longacre, RA Soslow. Vancouver General Hospital, Vancouver, BC, Canada; Royal Alexandra Hospital, Edmonton, AB, Canada; Stanford University Medical Center, Stanford, CA; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: There is conflicting data in the literature concerning the possible chemoresistance and prognosis of ovarian clear cell carcinoma (CCC). This could be due to interobserver variation in the diagnosis of CCC, particularly in mixed tumors.

Design: 32 cases originally diagnosed as CCC, mixed epithelial carcinoma with a CCC component, and serous carcinoma of the ovary were identified by reviewing pathology records from a single institution. These cases were reviewed by 4 gynecological pathologists using WHO criteria. Each pathologist was blinded to the original diagnoses and those of the other participants. Interobserver reproducibility was evaluated by kappa analysis. Representative paraffin tissue blocks from each case were assessed using immunohistochemical markers for WT-1, BRCA1, and ER.

Results: Responses from participating pathologists were separated into 3 categories: pure CCC, mixed CCC, and non-CCC. Most of the non-CCC cases were interpreted as serous carcinomas. Agreements among 4 pathologists are shown in Table 1. A full agreement was defined as 4 pathologists agreeing on the category; partial agreement refers to 3 pathologists reaching agreement. The reproducibility was greatest for pure CCC (kappa of 0.82), and the lowest for the mixed CCC (kappa of 0.32). Moderate agreement was obtained in the serous group (kappa of 0.59). The overall kappa was 0.62. The immunohistochemical profile for each tumor type is summarized in Table 2.

Conclusions: 1. Diagnosing mixed ovarian epithelial tumors with a CCC component is less reproducible than pure CCC or serous carcinoma. 2. The immunophenotype of mixed tumors is similar to that of serous carcinomas, and significantly different from that of pure CCC [WT1 (p=0.001), ER (p=0.001) and BRCA1 (p=0.029)].

Table 1. Interobserver Agreement

Original Classification	Full agreement	Partial agreement	No agreement
CCC	10	1	0
Serous	9	2	0
Mixed	2	3	5

Table 2. Immunohistochemical Profiles

Original classification	WT-1	ER	BRCA1
	N/W/S	N/W/S	N/P
CCC	9/1/0	9/0/1	0/10
Serous	1/2/8	1/6/4	4/7
CCC component of mixed tumor	1/2/7	1/3/6	4/6
Serous component of mixed tumor	1/0/9	1/3/6	6/4

N- negative, W-weak, S-strong, P-positive

911 TP53 Gene Status in Patients with Advanced Ovarian Cancer in Relation to Response to Paclitaxel Plus Platinum-Based Chemotherapy

D Hardisson, M Mendiola, A Mariño, A Dominguez-Caceres, G Hernandez-Cortes, A Suarez, J Palacios. University Hospital La Paz-UAM, Madrid, Spain; University Hospital Virgen del Rocio, Sevilla, Spain.

Background: Ovarian carcinoma (OC) is the most lethal cancer of the female genital tract. TP53 is mutated in more than 50% of ovarian carcinomas, particularly in the serous type. The relationship between TP53 mutation and response rates to platinum based therapies has been extensively studied in OC, with controversial results. The aim of this study was to assess whether TP53 gene status has any predictive or prognostic relevance in patients with advanced OC treated with paclitaxel plus carboplatin-based chemotherapy.

Design: The study included 72 patients with advanced OC. All patients underwent surgery followed by paclitaxel plus carboplatin-based chemotherapy. Median age was 53.50 yr (20-82). Mean follow-up time was 42 months. Histological types were as follows: serous, 46; clear cell, 5; mixed, 5; endometriod, 3; mucinous, 2; transitional cell, 1; undifferentiated, 10. DNA was extracted from formalin fixed paraffin embedded tumor samples. TP53 exons 5-8 were amplified by PCR, followed by DHPLC analysis to assess TP53 status. Abnormal traces were confirmed by direct sequencing.

Results: TP53 mutations were present in 23 tumors (32%). There was one novel mutation according to IARC database (213 CGA>TGA). Two samples showed a polymorphism not described before in OC (213 CGA>CGG). Mutations were present only in serous, undifferentiated and mixed histological types. Most of the mutations were single nucleotide substitutions (point mutations). 14 (61%) were missense mutations, 4 (17%) nonsense and 1 (4%) a silent mutation with no aminoacid change. We have found also 4 frameshift deletions. Mutations were located mainly within exons 5 (43%) and 7 (30%). Nucleotide transitions (78%) were found more frequently than transversions (12%). The most common nucleotide change was G to A transitions in 9 of 23 (73%). Kaplan-Meyer analysis showed no differences in survival between patients with or without TP53 mutation. Analysis of survival data stratified by patients' age showed differences in survival rates between tumors with or without TP53 mutation but results were not statistically significant.

Conclusions: TP53 mutations is a frequent alteration in serous OC. We report a novel missense mutation (213 CGA>TGA). TP53 status is not a predictive nor a prognostic variable in patients with advanced OC treated with a paclitaxel-platinum based regimen.

Supported by SAF2004-08258-C02-02 (MEC).

912 Uterine Malignant Müllerian Mixed Tumor (MMMT): A Morphologic and Immunohistochemical (IHC) Study with Emphasis on the Diagnosis of Heterologous Rhabdomyosarcoma (RMS)

JA Harris, JK McKenney. University of Arkansas for Medical Sciences, Little Rock, AR.

Background: The largest study of uterine MMMTs reported no adverse prognostic significance for heterologous differentiation, but more recent studies have questioned this concept. RMS is the most common heterologous element in this setting and the criteria employed for its diagnosis could greatly influence the results of future studies. We, therefore, studied a series of uterine MMMTs to evaluate the correlation of morphologic appearance with IHC evidence of skeletal muscle differentiation.

Design: All uterine MMMTs with available glass slides and blocks were identified, and all H&E-stained slides were reviewed to confirm the diagnoses. Each case was evaluated for the presence of neoplastic spindle cells with abundant eosinophilic cytoplasm (AEC) and/or definitive rhabdomyoblasts defined by cross-striations visible on H&E. Immunostains for desmin, myogenin, and MyoD-1 were performed on a representative block containing neoplastic cells with AEC when present and/or sarcomatous overgrowth.

Results: 35 cases of MMMT were identified. 23/35 (66%) cases contained neoplastic spindle cells with AEC. 3/36 (8%) contained rhabdomyoblasts with obvious cross-striations, and all 3 also had cells with AEC. Overall, 10/35 cases (29%) contained neoplastic spindle cells expressing nuclear myogenin, 9/35 (26%) expressed nuclear MyoD-1, and 8/35 (23%) expressed cytoplasmic desmin. Nuclear myogenin and/or MyoD-1 immunoreactivity was required for designation as skeletal muscle differentiation by immunohistochemistry, which was present in 12/35 cases (34%). Of the cases containing cells with AEC, 9/23 (39%) expressed myogenin and/or MyoD-1 at least focally (including the 3 cases with rhabdomyoblasts) and 7of these 9 co-expressed desmin. 3 cases were characterized by a neoplastic spindle cell component without AEC: 2 expressed both myogenin and MyoD-1, and 1 expressed myogenin alone.

Conclusions: 34% of MMMTs showed IHC evidence of skeletal muscle differentiation. The only specific morphologic feature for predicting IHC evidence of skeletal muscle differentiation was the presence of rhabdomyoblasts with obvious cross-striations. The presence or absence of neoplastic cells with AEC was not a specific predictor of skeletal muscle differentiation by immunohistochemistry. This study highlights the importance of immunohistochemistry in defining MMMT with heterologous RMS, as the morphologic appearance alone may be misleading.

913 Vascular "Pseudoinvasion" in Laparoscopic Hysterectomy Specimens: A Diagnostic Pitfall

AV Herdman, KA Moller, S Logani. Emory University, Atlanta, GA; Southwest Gynecologic Oncology Associates Inc, Albuquerque, NM.

Background: Laparoscopic hysterectomy has proven to be an equally effective and safe technique when compared to conventional abdominal surgery for endometrial carcinoma. The procedure, as performed at our institution, involves the use of a uterine balloon manipulator for surgical exposure. The fallopian tubes are either cauterized or ligated to prevent transtubal spread of the tumor. The inflated balloon manipulator thus creates a positive closed pressure system within the uterine cavity. We observed extensive tumor involvement of blood vessels in one case of grade 1, stage 1A endometrial carcinoma and postulated that the closed pressure system may cause tumor displacement into vascular spaces.

Design: Slides of hysterectomy specimens performed laparoscopically between August 2004 and March 2006 at Emory University Hospital were reviewed. Seven patients underwent laparoscopic hysterectomy for endometrial carcinoma or atypical complex hyperplasia (ACH) and 30 patients had surgery for benign uterine pathology. Six to 19 (average 12) slides from the tumor cases and 2 to 8 (average 4) slides from the benign hysterectomies were reviewed for the presence or absence of endometrial tumor/tissue in vascular spaces.

Results: Patients with endometrial carcinoma/ACH ranged from 54 to 72 years in age. These included 6 FIGO grade I endometrioid carcinomas (3 stage 1A; 3 stage 1B) and one patient with atypical complex hyperplasia. Tumor within blood vessels was noted in 5 of 7 (71%) cases. In 3 cases including the case of ACH, multiple small and large caliber blood vessels showed tumor. In the remainder, one case had 2 small vessels involved and in the other 7 small vessels showed tumor within vascular lumina. Patients who underwent hysterectomy for benign disease were relatively younger (median age 48 years). Benign endometrial glands and stromal tissue was noted within vascular spaces in 4 of 30 (13%) cases.

Conclusions: We describe a hitherto unreported artifact of vascular pseudoinvasion generated by the operative technique in laparoscopic hysterectomy specimens. This may have incorrect adverse therapeutic implications for the patient. We postulate that this artifact most likely results from the creation of a closed pressure system generated as part of the operative technique. Pathologists need to be aware of this potential artifact, especially if they encounter a significant number of laparoscopic hysterectomy specimens for endometrial carcinoma in their practice.

914 Immunohistochemical Characterization of Ovarian Carcinomas Based on Mucin Production Activity: Analysis of MUC1, MUC2, MUC4, MUC5AC and MUC6 Expression

K Hirabayashi, H Kajiwara, M Yasuda, RY Osamura. Tokai University School of Medicine, Isehara, Kanagawa, Japan.

Background: Mucins are high molecular weight glycoproteins with oligosaccharides attached to the apomucin protein backbone by O-glycosidic linkage and are synthesized by a variety of secretory epithelial cells. Mucin is classified into membrane-associated mucin and secretory mucin. MUC1 and MUC4 are membrane-associated mucin and MUC2, MUC5AC and MUC6 are secretory mucins. The aim of this study is to compare the expression of MUC1, MUC2, MUC4, MUC5AC and MUC6 in mucinous

adenocarcinoma (MC), clear cell adenocarcinoma (CC), serous adenocarcinoma (SC) and endometrioid adenocarcinoma (EC) of ovary from the analytic points of differential diagnosis among these histological types and association with the clinical outcome.
Design: Formalin-fixed paraffin-embedded tissue sections from 26 cases of MC, 35 cases of SC, 45 cases of CC and 25 cases of EC were immunostained using antibodies for MUC1 (Novocastra), MUC2 (Novocastra), MUC5AC (Novocastra) and MUC6 (Novocastra). MUC4 (Zymed) was only stained for MC. EnVision(DAKO) was used for immunodetection in a DAKO aoutostainer. Cases were scored based on the percentage of positive cells: 0 (<5% of positive cells), 1+ (5-10% of positive cells), 2+ (11-50% of positive cells), or 3+ (>50% of positive cells). Kaplan Meier analysis was performed to determine a correlation between mucin expression and patient survival.
Results: MUC1 was more frequently expressed in EC, CC and SC than MC ($p<0.05$). MUC2, MUC5AC and MUC6 were mainly expressed in MC and EC. In contrast, SC and CC revealed negative or low expression for MUC2, MUC5AC and MUC6. MC revealed more frequent expression for MUC2 and MUC5AC than the other histological types ($p<0.05$). Low expression of MUC2 (<50% of positive cells) and MUC4 (<25% of positive cells) in MC and high expression of MUC1 (>50% of positive cells) showed a better long-term survival rate ($p<0.05$).
Conclusions: The results reveal that there is little production of secretory mucin in SC and CC, but EC products secretory mucins as well as MC. Especially, frequent expression for MUC2 and MUC5AC is characteristic of MC. There are significant differences of survival rate in expression of MUC2 and MUC4 in MC and also in expression of MUC1 in EC. The results suggest that the difference in mucin production between ovarian carcinomas would be helpful for the differential diagnosis and may be useful for the prediction of clinical outcome.

915 Papillary Serous Carcinoma of the Uterine Cervix Is HPV and P16 Positive

X Hong, B Gilks, CP Crum, MR Nucci. Brigham and Women's Hospital, Boston, MA; Vancouver General Hospital, Vancouver, BC, Canada.
Background: Papillary serous adenocarcinoma (PSC) is a distinctive subtype of endocervical adenocarcinoma that occurs in women with a bimodal age distribution, with peaks occurring prior to age 40 and after age 65. Although primary cervical PSC is morphologically similar to PSC of the upper genital tract, its association with HPV infection, the most common cause of endocervical adenocarcinoma of the usual type, and its distinction from PSC of the upper genital tract is unknown.
Design: 11 cases of presumed primary papillary serous carcinoma of the cervix were immunohistochemically stained for p53 and p16; their HPV status was studied by PCR-RFLP when possible.
Results: Ten of 11 and 2 of 11 cases with evaluable tissue were diffusely positive for p16 and p53, respectively. All 6 cases that had amplifiable DNA were positive for HPV 16 or 18. Two HPV-positive cases, both in women over age 65, were concurrently positive for p16 and p53.
Conclusions: The majority of endocervical PSCs are HPV and/or p16 positive and do not exhibit the immunophenotype (p53+) of upper genital tract PSC. Moreover, the presence of HPV in the older age group indicates that the two groups cannot be distinguished by HPV status. In addition, the co-localization of all three biomarkers in two cases over age 65 raises the possibility that the pathogenesis of some PSCs in older women involves a unique combination of host gene alterations and HPV infection.

916 Human Placental Site Trophoblastic Tumor: Novel Genetic Mode of Oncogenesis That Excludes the Presence of Y Chromosome

P Hui, HL Wang, PG Chu, B Yang, J Huang, RN Baergen, XM Yang, RA Soslow. Yale University, New Haven, CT; Washington University, St Louis, MO; City of Hope National Medical Center, Duarte, CA; Cleveland Clinic Foundation, Cleveland, OH; University of Rochester, Rochester, NY; Weill Medical College of Cornell University, NY, NY; Northwestern University, Chicago, IL; Memorial Sloan-Kettering Cancer Center, NY, NY.
Background: Placental site trophoblastic tumor (PSTT) is a neoplastic proliferation of extravillous trophoblasts that are fetal in origin. Clinically, the patients are of reproductive age with a history of full term pregnancy, abortion or complete mole. The majority of the tumors are preceded by a female gestational event and our pilot investigation of a few cases suggested that the tumor development might require a paternally derived X chromosome. It is necessary to study a large number of cases.
Design: A total of 21 cases of PSTT from 8 major medical centers in the United States were included in this study. All were diagnosed by the primary institutions with histologic and immunohistochemical examinations. DNA was extracted from the tumor and paired maternal tissue sections. DNA genotyping at 15 tetrameric polymorphic loci and sex determination locus were performed by multiplex PCR coupled with capillary electrophoresis. X chromosomal polymorphism was determined by PCR amplification of human androgen receptor gene locus (HUMARA).
Results: Genotyping analysis was informative in all 21 tumors and unique paternal alleles were present in all, confirming the trophoblastic origin of the tumors. Presence of X chromosome and absence of Y chromosome were consistently observed in all cases. Among 14 informative cases by HUMARA analysis, all but one demonstrated two X chromosomes in tumor samples and 7 cases showed a unique paternal X allele to their paired maternal tissues.
Conclusions: Our results essentially confirm a novel genetic mode that excludes a Y chromosome contribution to the genome of PSTT and that the tumor arises exclusively from the trophectoderm of a female conceptus. As X chromosome compensation and its inactivation in female cells are biological relevant, PSTT provides an important model with which the proliferative advantage conferred by the paternal X chromosomes can be studied.

917 Molecular Confirmation of Complete Hydatidiform Mole: Practical Application of the Gold Standard

P Hui, C Johnson, H Kermalli. Yale University School of Medicine, New Haven, CT.
Background: Molar gestations are defined at the genetic level by the unique parental chromosomal contributions. However, current pathologic diagnosis of molar pregnancies relies largely on routine histological and immunohistochemical examinations of tissue sections, and a precise diagnosis of hydatidiform moles is frequently difficult. Although DNA ploidy analysis is useful in confirming a partial mole, practical application of the molecular markers are lacking for the confirmation of a complete mole. In this study, we have explored methodologies of DNA genotyping for the molecular confirmation of a complete hydatidiform mole.
Design: Thirteen archival cases of molar gestations were selected with a diagnosis of complete mole or consistent with complete mole. DNA extractions of the molar tissue and paired gestational endometrium were obtained from unstained sections. DNA genotyping was performed by a multiplex PCR reaction targeting 15 tetrameric polymorphic loci of the human genome, and the product was analyzed by capillary electrophoresis.
Results: Multiplex PCR was successful in all cases with input template DNA amount ranging from 1.5 to 2.5 ng, equivalent to 150 to 250 diploid cells. All 13 cases were confirmed to be complete mole by a demonstration of sole paternal contribution to the molar genome, including 12 cases of monospermic and 1 dispermic (Table).
Conclusions: This study demonstrates the importance of molecular confirmation of complete mole by DNA genotyping. The methodology uses minimal amount of template DNA extracted from a few hundred cells, and consists of one multiplex PCR amplification followed by high-resolution capillary electrophoresis, making DNA genotyping, the gold standard for confirming a complete mole, into a practical application.

CASE#	HISTOLOGIC DX	PLOIDY	DX AFTER PLOIDY	P57 STAIN	GENOTYPING	MOLECULAR DX
1	MOLAR PREGNANCY	DIPLOID	C/W COMPLETE MOLE	ND	Monospermic	COMPLETE MOLE
2	FAVOR COMPLETE MOLE	DIPLOID	C/W COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
3	C/W COMPLETE MOLE	ND	N/A	NEG	Monospermic	COMPLETE MOLE
4	MOLAR PREGNANCY	DIPLOID	C/W COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
5	COMPLETE MOLE	DIPLOID	COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
6	C/W COMPLETE MOLE	ND	N/A	NEG	Dispermic	COMPLETE MOLE
7	SUSPICIOUS FOR PARTIAL MOLE	DIPLOID	C/W COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
8	FAVOR COMPLETE MOLE	DIPLOID	C/W COMPLETE MOLE	ND	Monospermic	COMPLETE MOLE
9	COMPLETE MOLE	DIPLOID	COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
10	C/W COMPLETE MOLE	DIPLOID	C/W COMPLETE MOLE	ND	Monospermic	COMPLETE MOLE
11	FAVOR COMPLETE MOLE	DIPLOID	C/W COMPLETE MOLE	NEG	Monospermic	COMPLETE MOLE
12	COMPLETE MOLE	ND	N/A	ND	Monospermic	COMPLETE MOLE
13	COMPLETE MOLE	TETRAPLOID	COMPLETE MOLE	ND	Monospermic	COMPLETE MOLE

918 Immunohistochemical Comparisson of Serous Carcinomas of Ovary Versus Endometrium

DN Ionescu, I Chebib, A Alkushi, AM Gown, CB Gilks. BC Cancer Agency, Vancouver, BC, Canada; University of Calgary, Calgary, AB, Canada; PhenoPath Laboratories and IMPRIS, Seattle, WA; Vancouver General Hospital, Vancouver, BC, Canada; King Fahad National Guard Hospital, Riyadh, Saudi Arabia.
Background: Serous carcinomas of ovary are frequently associated with BRCA1 or BRCA2 mutations, while serous carcinomas of endometrium are not, suggesting that there are different molecular events during oncogenesis in these two morphologically similar tumors. The aim of this study was to characterize the immunoprofile of endometrial and ovarian serous carcinoma.
Design: A cohort of 212 sequential cases of ovarian serous carcinoma (with median follow-up of 12 years) and 35 endometrial serous carcinoma (with median follow-up of 8.0 years) were reviewed for this study. Tissue microarrays (TMA) of endometrial and ovarian serous carcinomas were constructed from formalin-fixed paraffin-embedded tissue. Immunostains for CK5/6, WT1, Mesothelin, podoplanin, p16, p53, estrogen and progesterone receptor (ER, PR), Her-2/neu, CK7, CK 20, TTF1, E and P Cadherin and beta-Catenin were performed.
Results: There were significant differences in staining of ovarian and endometrial serous carcinoma for 5 of 16 markers analyzed (Table 1). There were no differences in immunostaining for the remaining markers tested.
Conclusions: Immunohistochemical profiles of serous carcinomas of the ovary and endometrium are significantly different, however, no single immunohistochemical stain or combination of stains has the ability to differentiate ovarian from endometrial primary site with certainty in an individual case.

Immunostaining of Endometrial versus Ovarian Serous carcinoma

	ENDOMETRIUM		OVARY		
STAIN	#POSITIVE	#NEGATIVE	#POSITIVE	#NEGATIVE	p-VALUE
WT1	5	25	162	46	< 0.001
MESOTHELIN	15	19	165	44	< 0.001
CK5/6	17	16	10	199	< 0.001
HER2/NEU	8	22	15	187	< 0.001
ER	17	19	156	51	< 0.001

919 Granulosa Cell Tumors of the Ovary with a Pseudopapillary Pattern: A Study of 12 Cases of an Unusual Morphologic Variant Emphasizing Their Distinction from Transitional Cell Neoplasms and Other Papillary Ovarian Tumors

JA Irving, RH Young. Vancouver General Hospital, Vancouver, Canada; Massachusetts General Hospital, Boston, MA.

Background: Granulosa cell tumors of the ovary with a pseudopapillary pattern have received only brief mention in the literature. We have seen 12 cases in which the favored diagnosis by the referring pathologist was often that of a transitional cell or other papillary ovarian neoplasm.

Design: Twelve cases of juvenile granulosa cell tumor (JGCT; 9 cases) and adult granulosa cell tumor (AGCT; 3 cases) with a pseudopapillary pattern were retrieved from consultation files (RHY) and from the surgical pathology archives of the Vancouver General Hospital. A mean of 12 slides per case (range 1-24) were available for review.

Results: Patients with JGCT were 14 to 30 (mean 21) years; patients with AGCT were 24, 40, and 69 years. Clinical presentation was known in 10 cases, with patients presenting with a pelvic mass (6 cases); acute abdomen (2 cases), secondary amenorrhea, and infertility (1 case each). Two patients had extra-ovarian spread at the time of presentation; peri-operative tumor rupture was noted in 3 cases. All 12 tumors were unilateral, with a mean tumor size of 13.3 cm (JGCT) and 8.1 cm (AGCT). Six tumors were grossly cystic (3 unilocular, 3 multilocular), with multiple, fleshy, intracystic papillary projections ranging in size from 0.1 to 1.5 cm. Two tumors were multinodular, and 2 were solid-cystic; no gross descriptions were available for the remaining 2 cases. Microscopically, pseudopapillae were characterized by intracystic cellular projections with intervening necrosis and hemorrhage (7 cases), or by blunt to elongate, undulating folds of neoplastic cells without appreciable necrosis (8 cases); both patterns were present in 3 cases. The pseudopapillae often contained fibrovascular cores with dense stroma and dilated, congested vessels, and were lined by several to more than 10 layers of granulosa and thecal cells. Five cases showed either or both bizarre cytologic atypia and atypical mitoses. Cytologic features and/or areas with architectural patterns typical of granulosa cell tumor were identified in all cases.

Conclusions: A pseudopapillary pattern may be seen focally, or as the predominant morphologic pattern, in granulosa cell tumors of both adult and juvenile type. Diverse papillary ovarian neoplasms, in particular transitional cell tumors, can enter the differential diagnosis and potentially lead to misinterpretation unless the pathologist is alert to this unusual morphologic variant.

920 Intraoperative Reporting of Ovarian Tumors with Borderline Features

N Ismiil, S Nofech-Mozes, Z Ghorab, A Covens, R Kupets, MA Khalifa. University of Toronto, Toronto, ON, Canada.

Background: It has been recommended to report ovarian cystectomy specimens with frozen section (FS) features suggesting a borderline tumor as "at least borderline". This terminology is likely to trigger the surgeon to proceed with omentectomy. The aim of this study is to review our experience regarding the surgeons' response to the terminology used by pathologists intraoperatively.

Design: Surgical pathology database and medical records were searched in the period of July 1999 to June 2005 for all cases with intraoperative consultation on ovarian cystectomies or oophorectomies. A natural language search was performed once using the term "at least" and another time for the term "borderline". Pulled out data were then examined by two gynecologic pathologists to validate the specimens and to exclude any unrelated case. The complete operative procedure on each case was recorded. Data were entered in a Microsoft Access database and statistically tested.

Results: A total of 76 patients were identified. Thirty-six tumors were intraoperatively reported as "at least borderline" (AL) and 40 were diagnosed as straightforward "borderline tumor" (BL). Gynecologic oncologists operated on 61 patients, 19-88 years old (mean = 56.1; SD = 15.7) while general gynecologists operated on 15 patients, 29-76 years old (mean = 54.9; SD = 15.8). The age difference between the two groups of patients was not statistically significant by the *t*-test (p = 0.7936). Omentectomy was not performed in 5 patients. When the FS was reported as BL, general gynecologists were 41 times more likely to under manage patients and not proceed with an omentectomy (OR = 41.429; 95% CL = 1.543 – 988.018). The Odds Ratio dropped to 17.33 (95% CL = 1.653 – 174.398) when the AL terminology was used.

Conclusions: The intraoperative use of the term "borderline tumor" for diagnosing ovarian cystectomy or unilateral oophorectomy specimens carries a considerable risk for under treatment, especially when the operator is a general gynecologist. We document that the term "at least borderline" is superior as it increases the patient's chance for adequate management.

921 The Morphologic and Immunophenotypic Spectrum of a Candidate Precursor to Pelvic Serous Carcinoma

E Jarboe, Y Lee, R Drapkin, FD McKeon, CP Crum. Brigham and Women's Hospital, Boston, MA; Dana Farber Cancer Research Institute, Boston, MA; Harvard Medical School, Boston, MA.

Background: Recently a mucosal change characterized by strong p53 immunostainnig has been described in benign-appearing mucosa of the distal fallopian tube. This entity shares many characteristics with tubal carcinoma and is termed the "p53 signature". This report summarizes a series of tubes with p53 signatures and characterizes their morphologic and immunophenotypic characteristics.

Design: Consecutively accessioned fallopian tubes were examined in their entirety in hematoxylin and eosin stained sections and immunostained for p53, MiB1, and selectively, Cyclin E. The goal was to identify subsets of p53 signatures based on morphologic and immunophenotypic characteristics. A p53 signature was defined as 12 or more sequentially arranged secretory cells with moderate to intense p53 positivity.

Results: Fifty-two p53 signatures associated with non-neoplastic disorders, BRCA+ women, and tubal cancers, were studied. Four p53-positive epithelial patterns were identified: A) discontinuous p53 signatures, with intervening ciliated cells, low MiB1 and cyclin E staining; B) continuous p53 signatures of uninterrupted secretory nuclei, low MiB1 and cyclin E index; C) "proliferative p53 signatures", with epithelial stratification, moderate proliferative index (50% of nuclei), intervening ciliated cells and variable cyclin E staining; and D) tubal intraepithelial carcinomas (TIC), with homogeneous p53 staining, a high proliferative and cyclin E index (>80%). The nuclear morphology of lesions in groups A-C varied widely. Although nuclear enlargement relative to adjacent mucosa was seen in many signatures, most p53 signatures could not be consistently distinguished from adjacent tubal epithelium without p53 immunostaining.

Conclusions: p53 signatures may or may not displace ciliated cells early in their natural history. Proliferative signatures comprise a more advanced stage, are moderately proliferative and maintain cell polarity with variable preservation of intervening ciliated cells. However, TICs are homogeneous, with poorly polarized cell growth and high proliferative activity. P53 signatures are currently not clinically relevant. However, proliferative p53 signatures share properties with TIC and may signify an early form of TIC. Morphologic and immunohistochemical criteria for these distinctions are illustrated and their limitations discussed.

922 Evidence Supporting the Fimbria as One Site of Origin for Concurrent Ovarian and Endometrial Serous Carcinomas

EA Jarboe, M Hirsch, CP Crum, D Kindelberger, GL Mutter, MR Nucci. Brigham and Women's Hospital, Boston, MA.

Background: Serous adenocarcinomas involving endometrium and ovary may co-exist, and the site(s) of origin may be unclear. We recently showed that many ovarian serous carcinomas are associated with an early tubal carcinoma (tubal intraepithelial carcinoma or TIC) and share an identical cell of (by sequence-specific p53 mutation) origin. Because the presumed origin of multi-site serous carcinomas determines management, we analyzed a series of concurrent endometrial/ovarian serous carcinomas with attention to the distal fallopian tube.

Design: Ten consecutive cases of concurrent serous endometrial and ovarian carcinomas, including one case with mixed serous and clear cell features, were studied. In each case, fallopian tubes were submitted *in toto* according to the protocol for sectioning and extensive examination of the fimbriated end (SEE-FIM). Location and extent of the endometrial tumor and presence or absence of TIC were documented and compared.

Results: For the serous carcinomas involving the endometrium, myometrial invasion was either absent (5/10), superficial (<5%; 4/10) or deep (90%; 1/10). Six of ten serous carcinomas involving the endometrium were associated with endosalpingeal involvement; of these TIC was confirmed in four, of which three involved the fimbriated end. The remaining four cases, including one with deep myometrial invasion, did not involve the endosalpinx. In three cases, the carcinoma involved an endometrial polyp and in two of these the carcinoma was confined to the polyp. The endosalpinx was involved in two of these cases, including one with confirmed TIC.

Conclusions: Concurrent endometrial and ovarian serous carcinomas are heterogeneous, with more than one possible site of origin. This study shows, for the first time, that a candidate origin in the distal fallopian tube can be identified in a significant minority of cases. Assignment of the site of origin is particularly germane to management schemes (e.g. consideration of intra-peritoneal chemotherapy) that depend on this distinction. Therefore, we propose that the SEE-FIM protocol be applied to all endometrial serous carcinomas and that tumors with concurrent TIC be classified as a distinct subset pending confirmation of tumor origin.

923 The Clinical and Pathologic Features of Non-Invasive and Superficilally Invasive Adenocarcinoma of Endometrium. A Study of 44 Cases

MW Jones, GA Trucco. Magee Womens Hospital of UPMC, Pittsburgh, PA.

Background: The distinction between superficially invasive and non-invasive adenocarcinoma of endometrium is difficult due to irregularities of endometrial-myometrial junction, absence of desmoplastic response around early invasive foci, smooth-muscle-like alteration of endometrial stroma or presence of adenocarcinoma in superficial adenomyosis. We reviewed clinical and pathologic features of 44 superficially invasive and non-invasive adenocarcinomas of endometrium, endometrioid type in search for differences and similiarities between two groups.

Design: All cases of endometrial adenocarcinoma in files of Department of Pathology, Magee Womens Hospital diagnosed during year 1992 and 1993 were reviewed and 9 non-invasive and 35 superficially invasive adenocarcinomas were identified. High grade carcinomas including serous and clear cell type were excluded. The clinical information and follow-up were obtained from the Hospital Tumor Registry.

Results: Patients range in age from 30 to 77, mean 52.25. The tumors presented as endometrial polyps in 6 patients (including 3 non-invasive tumors) ranging in size from 1.0 to 7.0 cm , or diffuse endometrial thickenning in 38. Nine tumors showed either squamous, secretory or mucinous component in addition to endometrioid pattern. Twenty one tumors including 6 non-invasive were grade 1, 21 grade 2 and 2 grade 3. None showed lymphovascular invasion and none metastasized at the time of presentation. The follow-up ranged from two to 14 years, average 8.2 and was negative for recurrence or metastasis. All patients had several negative PAP smears, with the exception of one, who four years after hysterectomy had PAP smear suspiciuos for malignancy, not confirmed by biopsy. Four patients, all over 50 years old, were diagnosed with another malignancy. Three of them developed colon carcinoma and one invasive ductal carcinoma of the breast.

Conclusions: 1. Superficially invasive and non-invasive adenocarcinoma of endometrium, endometrioid type represents a low grade tumor with excellent prognosis and very low risk of metastasis, as supported by a long follow-up . 2. There is no significant difference in clinical and pathologic features of both tumor groups except for lower histologic grade (66%, grade 1) and more common presentation as endometrial polyps (33%) in non-invasive adenocarcinoma. 3. Since the presence of superficial myometrial invasion is often difficult to determine these tumors could be classified and treated as early endometrial adenocarcinomas.

924 Immunohistochemical Profile of Steroid Cell Tumors of the Ovary. A Useful Tool in Differential Diagnosis with Other Neoplasms with Clear Cell or Eosinophilic Features. Clinico-Pathologic and Immunohistochemical Study of 11 Cases

MW Jones, G Carter, DJ Dabbs, R Hari. Magee Womens Hospital, University of Pittsburgh, Pittsburgh, PA.

Background: Steroid cell tumors of the ovary may histologically resemble other neoplasms with abundant clear or eosinophilic cells, including metastatic endometrial, renal and hepatocellular carcinomas and hepatoid yolk sac tumor. Since the majority of steroid cell tumors have favorable prognosis, it is important to distinguish them from metastatic or primary malignant tumors with similiar morphology.

Design: Eleven cases of steroid cell tumor were selected from the files of Department of Pathology, Magee Womens Hospital. The clinico-pathologic information including patients' age, symptoms at presentation, tumor size, histologic pattern and mitotic activity were obtained. One section of each tumor was immunostained for inhibin, calretinin, CD99, androgen receptors and AE1/3.

Results: Women ranged in age from 32 to 84, average age 61. Three presented with elevated testosterone level and androgenic symptoms, 4 with adnexal mass, and in the remaining four the tumor was an incidental finding in surgical specimen removed for another reason. Average tumor size was 3.7 cm. Five were eosinophilic type, 3 clear cell type and 2 mixed. There was no significant cytologic atypia in any and mitotic activity was 0-1 per 10 HPF in 10. The single tumor that recurred had >3 mitotic figures per 10 HPF. All tumors show strong diffuse staining for inhibin and calretinin. Ten of 11 were positive for CD99 and 9 of 11 were positive for androgen receptor. The clear cell type showed weaker positivity for CD99 and androgen receptor compare to eosinophilic type. There was focal positivity for AE1/3 in three tumors.

Conclusions: Immunochistochemistry can be helpful in distinction between steroid cell tumors and other neoplasms with similiar histologic features. However, since occasional steroid cell tumors can be positive for AE1/3, and tumors originating in other sites including adrenocortical carcinomas can be positive for inhibin, the use of additional immunostains including calretinin, CD99 and androgen receptor is recommended.

925 Expression of ZBP1, a mRNA Regulating Protein, Is Associated with Progression of Ovarian Carcinoma

M Köbel, S Hüttelmaier, S Hauptmann. Martin-Luther-University, Halle, Germany.

Background: The mRNA regulating Zipcode binding protein ZBP1 controls the expression of β-actin and IGF-II on the translational level, while the expression of c-myc, CD44, and βTrCP1 are controlled by modulating mRNA turnover. Members of the ZBP protein family show an oncofetal pattern of expression with severely elevated protein levels in cancer. However, knowledge of a potential function in ovarian carcinoma progression is still lacking.

Design: In this study, expression of ZBP1 was determined in 106 ovarian carcinomas, 44 borderline tumors of the ovary and 5 normal ovaries by immunohistochemistry. Funtional consequences of ZBP1 knock-down by RNA interference were evaluated in ovarian carcinoma derived cells ES-2 and OVCAR-42.

Results: Normal ovarian surface epithelium was negative for ZBP1, serous borderline tumors showed a faint expression, and ZBP1 protein was strongly expressed in 69% of ovarian carcinomas preferentially in high-stage (P = 0.025) and high-grade (P = 0.045) cases. In Kaplan Meier survival analysis, ZBP1 expression was a significant prognostic indicator for reduced progression free survival (P = 0.0338) and overall survival (P = 0.0350). In ES-2 cells, the knock-down of ZBP1 caused severe defects in proliferation, c-myc expression, and organization of the actin cytoskeleton. The latter explains an increase of multinucleated cells and a decrease of invasion upon ZBP1 knock-down.

Conclusions: This indicates that ZBP1 besides controlling proliferation via the regulation of c-myc mRNA stability also modulates expression of factors essential for regulating actin organization, and thus inhibits cytokinesis and cell migration of ovarian tumor cells.

926 Molecular Analysis of Simultaneous Adenocarcinomas of the Ovary and Endometrium

M Kapali, S Dacic, N Esposito, G Mantha, MW Jones. Magee Womens Hospital of the University of Pittsburgh, Pittsburgh, PA.

Background: The distinction between synchronous and metastatic tumors of the endometrium and ovary can be difficult by morphology alone. We utilized LOH analysis to determine if molecular approach can distinguish synchronous tumors from metastases.

Design: Nine cases with simultaneous endometriod adenocarcinomas of the endometrium and ovary were retrieved from the archives of the department of Pathology, Magee Womens Hospital. The cases were reviewed and classified using clinicopathologic criteria. Microdissection-based loss of heterozygosity (LOH) analysis was performed in both the uterine and ovarian tumors. LOH analysis included 21 microsatellite markers situated on 16 chromosomal loci (chromosomes1, 3p,5q,9p,10q,11p,12q,13q,14q,17,18q,19 and 22q) For each patient the probability of the observed LOH pattern in the pair of tumors was calculated assuming an independent origin (PIO) or a common origin (PCO). The likelihood ratio (LR=PCO/PIO) was calculated in each case.

Results: LOH analysis correlated in 5 of 7 (71.4%) morphologically synchronous tumors. LOH analysis was inconclusive in 2 morphologically synchronous tumors due to low frequency of LOH. 2 cases which were indeterminate by morphologic criteria, the LOH analysis was supportive of synchronous tumor in one and metastatic in the other.Microsatellite instability (MSI) of analyzed tumor suppressor genes was also identified. Discordant/concordant MSI patterns were supportive of the LOH analysis results. See table.

Conclusions: Molecular analysis confirms that the morphologic criteria used to distinguish synchronous from metastatic disease are reliable in majority of cases. Clonality analysis using LOH approach coupled with MSI status of analyzed tumor suppressor genes can be an additional valuable tool in making a definitive distinction in cases which are indeterminate by morphologic criteria.

Case number	LOH analysis			Microsatellite instability		Molecular Dx	Morphologic Dx
	Discordant LOH	Concordant LOH	Liklehood ratio	Discordant MSI	Concordant MSI		
1	13	2	6.85	9	2	Synchronous	Synchronous
2	8	6	6.45	9	5	Synchronous	Indeterminate
3	9	1	2.99	10	6	Synchronous	Synchronous
4	0	1	14593	1	0	Inconclusive	Synchronous
5	4	4	0.16	5	0	Synchronous	Synchronous
6	3	0	0	0	1	Inconclusive	Synchronous
7	2	10	20265	2	5	Metastatic	Indeterminate
8	5	3	0.0003	1	1	Synchronous	Synchronous
9	9	5	4.13	8	5	Synchronous	Synchronous

927 Immunohistochemical and Ultrastructural Findings of Trophoblasts in Normal Full Term Placentas Showing Lineage Relationship

K-R Kim, Y Lee, DE Song, T-J Kwon. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea; Pochon CHA University, Bundang Hospital, Kyounggi-do, Republic of Korea; National Institute of Scientific Investigation, Seoul, Republic of Korea.

Background: Recently, intermediate trophoblasts (ITs) are subdivided into villous ITs, implantation site ITs (ISITs), and chorionic-type ITs (CTITs), depending on location, cytomorphologic features, growth patterns, and immunohistochemical phenotype, and the subtypes have provided a basic concept of cellular origin in various tumor and tumor-like conditions. The subtypes of ITs and their cellular characteristics are known to be closely related to their location, i.e.CTITs to fetal membrane and ISITs to basal plate. We recognized, however, that CTITs and ISITs were admixed in each anatomical compartment of the placenta, although one is more prevalent than the other in some areas, raising a question of site specificity of these subtypes.

Design: We observed histological and ultrastructural findings as well as immunohistochemical characteristics for p63, PLAP, Mel-CAM, hPL, and inhibin of various ITs in the fetal membrane, subchorionic area, intervillous septum, and basal plate in 13 normal term placentas.

Results: ITs of the fetal membrane consisted of two different types of ITs, each comprising characteristics of CTITs and ISITs. ITs of basal plate were predominantly composed of ISITs showing diffuse immunopositivities for hPL and Mel-CAM with smaller number of CTITs. ITs of the intervillous septum were mostly ISITs with small number of CTITs. In the subchorionic area, there were variable numbers of CTITs showing diffuse immunopositivities for PLAP and p63, as in the placental infarcts. The number of proliferating CTITs defined by Ki-67 labeling in the subchorionic area were correlated to the amount of subchorionic fibrinoids. Ultrastructurally, CTITs had abundant glycogen and rare intracellular organelles, indicating undifferentiated cellular components, whereas ISITs were differentiated cells with abundant intermediate filaments, rER, and some lipid vacuoles.

Conclusions: Our study suggests that CTITs and ISITs are not site specific, but both can be found in any placental compartments. Moreover, CTITs have cytomorphologic and immunohistochemical characteristics of undifferentiated cells, whereas ISITs have those of differentiated cells.

928 Myxoid Leiomyosarcoma: A Clinicopathologic Study of Ten Cases

D Kindelberger, M Hollowell, C Otis, M Nucci. Brigham and Women's Hospital, Boston, MA; Baystate Medical Center, Springfield, MA.

Background: Myxoid leiomyosarcoma (mLMS) is a rare variant of uterine leiomyosarcoma (LMS). Few studies have examined this subtype and the majority of these represent case reports. This study assembled the largest series to date of uterine mLMSs and examined their clinicopathologic, and immunohistochemical features.

Design: Cases were identified by searching our pathology database for "myxoid leiomyosarcoma". Clinicopathologic features were obtained by review of the pathology reports and electronic medical record. All cases were stained with Alcian blue, desmin, smooth muscle actin (SMA), h-caldesmon (hCD), CD10, and ALK-1 protein. Immunostaining was scored with 4+ indicating > 50% staining, 3+ indicating 25-50% staining, 2+ indicating 10-25% staining, 1+ indicating staining < 10%, and 0 indicating no staining. Cytologic atypia was graded as mild, moderate, or severe (1+, 2+, or 3+, respectively). Mitotic figures were counted per 10 high power fields.

Results: 10 cases of mLMS were identified. Patients ranged in age from 37 to 62. The tumors ranged in size from 1.5 to 16 cm and all were grossly described as gelatinous. Microscopically, all tumors were myxoid (with > 50% of the tumor having myxoid stroma), which was confirmed by Alcian blue staining. All had infiltrative borders. Cytologic atypia was 1+ in 3 cases, 2+ in 4 cases and 3+ in 3 cases. The mitotic rate ranged from 1 to 25 per 10 hpf; four cases had less than 3 per 10 hpf. 6 of 10 cases showed 2+ or less staining for SMA, desmin, and h-CD. Two cases showed 4+ CD10 staining, the remaining eight showed 2+ staining. ALK-1 was negative in all cases. Followup ranged from 1 to 5 years. Five of the patients died of their disease; three are alive with recurrent disease; two are alive with no evidence of residual disease.

Conclusions: mLMS is a rare variant of uterine LMS with a similarly poor prognosis. Tumors have an infiltrative growth pattern, and the majority exhibit at least moderate cytologic atypia. Although all tumors were positive for smooth muscle markers, confirming their smooth muscle origin, expression was typically limited, being present in less < 25% of cells in half of the cases. Negativity for ALK-1 helps distinguish mLMS from inflammatory myofibroblastic tumor, a mxyoid neoplasm recently described to also occur in the uterus. Similar to other uterine smooth muscle tumors, mLMS may be diffusely positive for CD10.

929 Estrogen and Progesterone Receptor Expression in Ovarian Carcinomas and Borderline Tumors

T Kiyokawa, T Hamada, H Hano. Jikei University School of Medicine, Tokyo, Japan.

Background: The role of receptor for estrogen (ER) or progesterone (PR) in the ovarian carcinogenesis is still controversial. Some recent studies suggest association between status of steroid hormone receptors and outcome in ovarian carcinoma. Although previous studies including those using immunohistochemistry have shown detection of ER and PR in ovarian tumors, the positive rate varies from one study to another. This study was carried out to explore expression of ER and PR in various histological types of ovarian carcinomas and in serous borderline tumors.

Design: Sixty-six cases of ovarian carcinomas (34 serous, 20 endometrioid, 12 clear cell type), and 20 of serous borderline tumors were immunohistochemically examined for expression of ER and PR using monoclonal antibody 6F11 and 1A6, respectively. Nuclear reactivity with strong intensity in more than 10% of the tumor cells was considered positive. Data was analyzed by means of chi-square test with or without Yates' modification.

Results: ER was expressed in 21 of 66 carcinomas (32%); in 9 of 34 serous (26%), in 10 of 20 endometrioid (50%), and in 2 of 12 clear cell type (17%). PR was expressed in 19 of 66 carcinomas (29%); in 4 of 34 serous (12%), in 15 of 20 endometrioid (75%), and none of 12 clear cell type (0%). Expression of both ER and PR was significantly more common in endometrioid carcinoma than in other types of carcinomas ($p<0.05$). Six of 20 endometrioid carcinomas contained foci of squamous differentiation, where neither ER nor PR was expressed. In 20 serous borderline tumors, expression of PR (positive in 9 cases, 45%) but not ER (positive in 5 cases, 25%) was significantly more common than in serous carcinomas ($p<0.05$).

Conclusions: Our results suggest expression of ER and PR is histological-type dependent in ovarian carcinomas. It was also confirmed that serous borderline tumor is distinct in PR expression from serous carcinoma.

930 Gastric Morphology and Immunophenotype Predict Poor Outcome in Cases of Mucinous Adenocarcinoma of the Uterine Cervix

A Kojima, Y Mikami, T Sudo, S Yamaguchi, Y Kusanagi, M Ito, R Nishimura. Ehime University, Matsuyama, Ehime, Japan; Kyoto University Hospital, Kyoto, Japan; Hyogo Medical Center for Adults, Akashi, Hyogo, Japan.

Background: Endocervical type mucinous adenocarcinoma (ECA) of the uterine cervix is defined as a tumor composed of cells resembling those of the endocervical glands. However, in reality it appears to be a heterogeneous group of neoplasms, and recent studies have demonstrated that a minority of ECA displays a gastric immunophenotype. The aim of this clinicopathologic study was to assess the significance of the gastric phenotype.

Design: Fifty three cases of mucinous adenocarcinoma of the uterine cervix (37 FIGO stage IB, 4 stage IIA and 12 stage IIB) were reviewed and re-evaluated using a newly established morphologic criteria for separating gastric type adenocarcinoma, which was defined as a tumor showing clear and/or pale eosinophilic and voluminous cytoplasm, with distinct cell borders. Clear cell adenocarcinomas were carefully excluded from the study. The results were correlated with immunophenotype, determined by HIK1083 and MUC6 immunostaining, and patient outcome.

Results: Following the current WHO classification (2003), 47 tumors (89%) were classified as ECA, one (2%) as intestinal type, one (2%) as mixed endocervical and intestinal type, and four (8%) as minimal deviation adenocarcinoma (MDA). Twelve of 47 (26%) ECAs and all four MDAs, reclassified as gastric type using the novel criteria, were frequently positive for HIK1083 with a rate of 75% (12/16), whereas only 11% (4/37) of non-gastric tumors were positive. There was no significant difference in MUC6 reactivity between gastric and non-gastric type tumors (31%, 5/16 versus 16%, 6/37; P=0.4). Patients with gastric type adenocarcinomas had a significantly decreased 5-year disease specific survival rate (30% versus 77%; P<0.0001), and the gastric type morphology was related to a significant risk for disease recurrence compared to the non-gastric type (P=0. 001; HR, 4.5; 95% CI, 1.42–14.2). HIK1083-positivity was also related to decreased 5-year disease specific survival rate (38% versus 74%; P<0.005).

Conclusions: Gastric type adenocarcinoma is considered to be a distinct subtype of mucinous adenocarcinoma of the uterine cervix in terms of immunophenotype and morphology, and shows an aggressive clinical course.

931 Thyroid Transcription Factor 1 (TTF1) Immunoreactivity in Ovarian Neoplasms

LA Kubba, J Liu, A Malpica, E Euscher, E Silva, MT Deavers. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Background: TTF1 protein expression is widely used in the diagnosis of lung and thyroid carcinomas. Although there have been reports of TTF1 immunoreactivity in tumors other than those originating from the lung or the thyroid, the expression of this marker has only been studied in a few cases of ovarian neoplasms. The purpose of our study was to investigate the expression of TTF1 in a variety of ovarian neoplasms.

Design: Tissue microarray paraffin blocks of 138 ovarian serous (Ser) adenocarcinomas, 65 endometrioid (EM) adenocarcinomas, 35 mucinous (Muc) adenocarcinomas, 30 mucinous neoplasms of low malignant potential (MucLMP), and 10 clear cell (CC) adenocarcinomas were selected from the archival material of MD Anderson Cancer Center. Sections were stained with anti-TTF1-antibody (8G7G3/1 antiTTF antibody, Dako, 1:25 dilution) and were reviewed for the presence or absence of immunoreactivity.

Results: TTF1 nuclear immunoreactivity was demonstrated in 3% (2/65) of the EM adenocarcinomas. No nuclear immunoreactivity was identified in the remaining ovarian neoplasms. Cytoplasmic immunoreactivity was demonstrated in 1.5% (1/65) of the EM adenocarcinomas, 0.7 % (1/138) of the Ser adenocarcinomas, 6% (2/35) of the Muc adenocarcinomas, 10% (1/10) of the CC adenocarcinomas, and none of the MucLMP.

Conclusions: TTF1 nuclear immunoreactivity can occur in some EM adenocarcinomas of the ovary. Cytoplasmic immunoreactivity can be demonstrated in a variety of ovarian neoplasms but is non-specific and of undetermined significance. Although TTF1 immunoreactivity is generally considered to be a specific marker for lung and thyroid neoplasms, the occasional immunoreactivity of ovarian EM adenocarcinomas should be considered in the evaluation of neoplasms of unknown primary site. It should also be taken into consideration when evaluating adenocarcinomas involving the lung in patients with a history of a gynecologic malignancy.

932 Conservative Therapy for Stage 1 Adenocarcinoma of the Endometrium in Young Women: Clinicopathologic Study

RS Kulkarni, EM Ongcapin. St Barnabas Medical Center, Livingston, NJ.

Background: Approximately 1600 women under the age of 50 will be diagnosed with endometrial cancer every year. For these women, preservation of fertility may be one of the most important factors when seeking treatment options. Hormonal treatments with progestins like medroxyprogesterone acetate (MPA) are widely applied in these young women provided the adenocarcinoma is well differentiated and there is no myometrial invasion. Treatment protocol consists of high dose MPA followed cyclic administration of low dose MPA. A retrospective review of all endometrial carcinomas diagnosed in younger women (less than 50yrs) at the Saint Barnabas Medical Center, Livingston, New Jersey. Patients were treated with medroxyprogesterone acetate for 6-10 months. Patient followup included response to therapy with diagnostic endometrial biopsies; successful pregnancies and recurrence of disease were done.

Design: A retrospective review of all endometrial carcinomas diagnosed in women younger than 50 years and treated with medroxyprogesterone acetate at the Saint Barnabas Medical Center, Livingston, New Jersey. Forty patients with endometrial carcinoma (20 with stage 1 Endometrioid Adenocarcinoma. 11 with stage 2 Adenocarcinoma and 9 with Stage 3 Adenocarcinoma) All of the stage 1 carcinomas were treated with high-dose medroxyprogesterone acetate alone as primary therapy and their clinical responses evaluated.

Results: Twelve of the twenty cases with grade I Adenocarcinoma and four of the ten with grade 2 carcinoma responded initially (follow-up curettage) to medroxyprogesterone acetate. The median length of treatment required for regression was 8 months. Two stage 2 patients who initially responded relapsed. Nine patients have conceived having healthy infants. Patients with other than stage 1 tumor had hysterectomy with radiotherapy.

Conclusions: This report demonstrates that conservative therapy in carefully selected young women with well-differentiated stage 1 endometrial cancer could be an alternative to hysterectomy. Recurrence was observed during long-term follow up in other stages even after pathological complete remissions.

933 Recurrence in Ovarian Cancer; a Transcriptomic Approach

A Laios, SA O'Toole, B Sheppard, N Gleeson, T D'Arcy, E McGuinness, M Ring, R Flavin, C Martin, J O'Brien, D O'Connell, O Sheils, D Cahill, E Gaffney, JJ O'Leary. Trinity College Dublin, Dublin, Ireland; University College Dublin, Dublin, Ireland.

Background: The majority of ovarian cancer patients present in advanced stages and are treated with surgery and combination chemotherapy. Approximately 80% respond to initial chemotherapy but eventually relapse. In cohort 1 of our study, we presented a distinct gene expression profile between primary and recurrent serous papillary adenocarcinomas and validated a panel of potential markers of recurrence, which included S100B, Claudin 16, BTC, CHORDC1 and IL27RA.

Design: Cohort 2 of our study consisted of 3 valuable paired ovarian cancers (primary and recurrent samples from the same patient but of different histology). Gene expression analysis was performed using the AB array 1700 system. R and Panther were used for statistical analysis. Validation of selected targets was performed using the TaqMan® Gene Expression Assays. Expression of genes identified in cohort 2 were also examined in cohort 1 samples. External validation of the identified genes in both cohorts was also carried out in an independent set of 11 primary and recurrent serous papillary adenocarcinomas of different histological grade.

Results: In the paired ovarian cancers 586 genes were differentially expressed between primary and recurrent tumours ($p<0.05$) and 75 genes at $p<0.01$. Some of the upregulated genes in the recurrent compared to primary that we chose to validate belong to the same gene families identified in cohort 1. These include TJP3, NRG2, FGF2, S100A8 and IL1R2. 9 targets were validated and these correlated with array results. Upregulated pathways mediated by these genes included angiogenesis, hypoxia and EGF receptor signaling pathways. NRG2 identified in cohort 2 was 6-fold upregulated on validation in the cohort 1 samples. IL27RA was the most differentially expressed gene (5-fold) in our external validation.

Conclusions: Our results suggest a multifactorial and coordinated mechanism for recurrence; the elevation of TJP3, enhancing the adhesive properties of tumour cells, the upregulation of EGFR ligands such as NRG2 and FGF2 and intracellular signalling via calcium binding protein S100A8. As all patients in both cohorts were treated with platinum based chemotherapy, some of the mechanisms involved in recurrence could be specific to the drugs used. We are currently extending our external validation on malignant, benign and normal ovarian tissues to assess tissue specificity.

934 Let-7 miRNAs Inhibit Leiomyoma Growth through down Regulation of HMGA2

J Laser, P Lee, K Mittal, JJ Wei. NYU Medical Center, New York, NY.

Background: Overexpression of HMGA2 due to chromosome 12q changes is a key molecular feature in many uterine leiomyomas (ULMs). HMGA2 functions as a mitogenic factor significantly associated with large ULMs. In our recent microRNA (miRNA) profiling analysis, let-7 miRNAs were highly dysregulated in most ULMs. Computer prediction analysis suggests HMGA2 is the top target genes by let-7 miRNAs. We hypothesized that let-7 miRNAs can inhibit ULM growth through down regulation of the HMGA2.

Design: 1) Compared the level of HMGA2 mRNA and protein in large (>10 cm) and small (<3 cm) ULMs by qRT-PCR (n=54) and immunohistochemistry (n=180); 2) Quantitatively analyzed HMGA2 protein in 10 HMGA2 mRNA positive ULMs (5 large and 5 small); 3) Set up primary tissue cultures in 3 ULMs with overexpression of HMGA2 mRNA and protein; 4) Conducted a transient transfection assay by introducing exogenous let-7c miRNA at the concentrations of 10nM, 20nM, 40nM and 60nM. Transfection efficiency was determined via fluorescent non-function RNA (Block-iT). 5) Analyzed of the levels of HMGA2 protein reductions at 48 and 72 hours by Western blot analysis. Semi-quantitation was performed by comparing levels of HMGA2 to control protein ERK.

Results: Overexpression of HMGA2 mRNA was identified in 38.8% (21/54) ULMs by qRT-PCR. There was no difference in HMGA2 mRNA levels between small and large ULMs. Overexpression of HMGA2 protein was detected in 37.8% (68/180) of the ULMs by immunohistochemistry via tissue microarray analysis. The levels of HMGA2 protein were strongly associated with ULM size: the larger ULM size, the higher levels of HMGA2 protein (r=0.81). The correlation of ULM size and HMGA-2 protein level was further supported by Western blot analysis (n=10). To test if differential expression of HMGA2 protein in ULMs was regulated by let-7 miRNAs, a transfection of exogenous let-7c miRNA in ULM primary culture cells was performed (n=3). A reduction of HMGA2 was found in a dose-dependent manner.

Conclusions: *In vivo*, 1) protein levels, but not mRNA of HMGA2 are positively correlated with ULM size, and 2) the levels of let-7 miRNAs are inversely correlated with ULM size. *In vitro, 3)* exogenous let-7 miRNAs can suppress HMGA2 protein production, suggesting a biological role of let-7 miRNAs as negative regulators in leiomyoma growth through downregulation of the target gene HMGA2.

935 Evidence Supporting a Multi-Step Model of Pelvic Serous Carcinogenesis That Originates in the Tubal Fimbriae

Y Lee, R Drapkin, A Miron, D Kindelberger, FD McKeon, CP Crum. Brigham and Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA.

Background: Recent reports have identified the distal fallopian tube as an important site of early serous carcinoma (tubal intraepithelial carcinomas or TIC) in BRCA+ women. Emerging data suggest that TICs are commonly associated with tumors otherwise fulfilling the criteria for ovarian and peritoneal serous carcinomas. Moreover, p53 positive segments of weakly proliferative, benign-appearing tubal secretory cells – termed p53 signatures – have also been identified and proposed as possible precursors to serous cancer. This study analyzed the frequency of p53 signatures in women with TIC and studied normal mucosa, p53 signatures, TICs and ovarian cancers (when present) for p53 mutations.

Design: Fallopian tubes were fully sectioned by the SEE-FIM protocol from 58 consecutive women without ovarian malignancy and 12 with TICs, 10 of which had a co-existing ovarian cancer. All were stained for p53. 14 p53 signatures, 13 TICs and 8 co-existing ovarian cancers were fully analyzed for p53 mutations by laser capture micro-dissection, PCR amplification and replicate sequencing of exons 1-11.

Results: p53 signatures were identified in 26 and 53 per cent and were multiple in 32 and 67 percent of tubes classified as normal and TIC respectively. Of 14 p53 signatures analyzed, 8 contained p53 mutations, many linked to ovarian cancer. In one TIC, direct continuity between a p53 signature and TIC was seen. All 13 TICs contained p53 mutations and 8 of 8 paired tubal-ovarian tumors shared the same p53 mutation.

Conclusions: The strong association between frequency and multiplicity of p53 signatures and TIC, the presence of serous cancer-related p53 mutations in both p53 signatures and TIC, the existence of continuity between p53 signatures and TIC, and the sharing of identical mutations between TIC and ovarian cancer are compelling evidence for a continuum that initiates with the p53 signature and (in some) progresses to TIC. In this model TIC, once established, spreads rapidly to the ovary and peritoneum. The fimbrial p53 signature warrants designation as a candidate precursor to both tubal and ovarian serous carcinomas and as a potential surrogate marker for serous cancer risk.

936 The Diagnosis of Hydatidiform Moles Using P57 Immunohistochemistry and Her2 Flourescent In Situ Hybridization

RD Legallo, EB Stelow, KA Atkins. University of Virginia, Charlottesville, VA.

Background: The identification of a hydatidiform mole is critical for management following miscarriage or pregnancy termination. Currently, routine histopathologic examination is the most commonly used technique for diagnosis but has been shown to be subject to great inter- and intraobserver variability. P57 immunohistochemistry (IHC) has shown to improve accuracy in the diagnosis of a complete mole (CM) but cannot discriminate a partial mole (PM) from hydropic abortus (HA). Her2 fluorescent in situ hybridization (FISH) is routinely performed on paraffin-embedded tissue, and we postulate it can act as a surrogate marker for ploidy and aid in the diagnosis of PM.

Design: Thirty-nine cases were selected based on histologic or clinical features concerning for or diagnostic of hydatidiform mole, 11 of which had cytogentics. All H&E slides were reviewed by 3 pathologists and a consensus diagnosis was rendered. P57 IHC and Her2 FISH were performed using archival, formalin-fixed, paraffin-embedded tissue. P57 staining was considered negative when cytotrophoblasts and villous stromal cells showed complete loss of nuclear staining. Her2 FISH was scored as triploid if more than 20% of nuclei showed 3 signals. A final diagnosis was assigned using the results from all ancillary studies including cytogenetics.

Results: There was disagreement between original and consensus H&E diagnosis in 30% of cases, all of which involved the differential of PM and HA. P57 was absent in all 8 histologically diagnosed CMs. One histologically diagnosed CM was changed to HA based on diffuse stromal and cytrophoblastic reactivity towards p57. Her2 was interpreted as triploid in 4 cases diagnosed as PM in both the original and consensus review as well as in 3 of the 4 cases assigned a final diagnosis of PM. In 7 cases that had either an original or consensus diagnosis of PM, Her2 was interpreted as diploid and agreed with the final diagnosis of HA. All final diagnoses correlated with cytogenetic analysis except for one cased diagnosed as HA found to be triploid by cytogenetics.

Conclusions: The diagnosis of CM can usually be made by routine histology, however, p57 IHC can helpful in distinguishing CM from PM or HA. The distinction of PMs and HAs is often difficult by routine histology, however. Her2 FISH may be a useful ancillary tool for the identification of triploid cases and appears, in our small study, to be both sensitive and specific for the diagnosis of PM. The use of p57 IHC with Her2 FISH may allow for the correct classification of most molar disease.

937 The Impact of Endocervical Cytologic Sampling (Endo Pap) on Cytologic-Histologic Correlation (CHC) Error Rates

CL LeMaster, L Dahmoush, MS Zaleski, C Vrbin, SS Raab, CS Jensen. University of Iowa Hospitals and Clinics, Iowa City, IA; University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: CHC is a federally mandated part of every cytology lab's quality control/quality assurance (QC/QA). As part of a multiinstitutional study sponsored by AHRQ, CHC was standardized in four labs and a database was created to study errors. Efforts to standardize the CHC process show different rates of discrepancies between labs, as well differences in the attributed cause of the discrepancy. Although differences between labs may be seen as differences in "quality" or attributed to reluctance on the part of some labs to attribute errors to the laboratory, it is likely that variations in clinical practice also account for differences. As such, close scrutiny of the overall practice parameters is important when comparing CHC data and attempting to identify "best practices". Endocervical sampling technique is one clinical practice variable noted and this study was designed to assess the impact on CHC at one institution.

Design: 371 discrepant CHC pairs were identified in the database from one laboratory in a two year period (2004-5) including 219 endo paps and 152 non-endo paps. All discrepant pairs were reviewed by a pathologist to determine the source of the error: cytologic/surgical sampling or cytologic/surgical interpretation. Discrepancy rates and frequencies for each source of error were compared for endo pap cases and non-endo pap cases.

Results:

Endo pap and non-endo pap discrepancy rates within CHC

Sampling Method	Discrepancy Rate	Distribution within CHC	Cytologic Sampling Error Rate	Interpretation Error Rate
Endo Pap	11.4%	59%	86.8%	2.7%
Non-Endo pap	.4%	41%	59.2%	7.9%

Significantly more discrepancies were related to endo paps than non-endo paps ($p<.004$). The discrepancy rate for endo paps was significantly higher than non-endo pap specimens ($p<.001$). Endo pap discrepancies are a result of sampling error significantly more often than are non-endo paps ($p<.01$). The interpretation error rate was significantly higher for non-endo paps than endo paps ($p<.001$).

Conclusions: 1) The majority of CHC discrepancies from one laboratory were from endo pap samples, although endo pap samples comprise only 5% of all gyn cytology.2) Most endo pap cases are classified as sampling errors reflecting that the sampling site of the endo pap is different from the corresponding histologic sample. 3)Cytologists should be aware of the impact that endo paps may have on CHC when comparing data with other laboratories.

938 Immunohistochemical Analysis of IMP3 in Ovarian Carcinomas: A Clinicopathologic Study

C Li, ML Anderson, M Lisovsky, A Sienko, P Cagle, A Fraire, D Lu, K Dresser. University of Massachusetts Medical School, Worcester, MA; Baylor College of Medicine, Houston, TX.

Background: IMP3, also known as L523S or KOC, is an oncofetal mRNA binding protein that is over-expressed in several malignant neoplasms but not in most normal adult tissues including ovary. Recently, it has been demonstrated that IMP3 expression is an independent prognostic factor for renal cell carcinoma. The aim of present study was to evaluate the potential of IMP3 as a diagnostic and/or prognostic factor for ovarian carcinomas.

Design: We have constructed a tissue microarray containing 141 samples collected from 47 ovarian cancers of different histologies, including serous (n=35), endometrioid (n=4), clear cell (n=3), and mixed (n=5) carcinomas. After obtaining IRB approval, patient demographics were collected anonymously and abstracted. Positive IMP3 staining was scored as positive for these cases in which 50% or more of cells demonstrated intermediate or strong immunoreactivity.

Results: IMP3 expression was identified in 19 out of 35 serous, 1 of 4 endometrioid, 2 of 3 clear cell, and 2 of 5 mixed ovarian carcinomas, making overall positive IMP3 stain seen in 24 out of 47 (51%) cases in this study. Among these 24 IMP3 positive cases, 2, 17, 2, 1, and 2 cases were diagnosed with stage IVA, IIIC, IIIA, IIB, and IIA disease, respectively. Three cases showed no response to primary therapy, 4 partial responses, and 16 complete responses. IMP3 negative cases were diagnosed at stage IVA (n=2), IIIC (n=9), IIIB (n=1), IIIA (n=1), IIC (n=2), IIA (n=1), IC (n=1), and IA (n=5). Among these negative cases, all except one case responded to primary therapy completely.

Conclusions: We have demonstrated significant expression of IMP3 in ovarian carcinomas. In addition, IMP3 expression is associated with higher clinical stage (p-valve = 0.01) and poor response to the primary therapy (p-value = 0.024).

939 Therapeutic and Antiangiogenic Activities of Cyclooxygenase-2 Inhibitor in High Grade Squamous Intraepithelial Lesions of the Cervix – Histologic and Immunohistochemical Follow up

HC Li, H Scott, KR Geisinger, JO Cappellari, BE Miller. Wake Forest University Baptist Medical Center, Winston-Salem, NC.

Background: High levels of cyclooxygenase-2 (COX-2) protein expression have been reported in high grade squamous intraepithelial lesions of the cervix (HGSIL). The COX-2 inhibitor, Celecoxib (*Pfizer*) has been shown to reduce the development of preinvasive lesions of skin and colon. In a pilot study, we investigated the efficacy of Celecoxib in the regression of HGSIL, and associated parameters of proliferation and angiogenesis.

Design: 28 women (mean age 27, range 17-50) with HGSIL, were evaluated by serial colposcopy, cervical cytology, and cervical biopsy. 14 women received Celecoxib (400 mg, bid) for 12 weeks prior to LEEP surgery. 14 women were non-compliant and had treatment for less than a month. These two groups were similar in respect to age, ethnicity, percentage of involvement of the cervix at study, and histologic features on initial cervical biopsy. Pre- and post treatment histology were evaluated. Immunohistochemical stains for COX-2, bcl-2, MIB-1, and vascular marker CD31 were also analyzed.

Results: After a 12 week period of Celecoxib treatment, the average number and lesion size of the compliant group (n=14) decreased from 2.2 and 152 mm^2 to 1.2 and 36 mm^2, respectively($p<0.05$). Histologically, Celecoxib treatment increased the regression rate of both moderate and severe dysplasia from 25% in non-compliant group to 57% in the compliant group. Immunohistochemically, a marked decrease of CD 31 positive stained stromal area beneath the dysplastic epithelium was demonstrated in 65% of the compliant cases. The percentage of positive MIB-1 expression in lower, mid and upper one third thickness was significantly decreased from an average of 31.1, 36.2 and 18.6 to 15.3, 11.3 and 0, respectively ($p<0.05$).

Conclusions: Reduction in degree of and complete regression of HGSIL may be achieved with COX-2 inhibitor by reducing angiogenesis and decreasing epithelial proliferation. More prominent decreases in lesion size, stromal vascular density and epithelial proliferation, compared to the observation in morphology, suggest that a longer period of treatment might maximize therapeutic effect of Celecoxib in HGSIL.

940 Clinicopathologic Features of Endometrial Serous Carcinoma in Women Younger Than 56 Years of Age

S Liang, C Tornos, M Pearl, E Chalas, M Kelly, P Schwartz, T Zhang, W Zheng. State University of New York, Stony Brook; Yale University; Shangdon University; University of Arizona.

Background: Endometrial serous carcinoma (ESC) is a high-grade lesion that account for 10-15% of all endometrial carcinomas. In contrast to the most common endometrioid type, ESC usually occurs in old patients, arises in resting endometrium, is not associated with hyperestrogenism, and has a high frequency of p53 mutations with a poor prognosis. About 10% of ESC are seen in women younger than 56 years. As such, the clinicopathologic features of ESC in this age group have not been addressed and this was the purpose of this study.

Design: We identified 39 patients younger than 56 years with ESC (1995 – 2005). Clear cell carcinomas, carcinosarcomas or mixed type containing ≥25% serous component were also included. All cases were surgically staged and the medical charts were reviewed. The following features were recorded: age, stage, depth of myometrial invasion, histologic diagnosis, lymphovascular invasion, lymph node status, BMI, history of other cancers, medical conditions, menstrual and exogenous hormonal status and follow-up information.

Results: The median age was 52.54y (41 to 56). BMI was >26 in 13 patients, ≤ 26 in 10, and unknown in 16. At least 20 patients (90.9%) were postmenopausal (0-20 years, average 6.85 years). Eleven patients had other cancers (9 breast, 1 colon, 1 thyroid). In patients with breast cancer, 2 used Tamoxifen for 1-5 years. Medical conditions included hypertension (7), diabetes (3), hypo- or hyperthyroidism (5), and hypercholesteremia (2). 74% of patients presented with PMB, and 14.7% with abnormal pap smear. Histological types were: ESC 30, ESC/CCC 4, ESC/endometrioid 3, CCC1 and carcinosarcoma 1. The FIGO staging was: stage I–48.7%; stage II-15.4%; stage III-10.3%, and stage IV-25.6%. For patients with stage Ib and higher, 20/26 had myometrial invasion, and 6/26 had only extrauterine disease (3 stage III and 3 stage IV). Lymphovascular invasion was found in 6/23 cases. The overall survival was 73% (average follow-up 32.2 months). The survival and follow-up for each stage were stage I-88.9%, 43.7 months; stage II–83.3%, 21.5 months; stage III–75%, 29.5 months; and stage IV–22.2%, 20.5 months.

Conclusions: ESC in patients of younger than 56 years has a high association with breast cancer (23%). Postmenopausal status is present in a majority of patients, suggesting that "physiological age" rather than actual age might contribute to ESC development.

941 Endometrial Serous Carcinoma Has a Strong Association with Breast Cancer, Particularly in Patients Younger Than 56 Years of Age

S Liang, M Pearl, C Tornos, E Chalas, M Kelly, P Schwartz, T Zhang, W Zheng. State University of New York, Stony Brook, NY; Yale University School of Medicine, New Haven, CT; Shangdon University, China; University of Arizona, Tucson, AZ.

Background: Endometrial serous carcinoma (ESC) bears a poor prognosis and usually occurs in elderly women with average age of 65 years. However, some cases are seen in younger women. It is unknown whether the younger patients have any specific risk factors and a different prognosis than the older group. The purpose of this study was to investigate these two issues.

Design: Three hundred and twenty eight ESC including mixed tumors with more than 25% serous component were identified from our database (1995 – 2005). All cases (except 6) were surgically staged. Medical charts were retrospectively reviewed. The following information was recorded: age, history of breast cancer, family history of breast cancers, history of oral contraceptive pill (OCP), Tamoxifen or hormonal replacement therapy (HRT), and clinical follow-up.

Results: Thirty-nine patients (group A) were 56 years or younger (41-56, average 52.54y), and 289 (group B) older than 56 years (56-92, average 71.06y). All patients were postmenopausal except 17 (2 perimenopausal and 15 unknown). History of OCP use was noted in 12 patients. Breast cancer was seen in 15.5% (50/323) of patients (group A: 23.1%, and group B: 14.4%; $p= 0.162$). HRT was noted in 15.8% (26/165) patient (group A: 19.4 %; group B: 14.7%). In group A, 6 of 27 non-breast cancer patients (while 1 of 9 breast cancer patients) used HRT ($p=0.399$). Family history of breast cancer was present in 16.2% (24/148), and mostly in group B patients (20). Overall survival was 65.6 % at 32.3 months follow-up (group A: 73%, 32.2 m; group B: 64.4%, 32.3 m; $p=0.307$). The stage-matched survival between group A and B was: stage I: 88.9% vs. 87.9% ($p=0.907$); stage II: 83.3 % vs. 79.2% ($p=0.819$); stage III: 75% vs. 63.2% ($p=0.634$); and stage IV: 22.2% vs. 23.3% ($p=0.941$).

Conclusions: 15.5% of patients with ESC have a previous history of breast cancer. This association is even stronger in patients who developed ESC before age 56 years. There is no statistically significant difference in survival in both age groups, although the younger group seems to have a slightly better outcome. However, larger scale studies and longer follow-up are needed to further confirm the relationship between breast cancer and the development of ESC at a younger age.

942 NF-kB p65 Expression Is a Favorable Prognostic Factor in High-Grade Serous Ovarian Carcinoma

J Liu, DG Rosen. M.D. Anderson Cancer Center, Houston, TX.

Background: Constitutive activity of NF-kB transcription factors has been detected in many human tumors, including ovarian cancer. The effects of NF-kB on tumorigenesis are largely attributed to its activation of multiple antiapoptotic target genes, but the role of NF-kB activation in ovarian cancer development and prognosis is poorly understood. We examined the correlation between NF-kB activation and clinical and pathologic parameters in a large cohort of patients with high-grade serous ovarian carcinoma.

Design: We performed immunoperoxidase staining against phosphorylated p65, the most commonly activated subunit of NF-kB, in a tissue microarray containing tissues from 275 patients with primary high-grade serous ovarian carcinomas who had surgery between 1990 and 2004 at The University of Texas M. D. Anderson Cancer Center. Differences in proportions between p65 expression and tumor grade, FIGO stage, tumor histotype, patient's age at time of diagnosis and response to primary therapy were calculated using chi-square analysis or Pearson correlation, as appropriate. Overall survival time and time to progression were assessed by Kaplan-Meier analysis.

Results: Nuclear expression of NF-kB p65 was correlated with late-stage disease (p=0.02). No correlation was found with grade, level of cytoreduction, or patient's age at time of diagnosis. Patients expressing p65 had longer overall survival (p=0.01) and later disease recurrence (p=0.01) than patients with low or no p65 expression.

Conclusions: Nuclear expression of p65 is a marker of favorable prognosis in high-grade serous ovarian cancer.

943 Endometrial Ablation for Dysfunctional Uterine Bleeding: Histologic Insights into Treatment Failures

R Livengood, N Bailey, JE Coad. West Virginia University, Morgantown, WV.

Background: Several hyperthermic endometrial ablation methods are clinically available for the treatment of dysfunctional uterine bleeding (DUB), ranging from the older lower energy surface rollerball (RB) to the newer higher thermal dosing water balloon (WB) and radiofrequency (RFA) devices. Up to 15% of these patients post-ablation proceed to hysterectomy for continued bleeding, which is generally attributed to residual dysfunctional endometrium. However, the histologic features of these treatment failures has not been well documented.

Design: 20 hysterectomy specimens were identified with a history of endometrial ablation (RB n=4; WB n=7; RFA n=9). The patients were treated according to the manufactures' recommendations. Blinded morphologic evaluation of the uterine sections was performed to identify the histologic cause(s) of the continued bleeding.

Results: Two primary bleeding sources were identified. Residual cycling endometrium and "hyperthermic fixation/necrosis" of myometrial arteries that preserved the vascular architecture, resisted tissue breakdown, and precluded thrombus organization with subsequent rebleeding. The uteri were subdivided into three failure groups: Group 1) residual cycling endometrium, Group 2) thermally-altered myometrial vessels and Group 3) combined etiologies. A minimal wound healing cleavage plane was present between the thermally-fixed and viable deeper myometrium.

Histologic Uterine Findings By Device Type

		Treatment	Treatment Failure Etiology			Thermally-Fixed
	Age	to Surgery	Group 1	Group 2	Group 3	Tissue Layer
RB	31 ± 7 yr	2 ± 1 mo	75 %	25 %	0 %	0.8 ± 1.5mm
WB	38 ± 11 yr	7 ± 10 mo	14 %	72 %	14 %	1.8 ± 1.2mm
RFA	34 ± 5 yr	4 ± 1 mo	22 %	45 %	33 %	3.4 ± 2.3mm

Conclusions: Active bleeding from non-healing thermally-fixed vessels contributed to 25% of the lower thermal dose (RB) and 81% of higher thermal dose (WB/RFA) treatment failures. The depth of tissue fixation also increased with greater thermal energy delivery (RB<<WB<RFA, p=0.06). In contrast to classical necrosis, the thermally-fixed tissue results from protein denaturation in such a fashion that it chronically resists breakdown by the body's repair pathways. This inhibits intrauterine cavity healing and thrombus organization with resultant prolonged bleeding. While reducing residual endometrium as the cause of failure, the higher energy devices (WB/RFA) appear to be more associated with an "etiology switch" from dysfunctional endometrium to treatment-related thermally-fixed vessels as the cause of continued post-treatment bleeding (p=0.08).

944 Differential Immunoreactivity of p16 in Uterine Leiomyosarcomas and Benign Variants of Leiomyoma

M Manduch, BR Gannon, TJ Childs. Kingston General Hospital, Queen's University, Kingston, ON, Canada.

Background: Several studies have now examined the cDNA expression profiles of normal myometrium, leiomyomas (LM), and leiomyosarcomas (LMS). This has produced a list of candidate genes, which might be useful tools for distinguishing these entities from each other. Potential candidates identified from this body of research include WT-1, IGF-1, h-caldesmon, cytokeratin 18 and the cyclin-dependent kinase-4 inhibitor, p16.

Design: To determine if immunohistochemical expression of these proteins could aid in the diagnosis of leiomyosarcomas and leiomyoma variants, we constructed a tissue microarray consisting of cases of normal myometrium (n=10), LM (not otherwise specified and variants; n=46) and LMS (n=8), and measured immunoreactivity for each of these proteins. The cases were scored based on staining intensity (weak, moderate, or strong) and extent (focal or diffuse), and assigned a final score ranging from 0 to +3.

Results: Immunostaining for p16 was statistically stronger in LMS then in LM and its subtypes ($p<0.001$). Specifically, p16 immunostaining in the LMS (n=8) was at least +2 while all of the LM (n=46) were either 0 (n=34) or +1 (n=12). The expression of the remaining antibodies did not show a statistically significant difference between the two groups. Furthermore, none of the markers studied showed any differences amongst the various LM variants.

Conclusions: The results of this study confirm the over expression of p16 in LMS and suggest that p16 can serve as a reliable immunohistochemical marker in distinguishing uterine LMS from LM and its benign variants.

945 Frequent LOH at Chromosome 1p36 in Ovarian Serous Borderline Tumors

TL Mao, RJ Kurman, TL Wang, IM Shih. Johns Hopkins Medical Institutions, Baltimore, MD; National Taiwan University Hospital, Taipei, Taiwan.

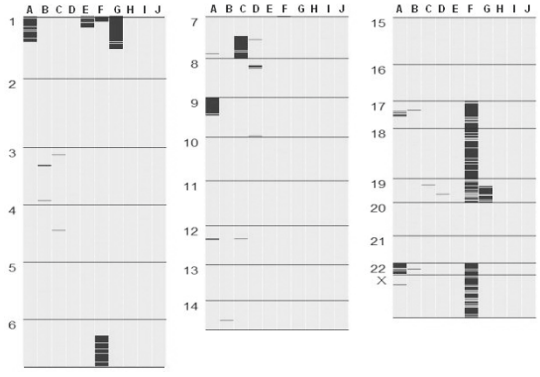
Background: Loss of heterozygosity (LOH) is a cardinal feature of human neoplastic disease. LOH is a result of chromosomal instability and loci of LOH may harbor tumor suppressor genes. Previous studies have demonstrated LOH in several loci in SBT genome but interpretation of the results are complicated by the fact that these studies did not analyze pure tumor cells and the results were not validated using an independent method.

Design: In order to characterize the LOH profiles in SBTs, we employed a genome-wide analysis, single nucleotide polymorphism (SNP) arrays. Purified (tumor cells >99%) tumor cells from10 cases of SBTs and paired normal tissue were analyzed by 10K SNP arrays. Nucleotide sequencing was used to validate the LOH results.

Results: Based on SNP arrays, the most striking finding is the LOH at the chromosome 1p36 region occurring in four out of 10 cases. No other chromosomal regions contained LOH in two or more cases. For validation, we genotyped four SNP sites (probe set ID: 1510643, 1515933, 1516342 and 1518917) within the minimal LOH region using nucleotide sequencing in those 10 cases and additional 8 SBTs. Four low grade (LG) serous carcinomas were also included because it had been shown that LG serous carcinoma has similar genetic changes as SBT. LOH was defined as presence of homozygosity in any of the four sites in which the matched normal tissue showed heterozygosity. All the tumors were found to be informative in at least one SNP marker. LOH was detected in 50% (11/22) of SBT/LG serous carcinoma.

Conclusions: The findings from this study provide cogent evidence that LOH at the 1p36 region is most common in SBTs. It occurs in 11 (50%) of 22 SBTs/LG carcinomas. LOH at 1p36 can be used as a molecular marker for clonality study in SBTs and future studies will be focused on demonstrating the candidate tumor susceptible gene(s) that contributes to tumor development in SBTs.

Fig. 1 LOH profile in 10 SBT and corresponding normal tissue (A to J). Chromosomal numbers are listed on the left of the panels. Blue boxes: LOH; yellow boxes: no LOH; grey boxes: un-informative regions.



946 The Histogenesis of Choriocarcinoma – An Immunohistochemical Study

TL Mao, RJ Kurman, CC Huang, MC Lin, IM Shih. Johns Hopkins Meidcal Institutions, Baltimore, MD; National Taiwan University Hospital, Taipei, Taiwan; Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

Background: Choriocarcinoma is defined as a trophoblastic tumor composed of cytotrophoblast and syncytiotrophoblast. Microscopically, these two types of cells are intimately associated with each other, forming the characteristic "biphasic pattern", however, a detailed analysis of trophoblastic cells in choriocarcinoma using immunohistochemical markers has not been performed.

Design: In this study, we applied immunohistochemistry using well-established and new trophoblastic markers to determine the trophoblastic subpopulations in 36 gestational choriocarcinomas. The antibodies included Mel-CAM, HLA-G, mucin-4, p63 and ß-catenin. A semi-quantitative assessment of positive cells and the cellular localization of these markers were recorded.

Results: 100% and 78.6 % of cases showed mostly diffuse and strong membranous and cytoplasmic staining for mucin-4 and HLA-G respectively. These markers are expressed in the trophoblastic cells of the trophoblastic columns (villous type intermediate trophoblast) and implantation site (implantation type intermediate trophoblast) of a normal placenta. The neoplastic mononucleate cells showed moderate reactivity in 75% of the cases for Mel-CAM, a marker specific to trophoblastic cells at the implantation site. The positively stained cells were larger with abundant cytoplasm, consistent with the morphology of intermediate trophoblasts. In contrast, only a very small portion (<5%) of mononucleate trophoblastic cells was positive for nuclear ß-catenin and p63, which are cytotrophoblast markers. The positively stained cells were scattered among the cytotrophoblastic cells clusters.

Conclusions: These results suggest that choriocarcinoma is composed of a mixture of syncytiotrophoblasts, intermeditate trophoblasts and, to a lesser extent, cytotrophoblasts. The presence of nuclear ß-catenin staining in the cytotrophoblast indicates that choriocarcinoma develops from neoplastic cytotrophoblastic cells which are the cancer stem cells that differentiate into either intermediate trophoblast or syncytiotrophoblast.

Immunohistochemical staining result of choriocarcinoma

antibody	No. of cases	positive cases	% of positive cells*		
		(%)	<10%	10-50%	>50%
p63	36	6 (16.7)	5	1	0
ß-catenin	36	11 (30.5)	10	1	0
HLA-G	28	22 (78.6)	0	2	20
Mel-CAM	28	21 (75.0)	10	8	3
MUC4	32	32 (100)	2	6	24

*: percentage of mononucleate trophoblasts

947 MicroRNA Signatures in Cervical Cancer: Suggests Hierarchical miRNA Control of Gene Expression

CM Martin, K Astbury, C Becker, R Henfrey, J Sherlock, S Guenther, O Sheils, JJ O'Leary. Trinity College, Dublin, Ireland; Applied Biosystems Europe, Warrington, United Kingdom.

Background: Cervical cancer, a potentially preventable disease, remains the second most common female malignancy worldwide. Human papilloma virus (HPV) has been implicated in the development of virtually all cases of cervical cancer. MicroRNAs (miRNAs) are a recently discovered family of small non-protein-coding RNAs known to negatively regulate expression of protein coding genes. Their expression profiles can be used for the classification, diagnosis, and prognosis of human malignancies.

Design: In this study, we examined the expression of 180 characterised miRNA molecules and carried out predicitive protein target analysis in cervical cancer cell lines: C33A (HPV negative), SiHA (HPV 16), CaSki (HPV 16 and 18) and normal cervical tissue using the Applied Biosystems TaqMan MicroRNA Assays Human Panel - Early Access Kit. Biological and technical replicate and serial passage analysis was carried out.

Results: C33A, SiHA and CaSki cells demonstrated distinct miRNA expression signatures in comparison with normal cervical tissue. In addition, differential miRNA expression patterns were observed between HPV positive and negative cell lines, with the predicted function of differentially expressed targets coinciding with a number of cell cycle regulatory molecules, including CDKN2a, CDK 6 and 8, Cyclin D2, Cdc14, p16, E2F and Rb. In particular CHEK 1, MYBL 2, cyclin E1 and E2 gene expression in cervical cancer appears to be selectively controlled by hierarchical miRNA species, suggesting 'master miRNA gene' control in specific hierarchical miRNA expression cascades. Expression profiles of miRNAs demonstrated high stability and reproducibility in serial passage experiments.

Conclusions: These findings highlight the potential importance of miRNA in the complex pathobiology of cervical pre-cancer and cancer. These miRNAs may serve as potential biomarkers of pre-invasive cervical disease and potential therapeutic targets. In addition, the control of RNA and protein expression via miRNAs is highly regulated and hierarchical. The research team are members of the Irish Cervical Screening Research Consortium [ICSRC].

948 Tubulo-Squamous Polyp: A Report of Ten Cases of a Distinctive Hitherto Uncharacterised Vaginal Polyp

WG McCluggage, RH Young. Royal Group of Hospitals Trust, Belfast, United Kingdom; Massachussetts General Hospital, Boston, MA.

Background: We report 10 cases of a morphologically distinct vaginal polyp which has hitherto not been characterised.

Results: The polyps occurred in women aged 39 to 78 years (most were postmenopausal) and were from 1.0 to 3.0 cm. Most whose location is known to us were situated in the upper vagina. Histologically all the polyps were remarkably similar and composed of well circumscribed expansile nests of epithelial cells embedded in a hypocellular fibrous stroma. The epithelial elements, which were morphologically bland, were predominantly glycogenated or non-glycogenated squamous cells but small tubules were present at the periphery of some of the nests in all the cases. In a few cases, some of the squamous nests contained keratin pearls and exhibited central necrosis with calcification. In occasional cases, small numbers of tubules were focally unassociated with the squamous component. In 3 of 4 cases tested, the cells lining the tubules were positive with prostatic acid phosphatase and in two of four with prostate specific antigen. The epithelial elements reacted with broad spectrum cytokeratins but the mesenchymal component was negative.

Conclusions: The histological features of this polyp, which we term "tubulo-squamous polyp of the vagina", are constant and distinctive and differ from mixed tumour of the vagina. Although not characterised previously, we believe several cases may have been reported in the literature as vaginal mixed tumour or Brenner tumour. Possible theories of histogenesis include a Mullerian origin, derivation from mesonephric remnants or derivation from urogenital sinus-derived epithelium. Positive staining of some cases with prostatic acid phosphatase and prostate specific antigen raises the possibility of some resemblance to ectopic prostatic tissue but the overall appearance is different from that entity, or derivation from paraurethral Skene's glands.

949 Molecular Cytogenetic Analysis of *HMGA1* and *HMGA2* Rearrangements in Mesenchymal Neoplasms of the Lower Genital Tract

F Medeiros, MR Erickson-Johnson, GL Keeney, AC Clayton, X Wang, AM Oliveira. Mayo Clinic, Rochester, MN.

Background: Chromosomal rearrangements of the chromatin remodeling gene *HMGA2* and its homolog *HMGA1* are commonly seen in a variety of benign mesenchymal tumors. Our group has recently shown that *HMGA2* rearrangements occur in approximately 35% of aggressive angiomyxomas. However, whether these abnormalities also occur in other histologically similar entities of the lower genital tract, such as angiomyofibroblastoma and cellular angiofibroma, is currently unknown. Herein, we evaluated *HMGA1* and *HMGA2* rearrangements in a series of benign mesenchymal tumors of the lower genital tract aiming to determine their frequency and possible diagnostic relevance.

Design: A total of 84 cases were analysed in this study, including 40 aggressive angiomyxomas, 17 angiomyofibroblastomas, 13 leiomyomas, 6 cellular angiofibromas, 5 fibroepithelial polyps, and 3 superficial angiomyxomas. All cases showed typical histologic features. Immunohistochemistry for smooth muscle actin, desmin, h-caldesmon, muscle-specific actin, S-100, and CD34 was done in all cases. Fluorescence in situ hybridization (FISH) for *HMGA1* and *HMGA2* loci was performed on paraffin-embedded thin tissue sections using custom-designed probes. An average of 200-400 cells was scored in each tumor by two independent investigators.

Results: Molecular cytogenetic analysis revealed *HMGA2* locus rearrangements in 15 of 26 aggressive angiomyxomas (37%) and in a single case of vulvar leiomyoma (8%). All rearrangements but one were apparently balanced in nature and were identified in 60-90% of the stromal cells. *HMGA1* rearrangements were not identified in any of the tumors studied in this series.

Conclusions: As previously shown, *HMGA2* rearrangements occur in approximately one third of aggressive angiomyxomas but can also occasionally be seen in leiomyomata of the lower genital tract. Other benign mesenchymal neoplasms of the lower genital tract are not characterized by *HMGA1* and *HMGA2* rearrangements. These findings indicate that aggresive angiomyxomas are genetically distinct from angiomyofibroblastomas and suggest that the identification of *HMGA2* may be of diagnostic value in separating aggressive angiomyxoma from other histologically similar entities.

950 Differential Expression of Vimentin in Ovarian and Uterine Adenocarcinomas

F Mohammadi, MC Ionescu. North Shore- Long Island Jewish Health System, Manhasset, NY.

Background: Vimentin has been reported to be reactive in most uterine adenocarcinomas, but few studies have evaluated vimentin reactivity in ovarian adenocarcinomas. Various investigators have shown the difference of WT-1 reactivity in differentiating ovarian from uterine carcinoma, however they have had different conclusions. Distinguishing between primary ovarian adenocarcinomas from those that are metastatic to the ovary of uterine origin has been a problem particularly for treatment purposes.

Design: We studied the expression of vimentin and WT-1 by immunohistochemistry in 32 cases of ovarian adenocarcinomas (25 serous, 3 clear cell, 3 endometroid, 1 mucinous) and 12 uterine adenocarcinomas (6 endometroid, 5 serous uterine, 1 carcinosarcoma).

Results: Ovarian tumors were negative for vimentin 28 of 32 (87.5%.) cases. Among uterine carcinomas vimentin immunoreactivity was seen in 11 of 12 (91.6%) cases. WT-1 was detected in 27 of 32 (84.3%) cases of ovarian carcinomas. None of the uterine adenocarcinomas was positive for WT-1 (table1).

Conclusions: The difference in vimentin and WT-1 expression was highly significant between ovarian and uterine carcinomas. Our results suggest that vimentin and WT-1 are minimally related to the morphologic appearance of specific type of tumor and they might reflect the multipotential differentiation of mullerian- duct derived epithelium. Use of vimentin and WT-1 as a immunohistochemical panel might be of value in discriminating between carcinomas that are primary ovarian versus uterine origin.

Table 1. Expression of Vimentin and WT1 in different types of ovarian and uterine carcinomas

Type of carcinoma	ovarian serous	ovarian clear cell	ovarian endometrioid	ovarian mucinous	uterine serous	uterine endometrioid	uterine carcinosarcoma
Vimentin-positive cases	2/25	1/3	1/3	0/1	4/6	6/6	1/1
WT1-positive cases	23/25	2/3	2/3	0/1	0/6	0/6	0/1

951 Where Is the High Grade Dysplasia Now? Impact of the Bethesda 2001 Classification and Liquid-Based Pap Tests on Cytohistologic Correlations

MY Ngae, K Kjeldahl, SG Miller, DM McKeon, TM Ten Haken, HE Gulbahce, SE Pambuccian. University of Minnesota, Minneapolis, MN; University of Minnesota Medical Center-Fairview.

Background: Cytohistologic correlation is essential for quality assurance in cervical cancer screening programs. In an influential study performed on conventional Papanicolaou (CP) smears classified by the Bethesda II (1991) system, Kinney et al (1998) observed that the largest proportion (38.8%) of CIN2+ (i.e. CIN 2, CIN 3 and squamous cell carcinoma) occurred in follow-up biopsies of women with Papanicolaou tests (PT) showing atypical squamous cells of undetermined significance (ASCUS). Our study aims to assess if this is still true with liquid-based PT (LBPT) classified by the Bethesda 2001 (B2001) system.

Design: Our multi-hospital departmental computer database was searched for all PT that had cervical biopsy correlates within 6 months of the PT from 01/01/04 to 06/30/06. The patient's first abnormal PT was correlated with the diagnosis from the first biopsy or the most severe lesion when multiple biopsies were available. The PT were screened and diagnosed according to B2001 (with the additional diagnosis of LSIL-H) by 12 cytotechnologists and 14 pathologists, while 16 pathologists diagnosed the biopsies. The corresponding PTs were reviewed when available during sign-out of the biopsies.

Results: 6,456 biopsies and 150,962 PT (1.5% CP, 97% Surepath and 1.5% ThinPrep) were accessioned in the period of the study.

Cytohistologic correlations 01/01/2004 to 6/30/2006

	NILM	ASC-US	ASC-H	AGC	LSIL	LSIL-H	HSIL+
ALL PAP TESTS	136986	8423	840	363	2970	350	652
% OF ALL PAPS	90.74%	5.58%	0.56%	0.24%	1.97%	0.23%	0.43%
# BIOPSIES	1667	1770	507	250	1598	164	483
% BIOPSIED	1.22%	21.01%	60.36%	68.87%	53.80%	46.86%	74.08%
CIN2/3+ BIOPSIES	25	219	136	13	265	49	316
% CIN2/3+ OF ALL BX	1.5%	12.37%	26.82%	5.2%	16.58%	29.88%	65.42%
% OF ALL CIN2/3+	2.44%	21.40%	12.70%	1.33%	25.9%	4.79%	30.89%

1026 biopsies were found to have diagnoses of CIN2+ (15.89%). The largest contribution of CIN2+ histologic diagnoses was from women with PT diagnoses of HSIL or higher (30.89%).

Conclusions: Our study shows that the B2001 category of ASC-US, while still the most frequent diagnosis made on PT is no longer the most important contributor of CIN2+ cases diagnosed on follow-up biopsies, while the new categories of ASC-H and LSIL-H contribute a relatively high proportion of CIN2+ diagnoses.

952 Immunohistochenical Analysis of Mismatch Repair (MMR) Genes in Premenopausal Women with Endometrial Endometrioid Adenocarcinoma (EEA)

S Nofech-Mozes, Z Ghorab, E Hsieh, I Ackerman, G Thomas, A Covens, MA Khalifa. University of Toronto, Toronto, ON, Canada.

Background: Deficiency of DNA MMR gene expression, most commonly of MLH1, MSH2 or MSH6 and Microsatellite instability (MSI) are found in EEA from patients with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. These patients often present at a younger age compared to sporadic EEA patients. MSI occurs in 15-30% of sporadic endometrioid carcinoma in all age groups, however, in this setting it is associated with MLH1 methylation with very few cases of MMR gene mutations. The data on the prevalence of MMR gene expression in EEA from premenopausal women is limited.

Design: Between 1999-2004, 43 hysterectomy specimens from premenopausal women 45 years of younger were diagnosed with EEA in our department. Pathology review of all slides was undertaken and the clinical data were retrieved from the patients' electronic charts. A panel of monoclonal antibodies MLH1 (CM220C, Biocare,1:25), MSH2 (FE11, Calbiochem, 1:2000), and MSH6 (Clone 44, BD Transduction Laboratories, 1:150) were used for immunohistochemistry. Sections were scored as either immunodeficient (complete absence of detectable nuclear staining of cancer cells) or intact (any nuclear staining).

Results: Median age at diagnosis was 40.1 years (range 30-45). The mean follow up was 13.6 months. All patients were treated primarily by hysterectomy, 43/44 with bilateral salpingo-oophorectomy and one patient choose to preserve her ovaries. Associated neoplasia was noted in the family history of 11 cases and in the same patient in 6 cases (3 invasive mammary carcinomas, 2 endocrine tumors and 1 small cell lung carcinoma). None of these cases were known HNPCC patients. Paraffin blocks were available for immunostaining in 39 cases. Immunohistochemistry revealed one MLH1 immunodeficient case, and two cases were both MSH2 and MSH6 immunodeficient. MLH1 deficiency was noted in a 37-year-old woman with FIGO grade 1, stage III EEA. MSH2 and MSH6 were deficient in a 43-year-old woman with FIGO grade 1, stage IA tumor, and a history of uterine malignancy in her sister and unknown malignancy in her father; and in a 45-year-old woman with FIGO grade 3, stage IB tumor and concurrent benign ovarian Brenner tumor.

Conclusions: MMR genes related proteins are immunodeficient in 7.7% of endometrial cancer seen in premenopausal patients regardless of the history of associated neoplasia. Other molecular pathways may contribute to the occurrence of EEA in younger women.

953 Lymphovascular Invasion (LVI) Is a Significant Predictor for Recurrence in Patients with Early Stage Endometrial Endometrioid Adenocarcinoma (EEA)

S Nofech-Mozes, Z Ghorab, N Ismiil, I Ackerman, G Thomas, A Covens, MA Khalifa. University of Toronto, Toronto, ON, Canada.

Background: EEA is the most common cancer of the female genital tract. Adjuvant treatment following hysterectomy is prescribed to patients with adverse pathologic features. The aim of this study is to evaluate the prognostic significance of the various histopathologic features of EEA. We also tried to identify the subset of patients with a high risk for recurrence.

Design: A group of gynecologic pathologists assessed the histopathologic features of 520 consecutive patients. The analysis of this study was limited to patients treated with hysterectomy, having a known follow up, and lacking evidence of serosal or extrauterine spread. Cases were accessioned in the Department of Anatomic Pathology in Sunnybrook Health Sciences Center between July 1999 and June 2004. Tumor grade was reported in a 2-tiered system, with a "high grade" defined as having > 50% solid areas. Grade, depth of myometrial invasion, presence of cervical involvement, and LVI

were evaluated. LVI was defined by the presence of malignant cells within endothelial-lined spaces on H&E-stained sections. Multivariate analysis using Cox proportional hazards model (SAS 8.2 software) tested the associations between time to recurrence, age, and pathologic features.
Results: The median age at diagnosis was 62.7 years (range 30-94). In 52 patients with lymph node sampling, nodes were negative. 455 patients (87.5%) were diagnosed with low-grade and 65 (12.5%) with high-grade tumors. The cervix was involved in 129 cases (24.8%;10.8% glandular only and 14% glands and stroma). The tumor was confined to the endometrium in 83 cases (16%), invaded the inner half of myometrium in 251 (48.3%) cases and the outer half in 186 cases (35.7%). LVI was noted in 116 (22.3%) patients (25% in low grade and 75% in high grade). After a median follow-up of 27 months (range 2-96), the tumor recurred in 65 cases (36 loco-regional, and 29 distant). The mean time to recurrence was 15 months (range 3-48). On multivariate analysis LVI was the only independent significant histologic feature associated with recurrence, hazard ratio 2.449 (CI = 1.419-4.227).
Conclusions: In our cohort of EEA, LVI is associated with tumors of high grade and is an independent predictor for recurrence. In patients with LVI who are significantly at higher risk of recurrence adjuvant therapy may be warranted.

954 Uterine Leiomyosarcomas Are Characterised by High p16, p53 and MIB1 Expression in Comparison to Usual Leiomyomas, Benign Leiomyoma Variants and Smooth Muscle Tumours of Uncertain Malignant Potential

CJ O'Neill, HA McBride, LE Connolly, WG McCluggage. Royal Group of Hospitals Trust, Belfast, Northern Ireland, United Kingdom.
Background: It has been suggested that p16 is overexpressed in uterine leiomyosarcomas in comparison to leiomyomas. In this study, we assess p16 immunohistochemical expression in a variety of uterine smooth muscle tumours, including usual leiomyomas, benign leiomyoma variants, smooth muscle tumours of uncertain malignant potential (STUMPs) and leiomyosarcomas. We aim to ascertain whether there are differences in p16 expression between these groups and whether p16 is of potential value in the assessment of problematic uterine smooth muscle neoplasms. We compare p16 expression with that of p53 and MIB1.
Design: Cases of usual leiomyoma (n=10), benign leiomyoma variants (n=27), STUMP (n=4) and leiomyosarcoma (n=22) were stained with p16, p53 and MIB1. For p16, cases were evaluated both with respect to staining distribution and intensity.
Results: There was a statistically significant difference in p16 distribution ($p<0.001$) and intensity ($p=0.001$) between leiomyosarcomas and the other groups. There was no difference in p16 expression between usual leiomyomas, benign leiomyoma variants and STUMPs. There were also statistically significant differences in p53 ($p=0.014$) and MIB1 ($p<0.001$) staining between leiomyosarcomas and the other groups.
Conclusions: p16 is overexpressed in uterine leiomyosarcomas compared to leiomyomas, benign leiomyoma variants and STUMPs, suggesting that p16 may be implicated in the pathogenesis of malignant uterine smooth muscle neoplasms. p16, in combination with p53 and MIB1, may be of value as an adjunct to morphologic examination in the assessment of problematic uterine smooth muscle tumours, although further large scale studies with follow-up are necessary to confirm this.

955 miRNA Signatures in Primary Versus Recurrent Ovarian Cancers

SA O'Toole, A Laios, B Sheppard, N Gleeson, T D'Arcy, E McGuinness, M Ring, E Gaffney, C Martin, R Flavin, D Cahill, D O'Connell, J O'Brien, O Sheils, JJ O'Leary. Trinity College Dublin, Dublin, Ireland; University College Dublin, Dublin, Ireland.
Background: MicroRNAs (miRNA) are endogenous RNAs, ~22 nucleotides long, which play important regulatory roles in animals and plants by targeting mRNA transcripts for cleavage or translational repression. An accumulating body of evidence reveals that miRNAs have critical functions in various biological processes and given the diversity and abundance of miRNA targets, miRNAs appear to functionally interact with various components of many cellular networks. In an attempt to understand the biology of recurrent ovarian cancer we have previously validated potential mRNA targets involved in recurrence and in this study we examined the expression of 180 miRNAs in the same sample cohort.
Design: miRNA was isolated from a set of 3 primary (FIGO stage III and grade 3) and 3 recurrent (grade 3) serous papillary adenocarcinomas of the ovary using the Ambion mirVana™ miRNA isolation kit. miRNA expression levels were examined using the Applied Biosystems TaqMan® MicroRNA Assays Human Panel-Early Access Kit consisting of 180 miRNAs. miR16 and let-7A were used as endogenous controls. Quantification of primary samples was carried out relative to recurrent using the delta delta CT method.
Results: Distinct expression patterns were identified between primary and recurrent ovarian cancers. 60 miRNAs were greater that 2 fold dysregulated between primary and recurrent specimens. 12 miRNAs were not detectable in the ovarian samples. miR147 was detected only in recurrent specimens and is predicted to target genes which were identified in our transcriptome studies, such as neuregulin (a ligand for the ErbB receptors) and some of the claudin family of genes. Other interesting findings which correlate with our transcriptome results include the downregulation of miR10b and miR9 which are predicted to target PIK3CA which promotes cell survival, the zinc finger family of proteins and other intracellular signaling molecules identified in our study.
Conclusions: Distinct miRNA patterns were identified between primary and recurrent ovarian cancer samples. Some of the differentially regulated miRNAs identified correlate with our transciptome findings and further work is needed to determine if the miRNAs are responsible for the altered expression seen at the mRNA level. miRNAs may have potential as biomarkers or therapeutic targets in recurrent ovarian cancer.

956 c-Met Expression by Intermediate Trophoblasts and Maternal Vasculature in Preeclampsia

AS Paintal, C Gong, M Tretiakova, AN Husain. University of Chicago Hospitals, Chicago, IL.
Background: Defective trophoblastic invasion has been implicated in the pathogenesis of preeclampsia (PE). Along with the extravillous cytotrophoblast which colonizes the maternal vessels, the intermediate trophoblast (IT) is also involved in invasion of the endometrium although its role in PE is not well understood. Hepatocyte growth factor (HGF) production by the villous mesenchyme promotes cytotrophoblast invasion via the receptor c-Met. Attenuation of this signalling pathway through downregulation of HGF is thought to play a central role in the shallow cytotrophoblast invasion seen in PE. Our goal was to evaluate the expression of c-Met by the vascular lining of unconverted maternal arterioles(endothelium) and converted vessels(cytotrophoblast derived) in placentas from women with PE as compared to normal subjects. Additionally, we evaluated expression of c-Met by ITs in both groups.
Design: We examined c-Met expression by the vascular lining and ITs along the maternal surface in placentas from subjects with PE (n=8) and from normal subjects(n=9). Unconverted maternal arterioles, converted vessels, and ITs were identified on H&E sections and expression of c-Met was assessed in each of these elements in corresponding IHC stained sections. Expression was classified as present or absent in both groups of vascular structures and as weak or strong in ITs.
Results: In all placentas from both groups, expression of c-Met by the unconverted maternal arterioles was absent whereas staining was present in the majority of converted vessels. Strong staining for c-Met by ITs was present throughout the majority of placentas from both patients with PE and normal subjects (63% and 67%; p=0.86).
Conclusions: The absence of expression of c-Met by unconverted maternal arterioles in both groups is consistent with the failure of the cytotrophoblast to colonize these structures. The presence of c-Met expression in the lining of the majority of converted vessels throughout both groups most likely reflects their derivation from the extravillous cytotrophoblast. c-Met expression by ITs was not altered in placentas from women with PE. This could indicate that the c-Met pathway does not play a major role in altering IT invasion in the setting of PE. Alternatively, downregulation of HGF expression with a subsequent reduction in signaling though c-Met may be responsible for decreased IT invasion in PE. To assess this possibility, future studies examining phosphorylated c-Met expression in this setting would be useful.

957 Reduced Expression of RASSF1A and Spry-2 in Endometrial Carcinoma

J Pallares, A Velasco, X Dolcet, D Llobet, M Encinas, S Puente, V Palomar, X Matias-Guiu. Hospital Universitari Arnau de Vilanova/University of Lleida, Lleida, Spain.
Background: Alterations in the regulation of the Ras-MAPK pathway are frequent in endometrial carcinoma (EC). RASSF1A and Spry-2 are negative regulators of these pathways. RASSF1A and Spry-2 have been found to be inactivated by promoter methylation in some human tumors.
Design: RASSF1A and Spry-2 immunostaining was evaluated in two tissue-microarrays constructed from paraffin-embedded samples of normal endometrium (80 cases) and EC (95 cases). RASSF1A and Spry-2 immunostaining was correlated with cell proliferation (Ki67) and clinicopathological data. Promoter methylation of RASSF1A and Spry-2 was assessed by Methylation-Specific PCR. Moreover, four different endometrial adenocarcinoma cell lines, (Ishikawa, KLE, HEC1A, and RL-95) were subjected to down-regulation of Spry-2 by siRNA.
Results: RASSF1A and Spry-2 were expressed in normal endometrium; and there was a very significant decrease in Spry-2 immunoexpression in the proliferative phase ($p = 0.000$). Reduced immunostaining of RASSF1A and Spry-2 was frequent in endometrial carcinoma (40% and 17%). In EC, reduced Spry-2 immunostaining strongly correlated with increased cell proliferation (Ki67) ($r = -0.367$; $p = 0.001$). Reduced expression of RASSF1A and Spry-2 was associated with promoter methylation, which was heterogeneously distributed in some tumors. Down-regulation of Spry-2 in endometrial carcinoma cell lines produced a significant increase in MAPK signaling pathway.
Conclusions: Reduced expression of RASSF1A and Spry-2 may play a role in endometrial carcinogenesis by controlling cell proliferation and MAPK signaling pathway.

958 Evidence That High and Low Grade Ovarian Serous Tumors Reflect Different Cells of Origin

MM Parast, R Drapkin, F McKeon, D Kindelberger, CP Crum, TA Ince, MS Hirsch. Brigham and Women's Hospital, Boston, MA; Harvard School of Medicine, Boston, MA.
Background: Recent data indicate that many high-grade serous tumors of the upper genital tract arise from the distal fallopian tube, whereas most borderline and low-grade malignant serous tumors develop in the substance of the ovary, potentially within ovarian inclusion cysts. This implies that serous tumor origin is influenced not only by the genetic alterations attributed to each, but to specific target cell types. We explored this possibility by comparing the immunoprofiles of normal tubal epithelium, ovarian cortical surface, and inclusion cyst epithelium, and their respective neoplasms.
Design: 10 normal fimbrial mucosa, 13 ovarian cortical inclusion cysts (CICs), 8 serous cystadenofibromas, 14 serous borderline tumors, 4 well-differentiated serous carcinomas, and 41 high grade ovarian serous carcinomas from a tissue array, were stained with antibodies targeting cells with ciliated (p73, Lh528) and secretory differentiation (HMFG2), as well as phosphorylated histone H2A as a marker of DNA damage (H2A.X). The staining was scored as 1+ (<5% of lesional cells), 2+ (5-25%), 3+ (25-50%), or 4+ (>50%).
Results: Both p73 and Lh528 antibodies consistently stained nuclei and cytoplasm, respectively, in ciliated cells in the fallopian tube, whereas HMFG2 was specific for secretory cells. High-grade serous carcinomas were strongly positive for H2A.X and

HMFG2 and negative for the ciliated cell markers. Similar to the fallopian tube, two cell populations could be distinguished in ovarian inclusion cysts and cystadenomas following staining with HMFG2 and Lh528, however; a much broader range of p73 immunostaining was seen. The morphologic transition from borderline to low-grade serous malignancy was marked by a loss of Lh528; however, strong (4+) p73 staining persisted.

Conclusions: High and low grade pelvic serous neoplasms can be distinguished by the site of origin (ovary and fallopian tube vs principally ovary) and the genetic pathway involved (p53 vs. k-RAS/BRAF mutations), as well as by the differentiation pathway of tumor cells. Although high-grade serous tumors are typically secretory cell specific, lower grade tumors express markers of a "hybrid" cell capable of both secretory and ciliated differentiation. This emphasizes the importance of target cell in tumorigenesis, specifically its role in governing susceptibility to the genetic alterations that produce this range of serous neoplasms.

959 Multifocal Mucosal Hyperplasia of Fallopian Tube Is More Common with Ovarian Surface Epithelial Carcinomas and Borderline Tumors, and in BRCA Mutation Patients with No Evidence of Neoplasia

R Pathi, MR Quddus, CJ Sung, MM Steinhoff, R Moore, F Liu, WD Lawrence. Women & Infants Hospital/Brown Medical School, Providence, RI.

Background: Mild mucosal hyperplasia has been reported to be present in ~ 4% fallopian tubes in the general population. Doucet C (Mod Pathol 2006 19: 94) reported mucosal hyperplasia in 78.3% of tubes of BRCA mutated patients. To our knowledge, no single institutional study has compared the association of fallopian tube mucosal hyperplasia (TMH) among women in the general population with and without mullerian malignancies. Morphologic studies of TMH are few and studies of its extent and multifocality have not been reported. In our study, TMH was defined as reported by Yanai-Inbar I and Silverberg SG (Int J Gynecol Pathol 2000 19:139).

Design: A retrospective review of fallopian tube sections was performed in 9 prophylactic adnexectomies from BRCA positive patients; 25 ovarian surface epithelial carcinomas; 25 endometrial carcinomas and 80 ovarian surface epithelial borderline tumors. Control cases included100 routine tubal ligations and the tubes from 50 benign gynecologic diseases. The entire tubes were submitted in BRCA positive cases and between 3 and 9 sections of each tube were submitted in other groups. The extent of TMH was categorized according to laterality and degree of focality (unifocal=one site in each tube; bifocal=two sites in each tube; or multifocal).

Results:

Groups	Mucosal Hyperplasia of Fallopian Tubes			
	Bilateral		Unilateral	
	Unifocal	Bifocal/Multifocal	Unifocal	Bifocal/Multifocal
Tubal Ligation (n=100)	4	2	17	1
Benign Uterine Diseases (n=50)	3	0	11	0
Ovarian Borderline tumors (n=80)	7	10	16	9
BRCA Mutation (n=9)	2	4	0	0
Ovarian Carcinoma (n=25)	3	2	7	1
Endometrial Carcinoma (n=25)	4	3	1	7

Conclusions: Tubal mucosal hyperplasia appears to be more prevalent in patients with neoplastic mullerian disease than in non-neoplastic gynecologic conditions. In benign gynecologic conditions, TMH tends to be unilateral and unifocal. However, bilateral multifocal TMH was found in 67% of patients with BRCA gene mutation with no evidenece of neoplasia. 56% of patients with ovarian and 68% endometrial carcinomas and 51% of surface epithelial borderline tumors exhibit varying degrees of concomitant mucosal hyperplasia either in one or both tubes. These findings support the existence of a "field effect" of mullerian neoplasia in the primary and secondary mullerian systems.

960 Differential Expression of microRNAs let-7c and miR21 in Benign, Atypical and Malignant Uterine Smooth Muscle Tumors

L Peng, P Lee, K Mittal, JJ Wei. New York University School of Medicine, New York, NY.

Background: MicroRNAs (miRNAs) are group of small non-coding RNAs. miRNAs that control cell proliferation, differentiation and cell death through regulation of the specific gene expression in transcription and translation levels. Aberrant expression of miRNAs has been found in certain types of solid tumors including leiomyoma. miRNAs are tissue or tumor specific and are associated with many biomedical factors of the tumors. We previously found that let-7 miRNAs and miR21 were top dysregulated miRNAs in uterine leiomyomas (ULM). In this study, we further examined the differential expression of these 2 miRNAs between benign and malignant uterine smooth muscle tumors.

Design: Selected 120 ULM, 29 leiomyosarcomas (LMS), 9 leiomyosarcomas with mixed atypical leiomyomas (ALM). Matched myometrium as internal controls; Prepared high density tissue microarray (TMA); Performed miRNA in situ hybridization with LAN miRNA probes of let-7c and miR21; Analyzed the differential expression of the selected miRNAs in uterine leiomyomas, atypical leiomyomas and leiomyosarcomas.

Results: Among 120 ULM, overexpression of let-7c and miR21 were found in 60% (65/109) and 72% (78/109) cases. Among 29 LMS, overexpression of let-7c and miR21 were found in 57% (16/28) and 86% (24/28) cases. Although there was a similar numbers of cases showing overexpression of let-7c and miR21 between ULM and LMS, however, the net gain values of let-7c and miR21 were significantly high in LMS (+0.73, +1.25) in comparison to ULM (+0.40, +0.71) ($P<0.05$ respectively). By further analysis of these 2 miRNA expression in normal myometrium, ULM, ALM and LMS, there were steady increased levels of let-7c and miR21 expression (r=0.91).

Conclusions: Our previous findings of overexpression of let-7c and miR21 in ULM are further supported by in situ hybridization in a large cohort tissue samples. In addition, we illustrate that overexpression of let-7c and miR21 are also the characteristic features in LMS . The levels of overexpression of let-7c and miR21 seem to be linearly correlated with the grade of tumor progression in uterine smooth muscle tumors.

961 The Alleged Low-Grade Leiomyosarcomas of the Female Genital Tract: Metastatic Lesions or Independent Primary Tumors?

L Posligua, A Malpica, M Deavers, M Merino, E Silva. M.D. Anderson Cancer Center, Houston, TX; National Institute of Health, Bethesda, MD.

Background: Although the definition of low grade leiomyosarcoma of the female genital tract has not been well established, there are tumors in which this designation is used due to the presence of bland histological features associated either with tumor deposits at another site or tumor recurrence. In this study, we present the clinicopathologic features of 16 such cases seen in our institution in a 17 year-period.

Design: Sixteen cases originally diagnosed as smooth muscle tumors of uncertain malignant potential that recurred (10), low grade leiomyosarcoma (bland smooth muscle tumors with tumor deposits at another site) (3), and cellular leiomyomas that recurred (3) were retrieved from our department files. All these tumors were considered to be low grade leiomyosarcoma and were compared with 31 conventional leiomyosarcomas diagnosed according to the WHO criteria.

Results: Low grade leiomyosarcomas: the age of the patients ranged from 37 to 58 years, median 45 years, all tumors recurred in the pelvis, 31% in the lung and 6% in the liver. The median time to recurrence was 16 months, 7 patients died of disease, and the median survival was 161 months. Conventional leiomyosarcomas: the age of the patients ranged from 35 to 86 years with a median of 52 years. The most common site of recurrence was the lung, 77%, followed by the pelvis, 51%, and the liver, 22%. The median time to recurrence was 9 months, 27 patients died of disease, and the median survival was 38 months. Immunohistochemistry done in 4 cases showed that WT1 was different in the uterine tumors and their recurrences in 2 of 4 cases, and Ki-67 was different in 3 of 4 cases.

Conclusions: Based on the differences in age, site of recurrence and immunohistochemistry, most probably the "recurrences" of these uterine smooth muscle tissues represent independent primaries.

962 Ovarian Borderline Mucinous Tumor of Endocervical Type: A Clinicopathologic Study of 78 Cases

LE Posligua, MT Deavers, EG Silva, A Malpica. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: At the Borderline Ovarian Tumor Workshop sponsored by the National Cancer Institute in 2003, it was determined that the clinicopathologic data on ovarian mucinous borderline tumors of endocervical type (OvMBTE) is still limited. In this study, we present our experience with seventy-eight of such cases seen in a single institution in a period of 17 years (yrs).

Design: Seventy-eight cases of OvMBTE were retrieved from our department files. H&E slides were reviewed in all cases, ranging from 1 to 27 slides per case, and the diagnoses of OvMBTE confirmed according to the WHO classification. Cases with intestinal differentiation in the borderline component were excluded. Clinical information was obtained from the patients' (pts) charts or through the attending physicians. The following clinicopathologic features were recorded: tumor (tu) size, laterality, the presence of serous epithelium in the borderline component, the presence of microinvasion (MI), endometriosis, endosalpingiosis, intraepithelial carcinoma (IECa), invasive carcinoma (InvCa) or sarcoma, FIGO stage, lymph node involvement (LNinv) and the presence of any other primary. Follow-up (f/u) ranging from 6 to 195 months (mos), (mean 59.8 mos) was obtained in 48 pts.

Results: The pts' age ranged from 21 to 76 yrs (mean 40.9 yrs). The tu size ranged from 1.7 cm to 28 cm (mean 9.8 cm). Sixteen cases were bilateral. A mixture with serous epithelium within the borderline component of the tu was seen in 38 cases. MI, IECa and InvCa were present in 11 cases, 11 cases, and 6 cases, respectively. In a single case the tumor was associated with a high-grade sarcoma. Endometriosis was noted in 35 cases, 9 of them were atypical. Endosalpingiosis was seen in 17 cases. Pelvic-omental implants were present in 11 cases (8, non-invasive; 3, invasive). LNinv was identified in one case. Staging was performed in 43 cases and according to the FIGO staging, they were distributed as follows: stage I, 33 cases; stage II, 7 cases; stage III, 3 cases. In 3 cases, a synchronous endometrial adenoCa was also present. In the pts with only OvMBTE, no recurrences were detected. The only cases that recurred and caused pts' deaths were those with either InvCa or sarcoma.

Conclusions: Cases of OvMBTE appear to have a benign behavior. Recurrences and death are going to be seen in the rare cases associated with InvCa or sarcoma. The presence of implants or microinvasion appear not to have an impact in prognosis.

963 p16 Expression in Squamous and Trophoblastic Lesions of the Upper Female Genital Tract

MD Post, SG Carinelli, S Campbell, RA Soslow, E Oliva. Massachusetts General Hospital, Boston, MA; Fondazione Ospedale Policlinico, Mangiagalli e Regina Elena, Milano, Italy; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: p16, a surrogate marker for HPV infection, is uniformly present in HPV-related carcinomas. Less is known about its expression in squamous lesions of the upper female genital tract and little data exists for p16 expression in endometrioid adenocarcinomas with extensive squamous differentiation (EC-SD) or trophoblastic lesions. This study aims to further characterize p16 expression in lesions of the uterine corpus and adnexa.

Design: p16 immunostaining was done on sections from ichthyosis uteri (1), primary uterine corpus squamous cell carcinoma (UCSCC)(2), primary ovarian SCC (OSCC)(5; 2 associated with a dermoid cyst), endometrial EC-SD (5), ovarian EC-SD (4), placental site nodules (PSN)(5), and placental site trophoblastic tumors (PSTT)(6). We evaluated the percentage of positive nuclei (focal=<10%, 10-50%, diffuse=>50%) and staining intensity (weak, moderate, strong).

Results: The case of ichthyosis uteri, all UCSCC and 1 OSCC (arising in a dermoid) were negative; the other dermoid-associated OSCC showed focal moderate staining and the remaining 3 OSCC displayed strong diffuse p16 positivity. 3/5 endometrial

EC-SD showed strong diffuse staining of the squamous component; 2 displayed strong staining, but in <50% of nuclei. The glandular component focally showed strong p16 positivity (2), with variably intense focal staining in 3 cases. The squamous component of all ovarian EC-SD showed focal moderate staining, with variable staining of the glandular component. 5/5 PSN and 4/6 PSTT showed focal weak staining while the other 2 PSTT were p16 negative.

Conclusions: p16 is expressed in OSCC, as well as in the squamous and glandular components of endometrial and ovarian EC-SD. Marked differences exist in p16 expression in UCSCC versus OSCC, and between pure OSCC and dermoid associated OSCC. Therefore, p16 expression may help identify the origin of SCC diffusely involving the corpus and cervix and suggests different pathogeneses for SCC of the upper female genital tract, likely unrelated to HPV infection. In contrast to previous data, we found p16 expression in trophoblastic lesions, albeit focal and weak. Thus, when considering the differential diagnosis of cervical SCC and trophoblastic lesions, only strong diffuse p16 staining should be considered helpful. Alternatively, if the differential includes primary UCSCC and trophoblastic lesions, p16 is not useful.

964 Serous Borderline Tumors (SBT) Represent a Biphasic Population Different from Invasive Serous Carcinomas (ISC) of the Ovary

PK Puri, AM Schwartz, DE Henson. George Washington University Medical Center, Washington, DC.

Background: Pathologists distinguish between serous borderline tumors and invasive serous carcinomas of the ovary based on morphologic and clinical grounds. We have investigated the contribution of epidemiologic analysis of these tumors and demonstrate that population-based investigations support the pathologic data and supply additional information.

Design: Data included 50,390 cases of ovarian cancer reported to the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. All cases were diagnosed between 1973 and 2001. The analysis included a comparison of the age-specific incidence rates and age frequency distributions of the serous borderline tumors and invasive serous carcinomas.

Results: Distinct patterns were observed between serous borderline tumors and invasive serous carcinomas when age-specific incidence rates were graphically displayed on log-log plots. SBT minimally change over a 40 year age span whereas ISC monotonically rises, indicating the absence of progression from SBT to ISC. In addition, the graph of age-specific incidence rate for SBT lacks inflection over the menopause consistent with a minimal contribution of hormonal modification during carcinogenesis. The age frequency density function demonstrates non-homogeneous, multimodal distributions suggesting the presence of sub-populations within each tumor type.

Conclusions: Epidemiologic analysis of pathological data on SBT and ISC confirm the clinico-pathologic studies that these two entities are separate tumor types with different carcinogenic pathways. Distinct incidence rate plots and frequency distributions support the lack of progression of SBT to ISC. The absence of graphical pattern changes across the menopause suggest that hormonal changes play a minor role in the pathogenesis of these tumors.

965 The Role of PTEN in the Pathogenesis of Uterine Smooth Muscle Malignancies: A Comparative Immunohistochemical Study of 44 Cases of Low Grade, High Grade, and Epithelioid Leiomyosarcomas

MR Quddus, CJ Sung, MM Steinhoff, WD Lawrence. Women & Infants Hospital/Brown Medical School, Providence, RI.

Background: PTEN, a tumor suppressor gene, has been reported to be altered/inactivated (PTEN null) in a substantial number of endometrioid carcinomas and their precursors, but studies of PTEN in uterine mesenchymal neoplasms are few and contradictory. We recently reported that 70% of low grade endometrial stromal sarcomas are PTEN null (Ologun et al. Modern Pathol 2006;19:191A) and that the latter may play a role in their histogenesis. In order to investigate the role of PTEN alteration/inactivation in the pathogenesis of other uterine mesenchymal tumors, we studied PTEN alteration/mutation in a spectrum of uterine smooth muscle tumors, including low grade leiomyosarcomas (LMS), high grade LMS, and epithelioid types of uterine LMS.

Design: Forty four cases of uterine leiomyosarcoma were retrieved from the pathology archives of our institution. These included 10 cases of low grade LMS and 22 cases of high grade LMS of the usual type, as well as 12 cases of the epithelioid variety. Four µm sections were cut from each block and stained with antibodies to PTEN (Cascade Bioscience, Winchester, MA). An H&E section confirmed the presence of tumor and positive and negative control slides were run with each staining batch. The staining characteristics of concomitant benign uterine smooth muscle was noted for comparison and the absence of nuclear and/or cytoplasmic staining indicated the presence of a PTEN mutation (null).

Results:

Table 1

	High grade Leiomyosarcoma (n=22)		Epithelioid Leiomyosarcoma (n=12)		Low Grade Leiomyosarcoma (n=10)	
PTEN Expression Status	Over-expression	Weak/Null	Over-expression	Weak/Null	Over-expression	Weak/Null
	20	2	12	0	2	8

Conclusions: A PTEN NULL phenomenon similar to that reported in low grade endometrial stromal sarcomas (70% of cases) is present in low grade uterine leiomyosarcomas (80% of cases), suggesting a possible role of down regulation of PTEN in the histogenesis of both LGSS and low grade leiomyosarcomas. In contrast, 91% of high grade uterine leiomyosarcomas show PTEN overexpression and all of the epithelioid leiomyosarcomas exhibited diffuse overexpression of PTEN. The marked contrast between PTEN expression in low grade and high grade LMS (including epithelioid LMS) also supports the development of the latter as *de novo* high grade tumors rather than as a "dedifferentiation" of low grade LMS to high grade LMS.

966 High Grade or Low Grade VIN? The Role of Ki67 and P16INK in Assigning a Histologic Grade to Squamous Atypia "Less Than VIN 3" in a Binary Classification System

MR Quddus, CJ Sung, MM Steinhoff, C Zhang, WD Lawrence. Women & Infants Hospital/Brown Medical School, Providence, RI.

Background: Traditionally, classic or usual type vulvar intraepithelial neoplasia (VIN) has been divided into VIN1, VIN2, and VIN3. As in the histologic reporting of cervical squamous lesions, there has been a trend toward using the Bethesda type classification of low grade (LVIN) and high grade (HVIN). Use of such a binary classification, however, not uncommonly results in the diagnostic dilemma of whether a particular VIN is best regarded as a VIN1 or 2. The latter poses the potential for a difference in clinical treatment, since the usual treatment for HVIN is more aggressive. In current practice, the immunomarkers p16INK and Ki67 are used as an adjunct to clarify the diagnosis of cervical SIL in which the distinction between low grade and high grade is diagnostically difficult on conventional stains. The aim of our study is to examine the utility of p16INK and Ki67 in clarifying and distinguishing between LVIN and HVIN.

Design: Fifty cases of VIN, representing both biopsy and excisional specimens, were retrieved from the archival files of our institution. Cases were selected which showed relatively clearcut histologic changes that qualified for at least VIN1 ("flat condyloma") but also exhibited sufficient intraepithelial architectural and/or cytologic atypia to raise the legitimate question of a VIN2. Four micron sections were stained with antibodies to p16INK (Mouse anti-human monoclonal antibody, BD Biosciences, San Diego, California) and Ki67 (Mouse anti-human monoclonal antibody, DakoCytomation, Carpinteria, CA). The cases were graded according to the presence of Ki67 (nuclear staining involving middle 1/3rd) and p16INK (diffuse strong cytoplasmic staining).

Results:

H&E Diagnosis	Ki67 & p16INK Diagnosis	
	HVIN confirmed	HVIN not confirmed
VIN1-2/VIN2	38	12

Conclusions: A considerable proportion of our cases (24%) in which there was sufficient cytologic atypia to question the diagnosis of VIN2 (and therefore a HVIN in the binary classification), did not display either HR-HPV surrogate markers and/or proliferation markers to support that diagnosis. Conversely, the questionable diagnosis of HVIN was confirmed in the other 76%, further affirming the diagnostic utility of the immunomarkers. Because of the difference in clinical management of VINs, our study suggests that use of the abovementioned immunostains would prove useful in the distinction between low grade and high grade VIN.

967 FIGO Grade I Endometrioid Adenocarcinoma of the Uterine Corpus with Lymph Node Metastasis Closely Resembling Sinus Histiocytes: Reported Frequency in 79 Myoinvasive Cases

CM Quick, JK McKenney. University of Arkansas for Medical Sciences, Little Rock, AR.

Background: Although endometrioid adenocarcinoma of the uterine corpus (EAUC) with lymph node metastasis closely mimicking sinus histiocytes has been reported in a small series, the frequency of this intranodal pattern in a consecutive series of myoinvasive tumors has not been studied previously.

Design: 139 FIGO grade I EAUC were reviewed to identify cases with myoinvasion. In myoinvasive cases, all lymph nodes sampled were reviewed to identify possible metastatic disease and the histologic pattern of each intranodal focus was recorded. Intranodal tumor cells characterized by individual discohesive cells with round nuclei, variable small nucleoli, and a rim of eosinophilic cytoplasm were scored as histiocyte-like.

Results: 79 myoinvasive FIGO grade I EAUC were identified, 72 of which had lymph node sampling (91%). 8/72 cases (11%) had lymph node metastasis in 1-3 pelvic lymph nodes; the number of sampled nodes in positive cases ranged from 8-31 (mean = 16). 5 of 8 cases (63%) with positive lymph nodes had at least some intranodal tumor with histiocyte-like morphology, a pattern that was exclusive in 3 cases (4%). The two positive lymph nodes with other admixed patterns of nodal involvement contained solid epithelial nests with marked cytoplasmic eosinophilia. All 3 cases with a pure histiocyte-like morphology were initially unrecognized.

Conclusions: In this series, 11% (8/72) of myoinvasive FIGO grade I EAUC were associated with lymph node metastasis. 5 of the 8 had at least some intranodal involvement in which the neoplastic cells closely resembled histiocytes, and in 3 cases the histiocyte-like pattern was exclusive (4%). Since lymph node metastasis with the subtle histiocyte-like morphologic pattern may be easily overlooked, increased awareness of this pattern is important to proper patient staging and treatment.

968 CK17 and p16 Expression Pattern Distingishes Atypical Immature Squamous Metaplasia from High Grade Cervical Intraepithelial Neoplasia (CIN III)

S Regauer, O Reich. Medical University Graz, Graz, Austria.

Background: Atypical immature metaplasia (AIM) refers to a fullthickness intraepithelial basaloid lesion in the uterine cervix that features both metaplasia and atypia and therefore is difficult to distinguish from high-grade cervical intraepithelial neoplasia (CINIII). p16 is a marker for high risk HPV-induced dysplasia in the cervix. CK17 is a marker for cervical reserve (stem) cells, which give rise to metaplasias. We tested if lesions of AIM can be reliably reclassified into metaplasia and CINIII based on p16 and CK17 staining.

Design: We investigated 75 formalin-fixed and paraffin-embedded cervical specimens: cervical punch biopsies (n=30) , knife cone and LEEP (Loop Electrosurgical Excision Procedure) excision specimens (n=30) and endocervical curettings (n=15) with a varying proportions of mature metaplasia, immature metaplasia, CIN I, CIN II and CIN III and compared the staining pattern to 20 cases signed out as AIM. All cases were analyzed immunohistochemically using antibodies against CK17, p63 and p16 (Dako, Denmark).

Results: Neither exo- nor endocervical native epithelium reacted with antibody to p16. Endocervical epithelium showed no staining with antibody to CK17, while CK17 was inconsistently, focally and weakly expressed in the cytoplasm of basal keratinocytes in exocervixcal glycogenated squamous epithelium. Antibody to p63 reacted with basal keratinocyte nuclei in glycogenated squamous epithelium, but showed no reaction in columnar endocervical epithelium. Metaplastic epithelium was divided into mature and immature forms. The earliest phase of immature metaplasia was a proliferation of individual reserve cells which revealed a cytoplasmic staining with antibody to CK17 while antibody to p63 stained the nuclei. The immature metaplasia stained consistently positive for CK17 and p63 in proliferating cells, while the preserved endocervical cells were negative. p16 was negative. All dysplastic cells of CINIII demonstrated uniform staining with p16 and p63, and CK17 negativity. Based on the reciproke staining of p16 and CK17, 17/20 AIM were reclassified as metaplasia (10) and CINIII (7). Three AIM stained for both CK17 and p16 and were classified as CINIII.
Conclusions: "AIM" is a helpful histological descriptor for a troublesome squamous intraepithelial lesion in the cervix and it should be used sparingly as a final diagnosis. Immunohistochemical staining for p16 and CK17 allows distinction between metaplasia and high-grade dysplasia.

969 Mullerian Adenosarcoma: A Clinicopathologic and Immunohistochemical Study of 55 Cases

A Ribe, F Saro, F Peiro, J Prat. Hospital de la Santa Creu i Sant Pau. Autonomous University of Barcelona, Barcelona, Spain; Hospital General de Elda, Elda, Valencia, Spain.
Background: Adenosarcoma (AS) is a rare polypoid mixed tumor of Müllerian origin composed of low-grade malignant endometrial stroma and benign epithelial glands. Its pathogenesis is unknown.
Design: A clinicopathologic and immunohistochemical study of 55 AS (49 uterine, 3 ovarian, 1 vaginal, 1 tubal, and 1 peritoneal) was done. Expression of selected proteins involved in tumorigenesis by means of increase cell proliferation (p16, p53, pRB, PTEN, ki67, MLH-1, MSH-2, EGFR, PDGFR and Cerb-B2), alteration of differentiation (B-Catenin, E-Cadherin, CD10, VIM, ER and PR), and dysregulation of the apoptotic pathways (p53, BAX, Bcl-2, FAS-L, P-Akt) were investigated using a tissue array with 30 AS.
Results: Patients' age ranged from 13-86 years with a median of 50 years. History of hormonal therapy (5), tamoxifen (2), and radiotherapy (1) were recorded. The initial symptoms were vaginal bleeding (15), enlarged uterus (5) and abdominal pain (5). Sixteen AS showed sarcomatous overgrowth. Heterologous elements occurred in 13 cases and 9 tumors had myometrial invasion. Nineteen patients have been followed for 7 to 191 months. Five tumors metastasized and 2 recurred. Three patients died of tumor. PDGFR and EGFR were overexpressed in the stroma (70% and 27%, respectively) but were not detected in glands. pRb was frequently lost both in the stroma (57%) and glands (26%). p16 was overexpressed in the stroma (60%) and glands (29%). There were no significant alterations in proteins involved in apoptosis (Bax and Fas-L were detected both in glands and stroma (90%), whereas Bcl-2 was negative in both (>73%). The stromal proliferation index was >5% in 23% of cases. ER were detected in glands (60%) and stroma (30%) and PR were also expressed in both (60%). Nuclear staining for beta-catenin was seen in glands (42%), stroma (20%), or both (43%). E-Cadherine was only expressed in glands (40%). CD10 was only detected in the stroma (70%). MLH-1, MSH-2, and PTEN proteins were preserved in both components. Cerb-B2 was negative in both elements in all cases.
Conclusions: AS are highly differentiated tumors with a low proliferation index. They show a predominantly stromal growth characterized by overexpression of PDGFR, EGFR, and p16, loss of pRB, and nuclear accumulation of beta-catenin. There are no significant alterations in proteins involved in apoptosis and PTEN expression is preserved.

970 Significant Dysregulation of DNA Replication License and Replisome Proteins in Villoglandular Carcinomas of the Cervix Indicates Possible Aggressive Biological Behaviour

M Ring, S Crowther, C Martin, K Astbury, O Sheils, M Griffin, E Gaffney, D Gibbons, JJ O'Leary. Trinity College Dublin, Dublin, Ireland; National Maternity & St Luke's Hospitals, Dublin, Ireland.
Background: Villoglandular adenocarcinoma (VGA) of the cervix is reported as a variant of cervical adenocarcinoma with a good prognosis. The tumour usually occurs at a younger age than cervical adenocarcinomas in general, and typically exhibits mild cellular atypia and superficial invasion. The diagnosis of VGA is difficult. Current literature is not entirely consistent in relation to disease definition. Based on the favourable outcomes in most of the previously reported cases, it has been suggested that some patients with this subtype may be managed conservatively. However recently, it is recognised that VGA may have an aggressive biological behaviour. Because of the rarity of VGA and the difficulty but importance of the diagnosis, a molecular assessment of the condition is warranted. Using high density cDNA array analysis in cervical carcinoma cell line systems, we have identified a panel of biomarkers which can be used to assess the degree of DNA replication licence dysregulation in pre-cancer and cancer cells and to assess potential biological behaviour of lesions. This proteomic panel of markers includes: p16(INK4A), bcl-2, MIB-1, mcm 2,3,5,7 cdc 6, geminin, Topoisomerase IIα, survivin and nuf.
Design: In this study we examined the proteome expression of the above markers in 8 cases of histologically confirmed VGA. Slides were examined and scored as follows: 0= no staining, 1= 30% of abnormal cells positive, 2 = 30-60% of abnormal cells positive, 3 = 60-100% of abnormal cells positive. The age range of patients was 25-38 years with a median age of 30.8 years.
Results: All cases showed > 60% cellular positivity for mcm2, mcm3, Topoisomerase IIα, survivin, nuf, and geminin. 7/8 cases showed > 60% cellular positivity for p16(INK4A), mcm5, Cdc6. 5/8 cases showed > 60% cellular positivity for mcm7, and 6/8 cases were negative for bcl 2.
Conclusions: These findings indicate that there is significant dysregulation of DNA replication and replisome proteins in VGA of the cervix. In squamous cell and adenocarcinomas, similar proteomic profiles indicate a more biologically aggressive phenotype. The researchers are members of the Irish Cervical Screening Research Consortium [ICSRC].

971 Uterine Tumors Resembling Ovarian Sex Cord Tumors Frequently Have Incorporated Mature Smooth Muscle Imparting a Pseudoinfiltrative Appearance

SE Rollins, PB Clement, RH Young. Massachusetts General Hospital, Boston, MA; Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada.
Background: Awareness of sex cord or sex cord-like patterns in uterine mesenchymal tumors largely dates from a series characterized descriptively in 1976 as "uterine tumors resembling ovarian sex cord tumors" (UTROSCTs) (Am J Clin Pathol;66:512-25). That study included otherwise typical endometrial stromal neoplasms (ESNs); subsequently it was established that similar patterns occur in uterine smooth muscle tumors (SMTs). There remains a subgroup of enigmatic uterine neoplasms that lack a clear endometrial stromal or smooth muscle histogenesis, appropriately diagnosed as UTROSCTs. Recent immunohistochemical studies indicate these tumors may show bona fide sex cord differentiation. We reviewed a series of such cases to characterize their patterns and their differential features with ESNs and SMTs with sex cord or sex cord-like patterns.
Design: Cases which a diagnosis of UTROSCT was made or seriously considered were reviewed to discern their histologic spectrum; ESNs and SMTs with sex cord-like patterns were rigorously excluded, such tumors being more common in aggregate than UTROSCTs.
Results: 37 cases were identified after exclusion of tumors reinterpreted as ESNs or SMTs. Clinical data was available for 22. Patients ranged in age from 21 to 66 (mean 47) years, and the tumors ranged in size from 0.7 to 17.0 cm (mean 2.9 cm). The majority of tumors were submucosal and well circumscribed, usually with a yellow-tan cut surface. Histologically, UTROSCTs consisted of sertoliform tubules, sometimes in a retiform pattern, typically within a non-specific hyalinized stroma. A solid pattern was present focally in some cases. Tubules were lined by cells with abundant vacuolated cytoplasm and grooved nuclei. Foamy histiocytes were seen in 10 cases. 27 tumors contained foci of smooth muscle, usually having the appearance of entrapped myometrium, a finding that resulted in a pseudoinfiltrative appearance and in some cases, a suspicion for low-grade endometrial stromal sarcoma. The tumors had low mitotic activity with little nuclear atypia.
Conclusions: We reserve the diagnosis of UTROSCT for uterine tumors with predominant sex cord or sex cord-like differentiation after exclusion of tumors more appropriately classified as ESNs or SMTs. Many UTROSCTs have a component of smooth muscle (likely incorporated myometrial smooth muscle), which may incorrectly suggest a SMT and/or myometrial invasion by an endometrial stromal sarcoma.

972 Microtubule Associated Proteins Involved in Taxans Resistance

M Romero-Sanchez, R Nadal, BC Ojeda, C Pons, I Landin, I Boguna, V Artigas, A Barnadas, J Prat. Hospital de la Santa Creu i Sant Pau. Autonomous University of Barcelona, Barcelona, Spain.
Background: Current standard chemotherapy for ovarian carcinomas (OC) is based on antitubulin agents. Paclitaxel binds to microtubules subunits (tubulin), suppresses depolymerization, and blocks the metaphase/anaphase transition. The molecular mechanism of paclitaxel resistance is not clear.
Design: A panel of 70 archival paraffined OC, treated with CBCDA-Paclitaxel for FIGO stage II-IV, were grouped in 4 different tissue arrays according to their histology: 51% serous, 12% endometrioid, 13% poorly differentiated, and 24% clear cell. Tissue arrays included primary and metastatic tumors and normal tissues. Tumors were stratified into 2 groups according to onset of recurrence after chemotherapy: sensitive>6months and resistant<6months. Class III b-tubulin isotype and two RHO-GTPases effectors, Stahmin and mDIA, were analyzed. Immunohistochemical analyses for Stathmin and Class III b-tubulin with monoclonal antibodies were scored as negative=0; low level=1; intermediate level=2; and high level=3. mDIA mRNA levels were assessed by Relative Real Time RT-PCR in 24 cases.
Results: Class III b-tubulin: 50% of serous, 17% of endometrioid, 33% of clear cell, and 0% of undifferentiated tumors showed high expression level. There was no correlation with FIGO stage, histology, or resistance to paclitaxel. Expression was significantly higher in tumor than normal tissues (p=0.00). **Stathmin:** 62% of serous, 12% of endometrioid, 14% of clear cell, and 12% of undifferentiated tumors showed high expression level. A trend (p=0.07) was found when comparing protein level to tumor histology. Again, tumor tissues exhibited higher expression levels than normal tissues (p=0.00). In metastatic tumors, Stathmin levels significantly differed between resistant and sensitive tumors (p=0.05). In 24 cases, **mDIA** was overexpressed 10 fold in sensitive tumors and 17 fold in resistant tumors compared to normal tissue.
Conclusions: Class III b-tubulin, Stathmin, and mDIA expression levels are higher in primary tumors than corresponding normal tissues. Our results indicate that Stathmin is involved in taxans resistant ovarian carcinomas.

973 Analysis of Morphologic Parameters and Expression of β-Catenin in Endometrioid Carcinoma of the Ovary

DG Rosen, Z Zhang, J Liu. MD Anderson Cancer Center, Houston, TX.

Background: Currently, there are not reliable neither morphologic nor molecular prognostic indicators of tumor behavior for endometrioid ovarian carcinoma. Despite its widespread use the morphologic staging the Gynecologic Oncology Group (GOG) is not currently considered as a reliable factor on which to base a therapeutic decision. The purpose of this study was to assess the association of several morphologic characteristics and expression β-catenin in endometrioid ovarian carcinoma and determine which of the covariates alone or in combination based could best predict survival.

Design: A total of 58 cases of primary endometrioid carcinoma of the ovary that had primary surgery at M.D.Anderson cancer center were included in this study. The status of five morphometric parameters—including nuclear morphology (uniform or pleomorphic), mitotic count (10 high-power fields), glandular pattern (>50% or ≤50% of glands), presence of squamous differentiation (>20% or <20%), and presence of papillary pattern (no or yes)—and the expression of one β-catenin were visually analyzed by 2 independent pathologist.

Results: We found that of all the morphologic factors analyzed only a high mitotic count (>15 H.P.F.) and a low membranous expression of β-catenin were significantly associated with poor prognosis and early recurrence in disease in endometrioid ovarian carcinoma. The presence of squamous differentiation, papillary pattern or nuclear pleomorphism did not show any correlation with patient survival or recurrence free survival. Young age at time of diagnosis, advanced disease stage and sub-optimal debulking were among the clinical factors predicting poor survival and early recurrence.

Conclusions: With the exception of mitotic count no other morphologic parameter could predict survival by itself or in combination. The use of the actual grading system does not provide useful information on which to base anti cancer treatment. There is need to define a better grading system for endometrioid ovarian carcinoma. Molecular markers, such as β-catenin could aid in define this grading system.

974 Lichen Sclerosus and Squamous Cell Carcinoma of the Vulva

M Roychowdhury, AM Truskinovsky. University of Minnesota, Minneapolis, MN.

Background: Lichen sclerosus of the vulva is a chronic, progressive sclerosing skin disease that is thought to be lymphocyte-mediated. Squamous cell carcinoma of the vulva can arise in a background of lichen sclerosus, but the exact relationship between lichen sclerosus and vulvar carcinogenesis is unknown. We investigated what specific cutaneous findings in lichen sclerosus are associated with vulvar squamous cell carcinoma.

Design: We examined vulvar biopsies and excision specimens from 66 patients diagnosed with lichen sclerosus, 24 (36%) of whom developed squamous cell carcinoma of the vulva. We compared the skin affected by lichen sclerosus of the patients who developed vulvar squamous cell carcinoma and of those who did not in terms of multiple morphological and immunohistochemical parameters that were either measured or counted directly, or graded on a semiquantitative scale from 0 to 3.

Results: The patients with squamous cell carcinoma were older than those without carcinoma (mean age 72.6 vs. 66.0 years, p=0.03). The patients with squamous cell carcinoma had a significantly greater intensity of the dermal chronic inflammation (p=0.005) and a greater degree of acanthosis (p=0.02) than those without carcinoma. There was no significant difference between the two groups in terms of such parameters as the thickness of the epithelium affected by lichen sclerosus, the degree of homogenization of the dermal collagen, the number of dyskeratotic cells within the epidermis, hyperkeratosis, nuclear enlargement in the basal layer of the epidermis, the mitotic index and Ki67 index of the epidermis, and a focal vs. diffuse pattern of immunoreactivity for p53 within the basal layer of the epidermis.

Conclusions: Lichen sclerosus is a dynamic disease, the early stages of which are characterized by acanthosis and dermal chronic inflammation, while at the later stages the skin appears atrophic. Acanthosis and chronic inflammation likely denote active lichen sclerosus and may be associated with progression to squamous cell carcinoma. The increased duration of the disease may lead to a greater risk of carcinoma in older patients.

975 Lymphatic Vessel Invasion (LVI) in Ovarian Serous Tumors of Low Malignant Potential (S-LMP) with Stromal Microinvasion: A Case Control Study

AR Sangoi, JK McKenney, SS Dadras, TA Longacre. University of Arkansas Medical Sciences, Little Rock, AR; Stanford University, Stanford, CA.

Background: Stromal microinvasion (SMI) in ovarian S-LMP stratifies patients at long-term risk for disease progression independent of stage and primary ovarian histology. Despite the histologic impression and often quoted incidence of lymphatic vessel involvement (LVI) in S-LMP with SMI, the presence of LVI using D2-40 monoclonal antibody, a novel lymphatic endothelial marker to identify lymphatic invasion has never been formally evaluated in S-LMP.

Design: The presence and extent of LVI was assessed using immunohistologic staining with D2-40 antibody in 20 S-LMP with SMI and 20 S-LMP case controls without SMI. When possible, D2-40 staining was performed on two sections per case. The 20 S-LMP without SMI were case-matched for age and FIGO stage. S-LMP with typical and micropapillary ovarian histology were represented.

Results: Patients with S-LMP and SMI were 20-89 (mean, 48.2) years, while those with S-LMP without SMI were 22-64 (mean, 41.9) years. Patterns of SMI included individual eosinophilic cells and cell clusters, cribriform, simple and non-complex branching papillae, inverted macropapillae, and micropapillae. Immunohistologic staining with D2-40 monoclonal antibody identified LVI in 12/20 (60%) S-LMP with SMI, and 0/20 S-LMP without SMI, including two with micropapillary architecture. LVI ranged from focal (6 cases) to multifocal (6 cases, maximum of 5 discrete foci) in any one section and included isolated single cells, simple papillae, and in one case, cribriform glands. LVI did not correlate with age, stage, micropapillary architecture, pattern or extent of microinvasion. Focal LVI was identified in the one study patient who was pregnant. One of twelve patients with LVI had an intraabdominal recurrence with high grade disease at 16 months, while all other patients with follow up were free of disease.

LVI (Detected by D2-40)

Stromal microinvasion	Stage I	Stage II	Stage III	Total
Present	6/11 (55%)	2/4 (50%)	4/5 (80%)	12/20 (60%)
Absent	0/11 (0%)	0/4 (0%)	0/5 (0%)	0/20 (0%)

Conclusions: Lymphatic vessel invasion (LVI) in ovarian S-LMP is significantly associated with the presence of SMI, independent of age, stage, ovarian histology, implant status, and pattern or extent of microinvasion (p<0.0001). Although the number of cases is too limited to draw definitive conclusions, LVI may account for a higher risk of disease progression in patients with S-LMP and SMI.

976 Ovarian Clear Cell Carcinoma with Prominent Papillary Architecture: A Potential Mimic of Serous Tumor of Low Malignant Potential

AR Sangoi, RA Soslow, NN Teng, TA Longacre. Stanford Comprehensive Cancer Center, Stanford, CA; Memorial-Sloan Kettering Cancer Center, New York, NY.

Background: The differential diagnostic problems usually associated with ovarian clear cell carcinoma (CCC) have been well characterized and include primitive germ cell tumors, stromal tumors and metastases. Distinction from other types of surface epithelial carcinoma may also pose diagnostic problems, but the potential for misdiagnosis of a serous tumor of low malignant potential (S-LMP) is not well recognized.

Design: Ten ovarian CCC with prominent papillary architecture (CCC-P) were analyzed and compared to a series of well characterized S-LMP and CCC with mixed tubulocystic, solid and glandular patterns (CCC-M). Five CCC-P were assessed using immunohistologic markers for WT-1 and ER and compared to the S-LMP and CCC-M using tissue microarray technology.

Results: Each CCC-P was initially diagnosed as S-LMP at frozen section (n=6) and/or final diagnosis (n=6). Patients ranged in age from 39 to 65 (mean, 52.2) years. All patients presented with a pelvic mass; one was undergoing evaluation for infertility. Macroscopically, most CCC-P were described as multilocular cysts with internal papillary structures. Microscopically, each case had papillary architecture, 4 with hyalinized cores, comprising 30 to 95% of the tumor with clear to granular and eosinophilic cytoplasm. Most had a low mitotic index (7/10) and/or deceptively bland cytology (6/10); only careful attention to the cytologic features and/or mitotic index allowed correct identification of the tumor type in 3 cases. Psammoma bodies were identified in 50%. Hobnail cells were present in 9/10 (1 focal). Six were associated with pelvic/ovarian endometriosis. Seven were FIGO stage I, two were stage II and 1 stage III.

Ovarian Tumor Type	WT-1	ER
CCC-P	0/5	2/5
CCC-M	4/33	3/33
S-LMP	52/59	52/59

Conclusions: CCC-P is uncommon, but may pose significant diagnostic difficulty with S-LMP, resulting in inadequate staging and delayed treatment. Features most helpful in distinguishing CCC-P are monomorphous cytology, prominent, diffuse hobnail cells, and the presence of endometriosis. Increased numbers of mitotic figures may not be present and high grade cytology, which may be focal, may require careful examination of multiple tumor sections for detection. Since CCC-P and S-LMP exhibit significantly different immunoreactivity for WT1 (p<0.0001) and ER (p<0.0001), these markers may be useful adjunctive tests in problematic cases.

977 Comparison of Kurman-Norris and EIN Classification Systems for Predicting Myometrial Invasion in Endometrial Carcinoma

DM Selby, JC Shaw, CM Zahn, B Kendall. Wilford Hall Medical Center, Lackland AFB, TX; USUHS, Bethesda, MD.

Background: Proper preoperative classification of atypical endometrial proliferations is vital for appropriate therapy. Histologic criteria for stromal invasion, defined by Kurman and Norris (KN), are generally accepted as equating to carcinoma even though some of these proliferations do not show myometrial invasion (MI), which is possibly a better indicator of true malignancy. Endometrial Intraepithelial Neoplasia (EIN), a more recently described classification system, has not been well tested for it's ability to predict myometrial invasion.

Design: Pre-hysterectomy sampling specimens from 45 hysterectomy specimens diagnosed with endometrial adenocarcinoma were evaluated by a single pathologist blinded to follow-up, and diagnosed according to the KN and EIN categorizations. This pathologist had no formal training using the EIN system. Pre-hysterectomy diagnoses were condensed into two categories and compared: possible pre-malignant diagnoses (atypical hyperplasia [AH] and EIN) with carcinoma versus benign proliferations, and possible pre-malignant diagnoses with benign diagnoses versus carcinoma. The sensitivities and positive predictive values of these pre-hysterectomy diagnostic systems were compared relative to the presence or absence of myometrial invasion on the hysterectomy specimen.

Results: MI was present in 32 of the 45 hysterectomy specimens. Using KN criteria, AH or worse had a 90.6% sensitivity for MI and EIN or worse had a sensitivity of 96.9%; this difference was not significant (p=0.50). The positive predictive values of the KN and EIN systems for MI, using AH or worse and EIN or worse, were 74.4% and 70.5%, respectively (p=0.21). Using the criterion of carcinoma versus all other diagnoses, the sensitivities of the KN and EIN systems were 69% and 62.5%, respectively (p=0.63). The positive predictive values of the KN and EIN systems, considering carcinoma versus all other diagnoses, approached statistical significance (75.9% and 87% respectively, p=0.06).

Conclusions: There was no difference between the traditionally-applied endometrial sampling diagnostic system (Kurman and Norris) and the EIN classification system relative to predicting myometrial invasion in a subsequent hysterectomy specimen. While the EIN system has been suggested to be more accurate in predicting endometrial clonal proliferations, this system does not more accurately predict myometrial invasion.

978 Calponin h1 Expression Is an Independent Prognostic Factor in Uterine Leiomyosarcomas

C Serrano, M Tebar, I Abdulkader, E Lerma, J Ordi, E Serrano, M Abad, E de Alava. Centro de Investigación del Cáncer-Hospital Universitario de Salamanca, Salamanca, Spain; Leiden University Medical Center, Leiden, Netherlands; Santiago de Compostela, Spain; Hospital Sant Pau, Barcelona, Spain; Hospital Clínic, Barcelona, Spain.

Background: Microscopic findings and tumor staging remain the main prognostic/predictive factors in Uterine Leiomyosarcomas (UL). Nevertheless, multi-gene expression profile studies provide the chance to search for new markers useful for diagnosis and prognosis. Hierarchical clustering analysis from a cDNA expression microarray of soft tissue tumors performed by Nielsen TO. et al., classified leiomyosarcomas into two sub-groups depending on calponin expression. Our aim was to evaluate the clinico-pathological correlations of calponin expression in a well characterized series of 59 UL.

Design: We performed a tissue microarray from 59 UL with adequate follow up, including three 0.6-mm cores for each case. Immunohistochemistry for Calponin monoclonal antibody (DakoCytomation, CA M3556, 1:50) was performed with the IHC DAB MAP system (Ventana Medical Systems). The intensity and the extension of the immunostaining were both scored from 0 to 3. The multiplicative index of intensity and extension was considered for analysis as a new semi-quantitative variable ranging 0 to 9. This variable was also recoded into two new variables following: a two-tier distribution (low/high) and a three-tier distribution (low/intermediate/high expression). Final case score resulted by obtaining the mean of three cores corresponding to each specimen. The end point for this study was overall survival. Survival curves were generated using the Kaplan-Meier method. Multivariate analysis was performed using the Cox proportional hazards model.

Results: A better outcome was associated to calponin immunoreactive tumors, using both the two and three-tiered expression scoring systems. Results showed statistical significance (log-rank p=0.0185 and 0.0086 respectively). Multivariate analysis demonstrated that high calponin expression predicted a longer survival, independently of both stage and presence of metastasis at the time of the diagnosis (p = 0.025).

Conclusions: We have demonstrated that leiomyosarcomas actually fell into two distinct subgroups depending on calponin h1 expression, which arises as an independent positive prognostic factor in UL patients. Supported by the Ministry of Health of Spain (FIS-FEDER C03-10), and the Regional Government of Castilla y Leon.

979 Evaluation of p16, MCM 2, DNA Topoisomerase IIA, and ProEXC in Cervical Squamous Intraepithelial Lesions

J Shi, H Liu, M Wilkerson, S Meschter, W Dupree, F Lin. Geisinger Medical Center, Danville, PA; Lehigh Valley Hospital, Allentown, PA.

Background: p16, a cell cycle regulatory protein, has been shown to be overexpressed in nearly all high-grade squamous intraepithelial lesions (HGSIL) and approximately 70% of low-grade squamous intraepithelial lesions (LGSIL). Other cell cycle regulators, such as minichromosome maintenance protein 2 (MCM2), DNA topoisomerase IIA (TOP IIA), and ProEXC (a cocktail of MCM2 and TOP IIA), have also demonstrated some values in identifying squamous intraepithelial lesions (SIL). Data on direct comparison of those cell regulatory proteins in the detection of SIL are limited. In addition, increased diagnostic sensitivity in the detection of LGSIL with a cocktail of those primary antibodies has not been well studied.

Design: We evaluated the diagnostic value of p16, MCM2, TOP IIA, ProEXC, and a cocktail of p16 and ProEXC in 62 cervical biopsy specimens by immunohistochemistry. The 62 cases were divided into three groups: Group1 (G1) – 14 cases of benign squamous mucosa; Group 2 (G2) – 34 cases of LGSIL; and Group 3 (G3) – 14 cases of HGSIL. The staining intensity was graded as weak or strong. The distribution was recorded as negative (no staining), 1+ (basal 1-2 layers), 2+ (lower 1/3), 3+ (lower 2/3), or 4+ (full thickness).

Results: The results demonstrated that a nuclear and cytoplasmic staining pattern for p16 and the p16/ProEXC cocktail was observed in 14 of 14 (100%) cases of G3, with 3-4+ staining; whereas MCM2, TOPIIA, and ProEXC were positive (nuclear staining) in 11 of 14 cases (79%), 12 of 14 (86%), and 12 of 14 (86%), respectively, with 2-3+ staining in G3 cases. ProEXC and the p16/ProEXC cocktail showed positive staining of 2-3+ in all G2 (LGSIL) cases (n=34). In contrast, immunoreactivity for p16, MCM2 and TOP IIA was detected in 26 of 34 cases (76%), 28 of 34 (82%), and 26 of 34 (76%) in G2, respectively. Importantly, all 8 p16-negative cases in G2 were positively stained with ProEXC. The majority of cases in G1 showed 1+ staining for MCM2, TOP IIA, ProEXC, and the ProEXC/p16 cocktail.

Conclusions: Our data indicate 1) p16 is a more sensitive and specific marker for identifying HGSIL compared to other markers tested in this study; 2) ProEXC is a better marker for detection of LGSIL; 3) a cocktail of p16 and ProEXC provides the highest diagnostic value for detection of both HGSIL and LGSIL; and 4) caution should be taken when using a cocktail of p16 and ProEXC because positive staining is present in the basal layer of benign squamous epithelium.

980 Histologic Patterns of Low Grade Serous Carcinoma of the Ovary – A Tale of Two Concepts

E Silva, M Deavers, A Malpica. M. D. Anderson Cancer Center, Houston, TX.

Background: Conventionally, the diagnosis of ovarian low grade serous carcinoma (LGSCa) requires the presence of stromal invasion. The significance of micropapillary pattern is controversial. In this study, we investigated the presence of different histologic patterns in areas of invasion and in areas not diagnostic of invasion.

Design: We reviewed 60 cases of LGSCa of the ovary diagnosed based on the presence of stromal invasion. These cases were compared with 60 ovarian serous borderline neoplasms, stages II or III.

Results: All areas of invasion had a desmoplastic reaction. The most common pattern was invasion by small groups of cells, including micropapillae within empty spaces. Less frequently, the invasive foci were composed of macropapillae, glands and cribriform areas. Highly unusual components of the invasive areas were solid groups of cells, single cells and diffuse calcifications with very few cells. In 31 of 60 cases, adjacent to the areas of invasion, there were areas not diagnostic of invasion with marked epithelial proliferation, which in 14 cases were significantly larger than the invasive component. These were confluent areas measuring at least 2x2 mm and composed of different patterns including micropapillary (9 of 14), macropapillary, cribriform, glands and cysts. These areas were easily recognized because of 1- marked cellular proliferation, 2- almost a complete absence of stroma in the foci of epithelial proliferation, 3- total lack of organization of the papillae, 4- papillae of similar sizes and shapes, 5- similar interpapillary spaces. These features were present in the primary neoplasms as well as in the metastases and were absent in the borderline tumors, which were characterized by large amounts of stroma within the epithelial areas, hierarchical papillae, and variation in the sizes and shapes of the papillae and in the interpapillary spaces.

Conclusions: 1- The areas of invasion in LGSCa usually show small groups of cells in empty spaces, but additional patterns of invasion are: micropapillary, macropapillary, glandular, cribriform, solid, extensive calcifications with few epithelial cells, and single cell infiltration. 2- In addition to these patterns of invasion, we believe it might be possible to identify cases of LGSCa when a 2x2 mm area, not diagnostic of invasion, is occupied by macropapillae, glands, cysts, cribriform foci, or mainly uniform micropapillae, characterized by a marked disorganized cellular proliferation. 3- Micropapillary pattern of LGSCa is different from micropapillary pattern of serous borderline neoplasms.

981 IMP3: A Novel Molecular Marker That Distinguishes Endometrial Serous Carcinoma from Benign and Other Malignant Lesions of the Endometrium

S Singh, W Zheng, J Wang, SK Chambers, J McAlpine, Z Jiang. U of Arizona, Tucson; Shanghai Med College Fudan Univ, Shanghai, China; Tufts-New England Med Center, Boston; Yale Univ School of Med, New Haven; U of Mass, Worcester.

Background: IGF-II mRNA-binding protein 3 (IMP3) is a new oncofetal protein highly expressed in pancreatic cancer. We found that it is also expressed in endometrial lesions. The aims of this study were 1) to assess IMP3 expression in endometrial carcinomas, 2) to correlate IMP3 expression with the cancer types, grade, and stage, and 3) to evaluate the utility of IMP3 antibody in differential diagnosis.

Design: A tissue microarray (TMA) with 397 cases of endometrial lesions including 70 benign controls were used. The TMA slides were stained by IMP3 immunohistochemically (IHC). 315 cases were eligible for the analysis and IMP3 was scored by 2 pathologists. Final data set included endometrioid (n=204), mucinous (n=3), serous (n=51), clear cell (n=5), mixed (n=34), and other carcinomas (n=18). A renal cell carcinoma with known IMP3 expression was used as positive control. We used the criteria of at least 50% of the target cells with moderate staining as positive. The scores were validated by 50 cases.

Results: IMP3 expression was mainly present in endometrial serous, clear cell, and mixed carcinomas with positive expression rate of 73%, 71%, and 57%, respectively. Compared with endometrioid carcinoma of 10% expression, they had a significantly higher expression rate ($p <= 0.0001$). All benign cases were negative. No expression difference among endometrioid, mucinous, and other carcinomas was found. The percentage of IMP3 expression positively correlated with FIGO and nuclear grade (p trend = 0.005). Compared with stage 1 cancers, IMP3 expression was significantly higher in cancers of stages 2 ($p = 0.0004$), 3 ($p < 0.0001$), and 4 ($p < 0.0001$). The correlation coefficient value was 0.95.

Conclusions: We conclude that IMP3 is useful to differentiate between benign and malignant endometrium. IMP3 expression positively correlates with grade and stage. Strong and diffuse expression of IMP3 is highly sensitive to identify high-grade, particularly serous and clear cell carcinomas or mixed carcinomas. Therefore, it may be a useful diagnostic marker in endometrial cancers, particularly when limited material is available or when a tumor with low-grade architecture but with high-grade nuclei is seen.

982 Combined Transverse and Longitudinal Sectioning of Radical Hysterectomy Specimens Gives More Accurate Pathological Information for Invasive Cervical Cancer

DE Song, JY Ro, KR Kim. University of Ulsan College of Medicine, Seoul, Republic of Korea; The Methodist Hospital, Weill Medical School, Cornell University, Houston, TX.

Background: Depth of invasion, width, tumor volume, and presence or absence of parametrial invasion are important prognostic parameters in patients with invasive cervical carcinomas. For the diagnosis of invasive cervical cancers, the entire cervix of radical hysterectomy specimens are serially sectioned with a longitudinal incision, as in a cone biopsy specimen. However, it is difficult to measure the width or volume of

the tumor by longitudinal sectioning due to uneven thickness of tissue sections between the internal mucosal side and the external pericervical circumferences. Moreover, it is difficult to evaluate the relationship of the tumor with the surrounding structures in one plane.
Design: We have tried a modified sectioning method in 25 radical hysterectomy specimens and compared their advantages and disadvantages with those of traditional longitudinal sectioning.
Results: From the uterine fundus to the endocervix, where parametrial tissue is attached, transverse sectioning has several advantages over longitudinal sectioning: First, it is easy to obtain sections with even thickness. Thus, crude three-dimensional tumor volume can be calculated by multiplying digitally calculated tumor area by the thickness of each section. Second, it provides better overviews of the variable depth of invasion throughout the cervical wall in one plane, especially in the parametrial region. Third, it is easier to detect extension to the lower uterine corpus in a smaller number of sections. Fourth, the status of anterior and posterior margins in relation to the bladder or rectum can be easily evaluated, which is important in extensively infiltrating cases. Finally, correlations with radiological findings, i.e., CT or MRI, are easier. One critical disadvantage is a difficult evaluation of tumor involvement in the cervicovaginal junction. In that area, longitudinal sectioning is better to view the relationship of the tumor with the vaginal cuff. The ideal turning point from transverse to longitudinal sectioning seems to be 0.5 cm or upper from the cervicovaginal junction.
Conclusions: A combination of transverse and longitudinal sectionings of radical hysterectomy specimens in invasive cervical cancers provides more accurate pathological information, such as width, depth of invasion, tumor volume, and invasion into the surrounding structures.

983 Utility of Immunohistochemical Markers in the Differential Diagnosis of Uterine Papillary Serous Carcinomas and Endometrioid Carcinomas of the Endometrium

DE Song, KR Kim. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea.
Background: The histological distinction between uterine papillary serous carcinomas (UPSCs) and endometrioid adenocarcinomas (EMCs) is important because UPSCs show a more aggressive clinical course and a poor response to adjuvant treatment. Even a small percentage of UPSCs in a mixed form of carcinomas is correlated with a poor prognosis. Thus, definitive diagnosis of UPSCs in patients with low FIGO stage ensures optimal treatment. Although the UPSCs and EMCs are well known to have different pathogenetic mechanisms, biomarkers to differentiate these two tumors are not well established. We aimed to select useful immunohistochemical markers which can be applicable to the differential diagnosis between the two tumor types.
Design: We created three tissue microarrays, each containing three 1-mm-diameter cores from 19 low-grade EMCs (FIGO grade 1 or 2), 19 high-grade EMCs (FIGO grade 3), and 13 UPSCs. A panel of immunohistochemical stainings, including MLH1, MSH2, p53, PTEN, ß-catenin, c-erb B2, estrogen receptor (ER), progesterone receptor (PR), and E-cadherin was analyzed to examine any differences in intensity, percent positivity, and staining patterns among these three groups.
Results: Low grade EMCs expressed of p53 (5.3%), ß-catenin (31.6%), c-erb B2 (10.5%), ER (89.5%), and PR (89.5%) and loss of MLH1 (10.5%), MSH2 (15.8%), PTEN (42.3%) and E-cadherin (10.5%). High-grade EMCs showed expression of p53 (21.2%), ß-catenin (21.1%), c-erb B2 (21.1%), ER (15.8%), and PR (36.8%) and loss of MLH1 (31.6%), MSH2 (36.8%), PTEN (10.5%) and E-cadherin (36.8%). UPSCs showed expression of p53 (76.9%), ß-catenin (15.4%), c-erb B2 (15.4%), ER (7.7%), and PR (53.8%) and loss of MLH1 (15.4%), MSH2 (15.4%), PTEN (15.4%), and E-cadherin (38.5%). Only the p53 expression was significant ($p = 0.003$) in the differential diagnosis between the high-grade EMCs and the UPSCs. Between the low-grade EMCs and the UPSCs, p53 ($p < 0.001$), ER ($p < 0.001$), and PR ($p = 0.038$) showed significant differences.
Conclusions: Immunohistochemical expression of p53 is a useful tool for the differentiating UPSCs from EMCs regardless of FIGO grade. A panel of immunohistochemical results of p53 (+), ER (-), and PR (-) would best characterize the UPSCs, and the opposite results for low-grade EMCs. These immunohistochemical expressions can be helpful in the distinction of mixed UPSCs and high-grade EMCs from pure high-grade EMCs, and also in the distinction of EMCs with villoglandular features from UPSCs.

984 Re-Exploration of the Distinctive Ovarian Lesion Associated with Sclerosing Peritonitis: An Analysis of 25 Cases

PN Staats, WG McCluggage, PB Clement, RH Young. Massachusetts General Hospital, Boston, MA; Royal Group of Hospitals Trust, Belfast, Ireland; Vancouver General Hospital, Vancouver, BC, Canada.
Background: Sclerosing peritonitis (SP) may be associated with a distinctive, often bilateral, ovarian lesion composed of spindle cells and partially luteinized cells variably interpreted as luteinized thecoma or a non-neoplastic lesion related to massive edema-fibromatosis. The long-term outcome of these patients is unclear.
Design: Clinical, gross and microscopic features were evaluated in 25 consultation cases and immunohistochemical stains in 13 of them.
Results: Patients ranged from 10 months to 85 years (median 28 y) and had ascites, abdominal pain, and occasionally small bowel obstruction. The ovarian lesions (bilateral in 24 cases) were 2 to 31 cm and solid but often markedly edematous. In 18 cases the lesion replaced the entire ovary and in the remainder involved only the cortex. Microscopically, spindle cells in compact fascicles were admixed with varying numbers of weakly luteinized cells; prominent edema often created a microcystic pattern. Mitoses ranged from <1 to >80/10 HPFs, sometimes with markedly discrepant counts in bilateral lesions. Residual follicles or other normal structures were often present within the proliferation. No co-existent massive edema or fibromatosis was seen. Strong inhibin, calretinin, and/or CD56 positivity of the luteinized cells was found in all cases. The spindle cells were focally positive for SMA and desmin in most cases, and occasionally were focally positive for AE1/3, calretinin, and/or CD56, but not inhibin. Follow-up of up to 13 y (mean 5.5) in 17 cases found that 2 patients died of complications of peritonitis; all other patients are without evidence of disease.
Conclusions: The nature of the ovarian lesions associated with SP is unclear. As staining for CD56 is typical in ovarian fibromas and normal ovarian stromal cells (McCluggage WG, Intl J Gynecol Pathol, in press), CD56-negativity of the spindle cells in this study suggests they may not be of ovarian stromal type, although modulation of such cells with altered immunoreactivity is possible. It is also unclear if the lesions are neoplastic or non-neoplastic, but retention of the designation "luteinized thecoma" is justified pending contrary evidence. Studies to determine clonality are in progress and may help clarify this issue. SP in these cases may cause morbidity and mortality, but appears to resolve in most patients, with a generally good outcome.

985 Imbalances of Chromosomes 4, 9 and 12 Are Recurrent in Ovarian Stromal Tumors

RC Streblow, AJ Dafferner, M Fletcher, W West, RK Stevens, Z Gatalica, D Novak, JA Bridge. University of Nebraska Medical Center, Omaha, NE; Creighton University Medical Center, Omaha, NE.
Background: Conventional cytogenetic studies of ovarian stromal tumors are few, although extra copies of chromosome 12 have been frequently documented utilizing molecular cytogenetic techniques. Our preliminary cytogenetic studies of four ovarian stromal tumors and a review of the literature suggest that numerical abnormalities of chromosomes 4 and 9 might also be important, possibly as secondary changes. In the current study, FISH studies were employed in an effort to determine the frequency of chromosomes 4, 9 and 12 copy number abnormalities in a larger group of benign ovarian stromal tumors.
Design: Standard cytogenetic analysis was performed on four ovarian cellular fibromas. Interphase FISH studies were performed using centromeric enumeration probes specific for chromosomes 4, 9, and 12 on representative tissue sections from 17 ovarian stromal tumors (eight fibromas, three thecomas, one fibrothecoma, and five cellular fibromas). The number of hybridization signals for each specimen was assessed in a minimum of 200 interphase nuclei with strong and well-delineated signals by two different individuals. A specimen was interpreted as aneuploid (monosomic, trisomic, or polysomic) if the signal copy number was more than 2 SD above the average false-positive rate.
Results: Simple numerical abnormalities, including aneuploidies of chromosomes 4, 9, and 12, were detected in three of the four cellular fibromas analyzed cytogenetically. Molecular cytogenetic analysis revealed extra copies of chromosome 12 in all five cellular fibromas as well as in two fibromas and the fibrothecoma. Gain of chromosome 9 was confined to the cellular fibromas (4 of 6 cases; one case only analyzed cytogenetically). Loss of copies of chromosomes 4 and/or 9 were prominent in the fibromas (7 of 8 cases). FISH studies of the three thecomas were unsuccessful despite repeated efforts, possibly secondary to extended formalin fixation for these samples.
Conclusions: These findings confirm the presence of trisomy 12 as a nonrandom chromosomal abnormality in ovarian stromal tumors. Moreover, these conventional and molecular cytogenetic data suggest that gain of chromosome 9 in addition to gain of chromosome 12 is prominent in cellular fibroma. In contrast, loss of chromosomes 4 and/or 9 are recurrent in fibroma. In summary, imbalances of chromosomes 4 and 9 appear to represent important secondary abnormalities in ovarian stromal tumors.

986 ProExC™: A Helpful Tool for Difficult Cervical Specimens?

ME Sullivan, E Kilner, RE Meyer, H Du, CD Sturgis, M Milano, WG Watkin. Evanston Northwestern Healthcare, Evanston, IL.
Background: The interpretation of cervical specimens is often problematic. Interobserver variation in interpretation, sampling biases, and confounding factors such as reparative changes, metaplasia and atrophy can result in over or under diagnosis of dysplasia. ProExC™ antibody reagent contains antibodies against minichromosome maintenance (MCM2) and topoisomerase II alpha (TOP2A) proteins which are involved in cell replication. This novel immunohistochemical (IHC) marker has been developed to assist in the classification of abnormal cells in cytology and tissue specimens. The aim of this study was to determine if ProExC™ can help classify difficult cervical specimens.
Design: 108 cervical biopsy, loop electrosurgical excision procedure (LEEP), and conization specimens consecutively accessioned between 1/1/2006 and 3/31/2006 were identified. Nine cases were excluded due to insufficient tissue. 99 resultant cases were reviewed and diagnosed as negative (Neg), low grade dysplasia (LG) or high grade dysplasia (HG). There was discordance between the original and consensus review diagnosis in 24 cases; IHC staining for ProExC™ was performed on all cases and was scored for level of staining (lower, middle, or upper third of epithelium) and percent of positive nuclei (0-25, 26-50, 51-75, or 76-100%). The pattern seen with ProExC™ in adjacent uninvolved cervix was used to define negative staining as limited to the lower third of the epithelium and/or <25% nuclear positivity.
Results: Three cases were originally called negative but were thought to be dysplastic on review. All had a negative ProExC™ staining pattern. Twelve cases were originally diagnosed as dysplastic with a consensus diagnosis of negative. 12/12 showed lower third staining and 11/12 (91.7%) had <25% nuclear positivity. Four cases were upgraded from low to high grade dysplasia; all had middle to upper third staining but the percent of positive nuclei was evenly spread across the entire range. Five cases were downgraded from high to low grade; 4/5 (80%) had upper third staining and >50% nuclear positivity.

Conclusions: In difficult cervical biopsies, ProExC™ is a useful adjunct when attempting to distinguish negative specimens from true dysplasia. ProExC™ may be less helpful in distinguishing low grade dysplasia from high grade dysplasia in borderline cases as the staining pattern is more variable.

987 Calretinin and CD34 Immunoreactivity of the Endometrial Stroma in Dysfunctional Bleeding and in Hyperplastic and Neoplastic Endometrial Lesions

I Teo, A Elkeilani, EC Marginean, J Thomas, KT Mai. Ottawa Civic Hospital, Ottawa, ON, Canada; University of Ottawa, Ottawa, ON, Canada.

Background: We have recently demonstrated that calretinin immunostaining of the endometrial stromal cells (ESC) in the functionalis (FL) of premenopausal women shows a zonal reactivity. Calretinin reactivity increases during the proliferative phase from the superficial zone to full-thickness FL, reaches a maximum in secretory phase, then decreases to "zero" in menstrual period. CD34 reactivity was consistently observed in the ESC of the basalis layer (BL), decreasing in the peri-menopausal years. In this study, we investigate calretinin and CD34 reactivity in endometria in dysfunctional uterine bleeding (DUB), and in hyperplastic and neoplastic lesions.

Design: Immunostains for calretinin and CD34 were performed on 77 endometrial specimens.

Results: Polyps, hyperplastic and neoplastic endometria either had negative, or focal weak reactivity for calretinin in the ESC of the functionalis and basalis. Of these conditions, only polyps showed focal to extensive stromal reactivity for CD34. CD34 either did not stain, or only weakly, focally stained ESC in hyperplastic and neoplastic endometria. Regardless of hormone exposure or histologic appearance, ESC from women with DUB often showed weaker calretinin reactivity than normal endometrium, with focal to extensive loss of reactivity in the FL. All DUB cases demonstrated CD34 reactivity which appears to extend from the BL into the FL, and which overlaps areas of altered calretinin reactivity.

Conclusions: Alterations in calretinin and CD34 stromal reactivity occur in hyperplastic and neoplastic endometria. In DUB, the altered calretinin and CD34 stromal reactivity suggests expansion of the BL-type stroma into the FL, generating a "disordered endometrial stroma".

988 Loss of LKB1 Protein Expression Is Frequent in High Grade Serous Carcinoma of the Ovary

A Tone, H Begley, TJ Brown, PA Shaw. University Health Network, Toronto, ON, Canada; Mount Sinai Hospital, Toronto, ON, Canada.

Background: We have previously obtained gene expression profiles of tubal epithelium from *BRCA1* mutation carriers and women at low risk of carcinoma, and of serous carcinomas of tubal or ovarian origin. Significance analysis of microarray data demonstrated a reduced mRNA expression of the tumour suppressor LKB1 in carcinoma samples compared to benign epithelium. LKB1 is a serine/threonine kinase previously implicated in regulation of p53-dependent apoptosis, cell cycle arrest and regulation of cell polarity. The aim of this study is to clarify the expression of LKB1 in ovarian neoplasia, by performing immunohistochemistry on tissue microarrays representative of ovarian tumors of varying histological types.

Design: Archived sections of ovarian tumors were reviewed, paraffin blocks selected, and tissue microarrays created using triplicate 0.6 mm. cores. The major histological types included were: serous (n=90), non-serous (n=36), and micropapillary (n=12) carcinomas, and borderline serous tumors (n=19). Immunohistochemistry for LKB1 was performed using standard techniques. Percentage of tumor cells with positive cytoplasmic staining, staining intensity and histoscores were determined by viewing digitalized images with Aperio ImageScope software. A combined score of > 4 was considered positive. Fisher's exact test with 95% confidence intervals was used to determine the significance of results.

Results: 71/90 (79%) of serous carcinomas scored showed loss of cytoplasmic LKB1 expression compared to only 8/36 (22%) of non-serous carcinomas ($p<0.0001$). Within the serous histotype, loss of cytoplasmic LKB1 expression was observed in 76% of high grade carcinomas compared to 11% of tumors of low malignant potential ($p= 0.0009$) and 25% of micropapillary carcinomas ($p=0.03$). In addition, serous carcinomas associated with a known germline BRCA1/2 mutation showed a similar loss of LKB1 expression compared to serous cancers with negative mutation testing (89% vs. 83% respectively).

Conclusions: Cytoplasmic LKB1 protein expression varies according to histological type of ovarian cancer. It is frequently reduced in high grade serous carcinoma, but is uncommonly decreased in non-serous carcinomas and in borderline serous tumors. Hereditary and sporadic high grade serous cancers have a similar frequency of LKB1 loss. Reduced LKB1 protein expression in high grade ovarian serous carcinomas may indicate a role of loss of this kinase in serous oncogenesis.

989 Expression of Transcription Factor PAX2 in Ovarian Papillary Serous Carcinoma: An Aid in Differential Diagnosis with Peritoneal Malignant Mesothelioma

GX Tong, L Chiriboga, D Hamele-Bena, AC Borczuk. Columbia University Medical Center, New York, NY.

Background: Papillary serous carcinoma (PSC) of the ovary or the peritoneum can mimic peritoneal malignant mesothelioma (PMM). The differential diagnosis of PSC and PMM is important and often requires ancillary studies such as immunohistochemistry (IHC). Several IHC markers are avaiable, but some cases still cause diagnostic difficulty. PAX2 is a transcription factor expressed in specific types of tissue during embryonic stages, including the Mullerian system (MS), and is essential for the development of these organs. Its expression in the human MS is unknown. In this study, we investigated the expression of PAX2 in normal adult MS-derived tissues and compared its expression in ovarian PSC (OPSC) and PMM by IHC.

Design: Five tissue microarrays containing 54 PMM (38 men and 16 women), 36 OPSC, and 8 normal peritoneal biopsies were constructed with 3 or 4 representative cores from each case. Normal MS tissues included benign ovary (OV) (10), fallopian tube(FT) (7), endometrium (EM) (4), and cervix (CEV) (5). IHC of PAX2 (Zymed) was performed on formalin-fixed and paraffin embedded tissue sections with avidin-biotin peroxidase method after antigen retrieval. The PAX2 staining was scored semi-quantitatively by intensity and positive cells. All mesotheliomas were confirmed by calretinin, CK5/6, WT1, CEA, LeuM1, BerEP4 and B72.3 staining. ER, PR IHC was also performed.

Results: PAX2 was detected in epithelial cells of the FT, fimbriae, EM and CEV. In the OV, PAX2 was detected in the glands of endometriosis, rete ovarii, and in rare cysts, but not in the cuboidal surface epithelial cells. No PAX2 was detected in the stromal cells of the OV or EM, or in sex-cord derived structures. PAX2 was detected in 24 of 36 (67%) OPSC (weak, moderate, and strong nuclear staining in 4, 9, and 11 cases, respectively) and PAX2 positive cells varied from 5 to 100% (5-20%, 25-80%, and 100% in 11, 10 and 3 cases, respectively). PAX2 was not detected in 52 of 54 (96%) of the PMM with 2 exceptions, both were women in which moderate staining of PAX2 was noted. Normal mesothelial cells were negative for PAX2. ER and PR were detected in 33 (92%) and 15 (42%) of 36 of the OPSC, 2 (0.4%) and 1 (0.2%) of 54 of the PMM, respectively.

Conclusions: PAX2 is expressed in adult human MS-derived epithelia and OPSC. PAX2 may be a useful marker with moderate sensitivity (67%) and high specificity (96%) in the differential diagnosis of OPSC and PMM. The significance of PAX2 in 2 PMM requires further investigation.

990 The Morphologic Spectrum of Smooth Muscle Tumors in the Hereditary Leimyomatosis and Renal Cell Cancer (HLRCC) Syndrome. Its Clinical Significance

D Val, P Stratton, RD Neumann, WM Linehan, MJ Merino. NCI, Bethesda, MD; NIH, Bethesda, MD; CC, Bethesda, MD; NCI, NIH, Bethesda, MD.

Background: Hereditary leimyomatosis and renal cell cancer (HLRCC) is a recently identified autosomal dominant disorder in which affected individuals have a predisposition to develop uterine and cuteaneous smooth muscle tumors (SMT) as well as renal cell cancer. The morphologic spectrum of uterine SMT in this syndrome has not been fully described.

Design: The uterine SMT from 18 patients with confirmed germ line mutations in the FH gene were evaluated. Ages ranged 24 to 50 years (m 38). Patients presented with heavy bleeding, abdominal pain, or uterine tumors were identified as part of clinical screening. Multiple tumors were identified in all patients. 4 patients underwent myomectomy and 14 hysterectomy. One patient underwent subsequent myomectomies within three years, after the delivery of a child. One patient underwent prophylactic hysterectomy when her skin leiomyomas grew in size. Immunohistochemistry for proliferative markers was performed.

Results: All patients had multiple uterine SMT with maximum diameters ranging 1.8 to 8.9 cm (m 5.84 cm). Imaging of these tumors by F-18FDG PET/CT, show increased uptake in many of these tumors. Histologically, tumors were classified as follows: leiomyoma (7), cellular leimyoma (2) atypical leimyoma (5), SMT of unknown malignant potential (2) and leimyosarcoma (2). However, some patients with hysterectomy had coexistence of different types of SMT. A common histologic finding encountered in all the tumors, was prominent cellularity and pleomorphic cells with large prominent orangiophilic nucleoli with a surrounding clear halo. This was not observed neither in non-tumoral myometrial tissue nor in other SMT not associated with HLRCC. Mitotic counting as well as proliferation index (Mib-1) were assessed.

Conclusions: Recognition of the spectrum of HLRCC associated smooth muscle tumors is quite important in patients with this hereditary syndrome, considering that they occur predominantly in women of reproductive age that may have high predisposition for the development or progression to malignancy, and may want conservative therapy.

991 Molecular Genetic and Drug Resistance Data Support Classifying Moderately and Poorly Differentiated Ovarian and Peritoneal Serous Carcinomas into a Single Category

R Vang, IM Shih, R Salani, E Sugar, RJ Kurman. The Johns Hopkins Hospital, Baltimore, MD; The Johns Hopkins School of Medicine, Baltimore, MD.

Background: Serous carcinoma has been traditionally graded as well, moderately, and poorly differentiated. Recent morphologic and molecular genetic studies have shown that invasive well differentiated serous carcinoma, referred to by us as "low-grade micropapillary serous carcinoma (MPSC)," is distinct from moderately and poorly differentiated serous carcinoma from the standpoint of pathogenesis and clinicopathologic features. Since moderately and poorly differentiated serous carcinomas have overlapping features and their distinction is highly subjective, the goal of this study was to determine, based on molecular genetic analysis and drug resistance data, whether this distinction had biologic validity.

Design: 52 moderately and poorly differentiated (based on <50% or ≥50% solid architecture, respectively) ovarian and peritoneal serous carcinomas were analyzed for p53 mutation, amplification of cyclin E1 and AKT2, and extreme drug resistance to cisplatinin, carboplatin, taxol, and taxotere. Pure endometrioid, clear cell, mucinous, and transitional cell carcinomas were not included.

Results:

		Amplification		Extreme drug resistance			
Serous carcinoma grade	**p53 mutation**	*Cyclin E1*	*AKT2*	*Carbo-platin*	*Cisplatin*	*Taxol*	*Taxotere*
Moderately differentiated	26/34 (76%)	8/18 (44%)	7/18 (39%)	3/27 (11%)	3/28 (11%)	5/28 (18%)	3/27 (11%)
Poorly differentiated	13/18 (72%)	0/2 (0%)	1/2 (50%)	0/14 (0%)	1/14 (7%)	3/16 (19%)	1/14 (7%)
p-value	0.747	NC	NC	0.539	>0.99	>0.99	>0.99

Key: NC, not calculated.

Conclusions: Based on the molecular genetic and drug resistance data shown above, moderately and poorly differentiated ovarian and peritoneal serous carcinomas are indistinguishable. These findings are consistent with morphologic observations in which these tumors are often classified as "moderately to poorly differentiated." Although the numbers are small and more cases must be analyzed, this study suggests that the distinction of moderately from poorly differentiated serous carcinoma may not be relevant. For practical purposes, all of these tumors should be simply classified as "high-grade" serous carcinoma. A two tier classification of low-grade MPSC and high-grade serous carcinoma (Malpica et al, AJSP 2004) is therefore supported by this study.

992 Mitosis-Specific Marker Phospho-Histone H3 (pHH3) in the Assessment of Mitotic Index in Uterine Smooth Muscle Tumors

E Veras, A Malpica, MT Deavers, EG Silva. The University of Texas Medical School, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The assessment of an accurate mitotic index (MI) is of utmost importance in the proper classification of uterine smooth muscle tumors (USMTs). This assessment can be hampered by the presence of apoptotic bodies (ABs) or pyknotic nuclei (PN) which can mimic mitoses. Phospho-histone H3 (pHH3) is a recently described immunomarker specific for cells undergoing mitoses. In this study, we evaluated the MI of USMTs using pHH3 and H&E stained slides. A clinical correlation is also presented.

Design: Twelve cases of USMTs, six smooth muscle tumors of unknown malignant potential (STUMPs) and six leiomyosarcomas (LMSs), were retrieved from our department files. H&E slides were reviewed in all cases, diagnoses confirmed according to the WHO classification, and the most representative slide from each case was selected for MI. pHH3 immunostain was performed in all cases. Clinical information and follow-up were obtained from the patients' (pts) charts.

Results: Pts' age ranged from 35 to 62 yrs (mean, 44.7 yrs for the pts with STUMPs and 59.1 yrs for the pts with LMSs). STUMPs ranged in size from 1.2 to 12 cm (mean, 5.1cm). All pts with STUMPs are alive with NED after a follow-up ranging from 6 to 14 years (mean 9.5 yrs). LMSs ranged in size from 3.5 to 18 cm (mean, 8.45 cm). All pts with LMS, except one, are DOD 9 months to 4 years after diagnosis (mean 2.55 yrs). In the STUMPs, the MI on H&E ranged from 0 to 9 mitoses/10 high power fields(HPFs), (mean, 3.2) and in the LMSs, it ranged from 0 to 37mitoses/10HPFs (mean, 19). pHH3 demonstrated 0 to 16 mitoses/10HPFs (mean, 3.8) in the STUMPs and 0 to 61 mitoses/10HPFs (mean, 26) in the LMS. In 7 cases, the MI difference (MID) between H&E and pHH3 stains varied from 0 to 3 mitoses (mean, 1.6). In 4 cases the MI was higher for pHH3, and varied from 7 to 24 (mean, 14). In only one LMS, the pHH3 MI was lower than on H&E stained slides (MID, 7). The MI on H&E and pHH3 stained slides were comparable in 7 cases, 5 of which were STUMPs. In 4 other cases, 3 of which were LMSs, the pHH3 MI was significantly higher than the one obtained on H&E. In the latter cases there were increased ABs and PN among the mitotic figures on H&E.

Conclusions: pHH3 appears to be a useful tool in the assessment of the MI in USMTs, especially when ABs and PN are confounding factors. The higher MI obtained with pHH3 in some cases is most likely due to the recognition of true mitoses initially interpreted as ABs and/or PN.

993 P16/Ki67 Immunostaining Is Useful in Stratification of Atypical Metaplastic Epithelium of the Cervix

AE Walts, S Bose. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Cervical metaplastic squamous epithelium exhibiting atypia insufficient for a diagnosis of dysplasia is usually reported as "atypical squamous metaplasia" (ASQM). Stratification into reactive and dysplastic groups impacts treatment since the differential is often reactive vs. high (rather than low) grade squamous intraepithelial lesion (SIL). Diagnosis with H&E stains is associated with low levels of intra- and interobserver concurrence. P16/Ki67 immunostains are helpful to assess cervical and anal biopsies for HPV-associated lesions but staining in squamous metaplastic epithelium has received little attention. This study was designed to establish baseline staining characteristics of metaplastic squamous epithelium so as to determine if p16/Ki67 staining is useful in stratification of ASQM.

Design: Slides of 49 cervical biopsies containing squamous metaplastic epithelium were retrieved from our files. Patients ranged in age from 19 to 68 years (mean: 36 yrs). Based on H&E slides these cases were divided into four groups: nonatypical squamous metaplasia (BSQM) in benign cervices (12 cases), BSQM with SIL (low or high grade) elsewhere (9 cases), metaplastic epithelium involved by SIL (19 cases; 9 LG and 10 HG), and ASQM (9 cases). Sections of the biopsies and appropriate controls were immunostained for p16 (Biocare Medical) and Ki67 (Ventana). P16 was recorded as negative or band-like (positive in >90% of contiguous lesional cells) and Ki67 was recorded as positive when present in >50% of lesional cell nuclei. Presence of low risk and high risk (HR) HPV was detected by ISH utilizing the Inform HPV Family 6 and Family 16 probes (Ventana). Results were correlated with H&E diagnoses.

Results: In all cases, BSQM was negative for p16/Ki67 and HPV DNA irrespective of the presence of concurrent SIL. Metaplastic epithelium involved by SIL showed p16/Ki67 immunostaining similar to that previously reported by us in a study of anal biopsies. Band-like p16 staining and Ki67 positivity were present in 8 of 10 HSILs. One HSIL showed p16 band and HR HPV positivity with <50% Ki67 positive cells and one case (reported as CIN II) was p16/Ki67/HPV negative. Of the 9 ASQMs, 2 were p16/Ki67/HR HPV positive while 7 were p16/Ki67/HPV negative.

Conclusions: Observations previously reported by us on p16/Ki67 immunostaining are applicable to cervical metaplastic squamous epithelium irrespective of the presence of SIL elsewhere in the cervix, and these immunostains are a helpful adjunct in the stratification of ASQM as reactive or SIL. Additional studies are in progress.

994 Histopathologic Prognostic Factors in Stage I Uterine Leiomyosarcomas (Ut-LMS); a Clinicopathologic Study of 28 Cases

WL Wang, RA Soslow, M Hensley, H Asad, GF Zannoni, M de Nictolis, P Branton, E Oliva. Massachusetts General Hospital, Boston, MA; Memorial Sloan Kettering Cancer Center, New York, NY; Universita Cattolica, Rome, Italy; Istituto di Anatomia e Istologia Patologica, Ancona, Italy; Inova Fairfax Hospital, Church Falls, VA.

Background: Ut-LMSs have an overall 5-year survival of 15-25%. However, stage I Ut-LMSs are reported to have a better prognosis with a 40-70% 5-year overall survival. The purpose of this study was to evaluate histopathologic parameters that may influence outcome in stage I Ut-LMSs.

Design: Twenty eight stage I Ut-LMSs were identified from the files of 5 tertiary care hospitals between 1974 and 2006. Tumors were primary staged based on pathologic information and supplemented with radiology (10 cases) and clinical records (1 case). Stage I tumors with no additional clinical or radiologic staging information were included in the study if no recurrence was documented >6 months from the initial staging operation (17 cases). Clinicopathologic parameters evaluated included: age, tumor size, cell type, mitotic activity (MA), cytologic atypia, tumor cell necrosis (TCN), and lymphovascular invasion (LVI). Follow-up was available for all patients. Poor outcome was defined as either dead from disease or alive with disease.

Results: Thirteen patients (46%) with stage I Ut-LMSs had poor outcome with a follow-up ranging from 10 to 97 (mean 33) months; 7 died of disease and 6 were alive with disease. Fifteen patients (54%) with stage I tumors were alive and well with follow- up ranging from 8 to 148 (mean 54) months. The findings are shown in Table 1.

Conclusions: Clinically aggresive Stage I Ut-LMSs have more MA, more often an epithelioid component, extensive TCN, and LVI as compared to clinically indolent Stage I Ut-LMSs. Histological factors may contribute to some degree to stratify stage I Ut-LMSs into indolent and aggressive categories.

Table 1. Summary of Histologic Findings by Outcome

	Mean age (years)	Mean tumor size (cm)	Cell Type	Mean MA (range)	High grade atypia	TCN	LVI
Poor Outcome (n=13)	53 (37-73)	10 (5-12)		30 (6-69)	13	8	4
			6 S				
			4 E				
			1 E+S				
			2 M				
Alive and Well (n=15)	55 (35-72)	9 (5.5-13.5)		19 (4-66)	12	4	2
			12 S				
			1 E				
			2 M				

S= Spindled, E= Epithelioid, M= Myxoid, MA=Mitotic activity, TCN=Tumor cell necrosis, LVI=Lymphovascular invasion

995 Differential Expression of Insulin Liked Growth Factor 1 (IGF1) In Uterine Leiomyomas Is Regulated by MIR-29B

YH Wen, A Wei, J Laser, K Mittal, JJ Wei. New York University School of Medicine, New York, NY; University of Pennsylvania, Philadelphia, PA.

Background: IGF1 is one of the most potent mitogenic factors overexpressed in many human neoplasms. It is highly expressed in an autocrine fashion in both normal myometrium and uterine leiomyomas (ULM). Although overexpression of IGF1 mRNA in ULM has been suggested based on an observation of increase IGF1 protein in ULM, many global gene profiling analysis failed to detect an overexpression of IGF1 in ULM. Our recent study found miR-29b, a key predicted targeting microRNA for IGF1 is significantly down regulated in ULM. We hypothesized that increased level of IGF1 protein in ULM is due to a loss of IGF1 negative regulator miR29b.

Design: We 1) Collected fresh frozen tissues from 33 ULM and matched myometrium, extracted total RNA and performed semiquantitative RT-PCR for IGF1 mRNA; 2) Collected paraffin embedded tissues from 60 ULM and matched myometrium, constructed tissue microarray and examined IGF1 protein level by immunohistochemistry; 3) Extracted protein from 10 ULM and matched myometrium and examined IGF1 protein by Western blot; 4) Set up primary tissue cultures in 3 ULMs with high level of IGF1 mRNA and protein; 5) Conducted a transient transfection of exogenous miR-29b miRNA at different concentrations; 6) Analyzed of the levels of IGF1 protein after 48 hours by Western blot.

Results: IGF1 mRNA was detectable in all ULM and matched myometrium at moderate to high levels. There was no difference of IGF1 mRNA expression between ULM and matched myometrium ($p>0.05$). Immunohistochemistry on tissue microarray showed more than one third of ULM (24/56) had an increased IGF1 immunoreactivity. The level of IGF1 immunointensity in ULM was significantly higher than that of matched myometrium (paired t test, $p<0.05$). Increased IGF1 protein in ULM was further supported by a Western blot analysis of 10 ULM and matched myometrium. To test whether differential expression of IGF1 protein in ULMs was regulated by miR-29b miRNAs, exogenous miR-29b was transfected into ULM primary culture cells. A preliminary result revealed a reduction of IGF1 protein after 48 hrs and further quantitative analysis is under study.

Conclusions: IGF1 protein, but not mRNA, is elevated in ULM compared to normal myometrium. Our miRNA profiling analysis found IGF1 predicted targeting miRNA (miR-29b) was diffusely down-regulated in almost all ULM. A transfection assay of miR-29b into ULM cells supports the regulation of IGF1 by miRNA.

996 Expression of p63 in Brenner Tumors and Transitional Cell Carcinomas of the Gynecologic Tract

SF Wenson, Z Jiang, B Woda, K Dresser, CS Li. University of Massachusetts Medical School, Worcester, MA.

Background: Brenner tumor and transitional cell carcinoma (TCC) of the gynecologic tract are a group of neoplasms characterized by tumor cells showing transitional cell morphology. p63 is a homolog of the p53 tumor suppressor gene and is expressed primarily in the nuclei of basal cells in multilayered epithelia, including the urothelium of

the bladder. Loss of p63 expression has been shown to correlate with tumor progression in bladder cancer. Expression of p63 in both benign and malignant Brenner tumors as well as TCC of the gynecologic tract has not been previously reported.

Design: A total of 34 benign Brenner tumors, 2 malignant Brenner tumors, 3 ovarian TCC, and 3 fallopian tube TCC were selected from the surgical pathology files of UMMHC. Five-micron sections of formalin-fixed, paraffin-embedded tissue were routinely processed and stained with p63 antibodies. p63 expression was evaluated semi-quantitatively as negative (0% of cells positive), 1+ (<5% of cells positive), 2+ (>5% but less than 50% of cells positive), or 3+ (>50% of cells positive).

Results: As shown in Table 1, p63 was universally strongly positive in the transitional epithelium of benign Brenner tumors and more weakly positive in the lining epithelium of Brenner tumor microcysts. This expression pattern was similar to the p63 expression of normal urothelium in the bladder. In the malignant Brenner tumors and TCCs, there was much less p63 expression and most cases showed only focal positivity.

Table 1. p63 Expression

Tumor	3+	2+	1+	negative
Benign Brenner tumor epithelium	100% (34/34)	0% (0/34)	0% (0/34)	0% (0/34)
Mucinous microcysts w/in Brenner tumor	0% (0/34)	100% (34/34)	0% (0/34)	0% (0/34)
Malignant Brenner tumor and TCC of ovary and fallopian tube	12.5% (1/8)	12.5% (1/8)	50% (4/8)	25% (2/8)

Conclusions: p63 is universally strongly expressed in the transitional epithelium of benign Brenner tumors and more weakly expressed in the lining epithelium of Brenner tumor microcysts. In the positive malignant lesions most cases show only rare focal expression. Our findings indicate that p63 is strongly expressed in benign Brenner tumors but shows significantly reduced expression in malignant gynecologic transitional cell tumors.

997 No Metastatic Endocervical Carcinomas in a Series of p16^{INK4a}-Positive Mucinous Ovarian Adenocarcinomas

N Wentzensen, A du Bois, S Kommoss, J Pfisterer, D Schmidt, M von Knebel Doeberitz, F Kommoss. Institute of Pathology, Heidelberg, Germany; HSK, Wiesbaden, Germany; Faculty Mannheim, University of Heidelberg, Mannheim, Germany; Institute of Pathology, A2/2, Mannheim, Germany.

Background: Epithelial ovarian cancers may represent secondary manifestations of extraovarian carcinomas. Recently, several cases of p16-positive ovarian adenocarcinomas containing HPV DNA assumed to be metastatic endocervical carcinomas were reported (Elishaev et al., Am J Surg Pathol 2005;29:281-94). Since HPV-associated metastatic endocervical adenocarcinomas with occult primary tumors might be present in clinical trials of ovarian adenocarcinomas, we studied p16 expression and HPV DNA content in a series of mucinous ovarian adenocarcinomas from a large study.

Design: All 24 carcinomas with mucinous differentiation and available tissue blocks were selected from the AGO Ovar3 database, a large chemotherapy trial of advanced-stage ovarian carcinomas evaluating standardized postoperative platinum and paclitaxel chemotherapy after radical surgery. Recuts from archival paraffin tumor tissue blocks were stained with H&E. Standardized p16 immunostaining was performed using the p16^{INK4a} histology kit (DAKO), and both nuclear and cytoplasmic staining was considered specific. Staining was scored as negative, single cells positive, focal homogeneous staining, or diffuse staining. Total genomic DNA was extracted from paraffin blocks. HPV PCR analysis was performed using two consensus primer systems (GP5+/GP6+ and PGMY09/11), HPV16/18-specific primers (GP5+/GP6+-based), as well as E6-specific PCR for HPV16/18.

Results: 2/24 (8%) of tumors were p16-negative, 6/24 (25%) showed single positive cells, 9/24 (38%) showed focal homogeneous staining, and 7/24 (29%) showed diffuse staining. There was a strong correlation between tumor grade and p16^{INK4a} status. All 12 grade 2 or 3 tumors showed at least focal diffuse staining, while 8/12 grade 1 tumors showed no staining or only single positive cells (p=0.001). 23 tissue blocks yielded DNA sufficient for further analysis. None of the applied methods showed any presence of HPV DNA in any of the tumors.

Conclusions: No ovarian metastases of endocervical adenocarcinomas were found among mucinous adenocarcinomas from a large chemotherapy trial of advanced-stage ovarian carcinomas. p16^{INK4a} staining detected in many primary ovarian adenocarcinomas in the present series seems to be independent from HPV oncogene activity.

998 Mutations in the Wnt/β-Catenin and PI3K/Pten Signaling Pathways Underlie Ovarian Endometrioid Adenocarcinoma Development in Humans and Mice

R Wu, N Hendrix, R Kuick, DR Schwartz, Y Zhai, ER Fearon, KR Cho. University of Michigan Medical School, Ann Arbor, MI.

Background: One subtype of ovarian carcinoma, namely ovarian endometrioid adenocarcinoma (OEA) is characterized by frequent defects in the Wnt/β-catenin signaling pathway, usually as a consequence of *CTNNB1* mutations. Mutations predicted to deregulate the PI3K/Pten/Akt signaling pathway have also been reported in OEAs. These include inactivating mutations of *PTEN* and activating mutations of *PIK3CA,* which encodes the p100 catalytic subunit of PI3K. We conducted a mutational analysis of 72 primary human OEAs and found that Wnt/β-catenin pathway defects are significantly associated with concurrent *PTEN* and/or *PIK3CA* mutations. We wished to determine whether deregulation of these same pathways in the murine ovarian surface epithelium induces OEA-like tumors in mice.

Design: Using the method described by Flesken-Nikitin et al. (*Cancer Res*, 2003) and Dinulescu et al. (*Nature Med,* 2005), replication-deficient adenovirus expressing Cre recombinase (AdCre) was injected into the right ovarian bursa of *Apc*$^{loxp/loxp}$/*Pten*$^{loxp/loxp}$ mice. The left ovary was not injected. A group of 50 injected mice was followed to assess tumor development and progression.

Results: Tumors (APC^{-}/PTEN^{-}) developed in the AdCre injected ovaries with 100% penetrance and were grossly apparent by 6 weeks following injection. The mouse tumors pursued a course similar to untreated human ovarian carcinoma, with 75% of animals developing hemorrhagic ascites, sometimes accompanied by overt peritoneal dissemination of tumor. The APC^{-}/PTEN^{-} tumors are morphologically similar to human OEAs with formation of distinct glands and occasional foci of squamous differentiation. Based on immunohistochemical staining, the tumor cells are cytokeratin positive and, as anticipated, show nuclear accumulation of β-catenin protein and absence of PTEN. The APC^{-}/PTEN^{-} murine tumors display a gene expression profile more similar to human OEAs with Wnt/β-catenin and PI3K/Pten/Akt signaling pathway defects than to other ovarian carcinomas.

Conclusions: This new mouse model of ovarian cancer, based on conditional deletion of *Apc* and *Pten,* recapitulates the morphology, biological behavior, and gene expression profile of human OEAs with Wnt/β-catenin and PI3K/Pten/Akt signaling pathway defects. This model should help to advance understanding of the pathogenesis of human ovarian cancer and will likely prove useful for preclinical testing of therapies targeting these signaling pathways.

999 Prognostic Significance of Decreased GATA 4 Expression in Serous Tumors of the Ovary

JJ Xiong, CE Sheehan, JS Ross. Albany Medical College, Albany, NY.

Background: GATA 4 belongs to a family of zinc finger transcription factors, which regulates gene expression, differentiation and cell proliferation in a variety of tissues. The GATA 4 gene is located at one of 8p regions (8p21.1, 8p22-p23.1 or 8p23.1). Study showed allelic loss at distal 8p region in about 67% of ovarian serous carcinomas. Furthermore, GATA 4 transcription factor is involved in the control of cell-cell adhesion molecule expression, such as AlphaT-catenin. GATA 4 protein expression evaluation by immunohistochemistry in ovarian serous tumors has not been well documented.

Design: Formalin-fixed paraffin-embedded tissue sections from 56 primary ovarian serous tumors including 7 cystoadenomas , 7 tumors of low malignant potential (LMP) and 42 carcinomas - including 10 pairs of serous carcinoma consisting of both the primary and metastatic tumors - were immunostained by an automated method on the Xmatrx (BioGenex, San Ramon, CA) using mouse anti-human GATA 4 (Santa Cruz Biotechnology, Santa Cruz, CA). The nuclear immunoreactivity was semi-quantitatively scored based on intensity and percentage of positive cells in each case. Results were correlated with clinicopathologic variables.

Results: Nuclear GATA 4 expression was noted in 43% (3/7) cystoadenomas, 71% (5/7) LMPs and 55% (23/42) carcinomas. Expression of GATA 4 was significantly decreased from stage I to stage III (86% in Stage I, 50% in Stage II vs 43 % in Stage III, p=0.01). There is significant loss expression of GATA 4 in metastatic carcinoma compared to primary lesion (Matched pairs: p<0.001). No significant correlation was noted between GATA 4 expression and serous neoplasm, tumor grade and time of survival.

Conclusions: Our findings suggest that decreased nuclear expression of GATA 4 transcription factor may be a significant poor prognostic indicator, and GATA 4 may play a role in tumorigenesis of highly aggressive and metastatic ovarian serous tumors. These findings warrant further investigation of the role of GATA 4 with other cell-cell adhesion molecules in the evolution, progression and metastasis of ovarian cancers.

1000 Correlation Study of Dysregulated miRNAs and Their Predicted Target Genes in Uterine Leiomyomas

H Ye, TS Wang, L Chiriboga, H Yee, K Mittal, P Soteropoulos, JJ Wei. New York University School of Medicine, New York, NY; New Jersey Public Health Research Institute, Newark, NJ.

Background: MicroRNAs (miRNAs) tune embryonic development through regulation of the target gene activities. Aberrant expression of miRNAs is also involved in regulation of tumorigenic factors in human neoplasms. We recently identified a subset of miRNAs that are significantly dysregulated in human uterine leiomyomas (ULM). Some of the dysregulated miRNAs have the predicted target genes abnormally expressed in ULM. To examine if miRNAs expression is associated with the level of the selected gene translation in ULM, we initial the following study:

Design: 1) Collected paraffin embedded tissue samples from 36 ULM and matched myometrium, in which, miRNA expression had been examined; 2) Prepared a high density tissue microarray (TMA) in triplicates; 3) Performed immunohistochemistry for PD-ECGF, EGFR, ERα, PR-A, Ki67, GRIP, TGFα, RARα, RXRα, HMGA1, HMGA2, IGF1, IGF2, PI3K, TSC1, and TSC2; 4) Performed semiquantitative immunoscores by immunointensity and percentage of immunopositivity, Calculated the net gain or loss of immunoreactivity in ULM; 5) Analyzed the correlation strength (r value) of net values of 206 miRNAs with net values of 16 gene products. A global correlation was further characterized by cluster analysis.

Results: There was a strong agreement of the current immunoprofile with our previous two large cohort ULM population (n=180): increased immunoreactivity for PD-ECGF, EGFR, ERα, PR-A, Ki67, RARα, HMGA1, HMGA2, IGF1, IGF2, PI3K and TSC1, and reduced immunoreactivity for GRIP, RXRα and TSC2 in ULM. Among the 206 miRNAs, there was a total of 40 miRNAs showed moderate negative correlations (r = -0.4~-0.59) with dysregulated gene products. 22 miRNAs showed weak negative correlations (r = -0.2 ~ -0.39) with 10 gene products that contain predicted targeting sites at their 3' UTR. An unsupervised cluster analysis revealed a likely reversed expression pattern between miRNAs and the selected gene products.

Conclusions: This is the first attempt to examined both miRNAs and dysregulated gene products in a same set of human tumor samples. An inverse correlation of the protein levels with miRNAs can be appreciated. Our results indicate that miRNAs may play an important role in regulating some tumorigenic gene products in human ULM. As part of this study, we found that let-7 miRNAs negatively regulate their target gene HMGA2 *in vitro* (will be presented in a separate poster).

1001 "Macropapillary" Serous Carcinoma of the Ovary. A Distinctive Type of Low-Grade Serous Carcinoma

A Yemelyanova, TL Mao, N Nakayama, IM Shih, RJ Kurman. The Johns Hopkins Hospital, Baltimore, MD.

Background: Recent morphologic and molecular genetic studies have shown that noninvasive and invasive micropapillary serous carcinomas (MPSCs) are low-grade serous carcinomas of the ovary that arise from atypical proliferative (borderline) serous tumors (APSTs). All these tumors contain either mutant KRAS or BRAF in two thirds of cases. In the invasive neoplasms, invasion is characterized by small micropapillae that infiltrate the underlying tissue. Occasionally these tumors contain large "macropapillae" infiltrating the stroma, which have a haphazard distribution, but are lined by bland epithelium and therefore it is not clear whether they represent an invasive component. The current investigation was undertaken to determine whether the macropapillary pattern was another type of invasion in these tumors.

Design: Cases of low-grade serous carcinoma containing macropapillae were retrieved from the files of The Johns Hopkins Hospital and the epithelium lining macropapillae harvested using laser capture microdissection. The mutational status of KRAS, BRAF in the macropapillary, noninvasive and invasive MPSC components was analyzed by nucleotide sequencing.

Results: There were six cases of low grade serous carcinoma containing macropapillae. All tumors were bilateral, except one in which the status of the other ovary was unknown. The macropapillary component ranged from 30% to 100% of the neoplasms. The noninvasive component of the tumors was a MPSC in five cases and an APST in one case. In three cases typical invasive MPSC, without macopapillae, was metastatic to the peritoneum. In one case macropapillae admixed with invasive MPSC replaced multiple intraabdominal lymph nodes. In two cases there were no evidence of extraovarian involvement. In three cases, analyzed thus far, the identical (within each case) KRAS mutation (codon 12) was detected in the epithelium lining the macropapillae as well as in the APST, and noninvasive and invasive MPSC components of the tumors. No mutations of BRAF were found.

Conclusions: The presence of identical KRAS mutations in macropapillae and in the APST, and noninvasive and invasive MPSC components of these tumors indicates that they all share a common lineage. The finding of macropapillae within intraabdominal lymph nodes supports the interpretation that the macropapillary component is another manifestation of invasion in low-grade serous carcinomas. The recognition of this pattern is important, especially in cases when a tumor is composed entirely of macropapillae.

1002 Distinction of Primary and Metastatic/Secondary Mucinous Tumors Involving the Ovary: Performance of the Tumor Laterality and Size Algorithm in Diagnosis of 169 Rigorously Classified Tumors Categorized by Primary Site

A Yemelyanova, R Vang, K Judson, BM Ronnett. The Johns Hopkins Hospital, Baltimore, MD.

Background: Distinction of primary ovarian mucinous tumors from metastatic/secondary mucinous tumors involving the ovaries via intraoperative pathology consultation is required for proper surgical management. Previous studies have shown that an algorithm using tumor laterality and size can accurately distinguish a substantial majority of primary and metastatic tumors. Due to the occurrence of exceptions (large unilateral metastases), the performance of this algorithm in an expanded set of tumors subclassified by primary site was undertaken.

Design: Tumor laterality and size data (largest ovary for bilateral tumors) were analyzed for 169 mucinous tumors, rigorously classified as primary or metastatic/secondary based on clinicopathologic and immunohistochemical features. Per the algorithm: bilateral tumor, or unilateral tumor <10 cm = metastatic; unilateral tumor ≥10 cm = primary. Primary mucinous tumors included 29 atypical proliferative (borderline) tumors and 22 carcinomas.

Results:

Tumor type	N	Bilaterality	Mean/median size, cm (range)
Primary ovarian tumors	51	0	21.7/20.0 (10.0-36.0)
Metastases:	118	70%	8.9/9.0 (2.5-45.0)
Colorectum	35	54%	14.2/16.0 (2.5-45.0)
Appendix: low-grade adenomatous	16	88%	12.3/11.6 (3.0-25.0)
Appendix: carcinomas	17	88%	11.9/10.9 (3.5-24.0)
Pancreaticobiliary tract	19	95%	9.7/9.5 (2.5-21.0)
Stomach/small bowel	6	50%	9.9/9.0 (6.0-15.0)
Endocervix	6	66%	11.4/13.0 (3.0-20.0)
Unknown	19	63%	8.4/6.5 (2.5-19.0)

The algorithm correctly identified all primary ovarian tumors and 85% of metastatic/secondary tumors (colorectal: 66%; appendiceal: 88%/94%; pancreaticobiliary: 95%; gastric/small bowel: 83%; endocervical: 83%; unknown: 79%). Metastatic colorectal carcinomas provided the greatest number of exceptions due to greater frequencies of unilaterality and mean/median sizes >10 cm.

Conclusions: The algorithm has excellent diagnostic performance for distinction of primary and metastatic mucinous tumors at the time of intraoperative assessment. Recognition that metastatic colorectal carcinomas (the most common metastases) violate the algorithm more frequently than metastases from other sites should prompt lowering of the threshold for suggesting the possibility of metastatic carcinoma and for requesting evaluation of the bowel intraoperatively.

1003 Advanced Stage Mucinous Carcinomas of the Ovary Are Very Rare and Highly Lethal

RJ Zaino, M Brady, S Lele, H Michael. Hershey Med Ctr, Hershey, PA; Gynecologic Oncology Group, Buffalo, NY; U of Nebraska Med Ctr, Omaha, NE; Indiana Univ Med Ctr, Indianapolis, IN.

Background: Primary mucinous carcinomas of the ovary are uncommon, and their biologic behavior has been reported to be both better than and worse than that of serous carcinoma. During the past 10 years, several studies have shown that many mucinous carcinomas initially diagnosed as primary ovarian carcinoma were actually metastatic from another site.

Design: An international, phase 3 trial of about 4000 women with stage III or IV primary ovarian carcinoma with surgical staging followed by randomization to one of 5 chemotherapeutic arms was conducted between 2001 and 2004. Available H&E slides and pathology reports were reviewed (RJZ) from all US cases classified as primary mucinous carcinoma, with the following characteristics recorded: maximum diameter; bilaterality; hilar or surface involvement of ovary; nodular,expansile invasive or infiltrative growth patterns; small neoplastic glands; single neoplastic cells; signet ring cells; complex papillae; and necrotic luminal debris. Cases were re-classified as primary or metastatic to the ovary based on the proposed rule of Kurman et al, and with the use of the criteria of Lee and Young. Overall survival of groups were compared.

Results: Fifty four of 3698 cases were initially classified as mucinous, of which 10 were excluded from analysis (non-mucinous or missing slides) at review. Immunohistochemistry had been performed in only a few cases. Of the 44 cases, the median diameter was 16 cm, and most had bilateral disease, an infiltrative pattern, small neoplastic glands, and single neoplastic cells, although hilar involvement, luminal necrosis and nodular growth patterns were uncommon. Pseudomyxoma peritonei was present in 9 cases. At review, 17 cases (0.5%) were interpreted as primary mucinous carcinoma while 27 were judged as metastatic. The median survival did not differ significantly between these two groups, and was significantly less than that for women with serous carcinomas (14 vs 41 mo, $p<0.001$).

Conclusions: Advanced stage mucinous carcinoma of the ovary is actually very rare and highly lethal, with little response to conventional chemotherapeutic regimens. Alternative therapies with agents effective for other mucinous tumors should be pursued. Even with increased increased awareness by pathologists, mucinous carcinomas metastatic to the ovary continue to be overdiagnosed as primary ovarian neoplasms.

1004 WT1 Is a Useful Immunohistochemical Marker for the Diagnosis of Ovarian Pure Sertoli Cell Tumor and Facilitates Distinction from Other Non-Sex Cord-Stromal Tumors in the Differential Diagnosis

C Zhao, GL Bratthauer, R Barner, R Vang. Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, PA; Johns Hopkins Hospital, Baltimore, MD; Armed Forces Institute of Pathology, Washington, DC.

Background: WT1 has been shown to be expressed in various tumors, including some types of ovarian tumors. Of the latter, most studies have focused on surface epithelial-stromal tumors, such as serous carcinoma. However, expression in ovarian sex cord-stromal tumors, such as Sertoli cell tumor (SCT), and a potential application for distinction from non-sex cord-stromal tumors, have not received much attention.

Design: Immunohistochemical staining for WT1 (6F-H2, Cell Marque) was performed in 108 ovarian tumors: pure SCT, endometrioid borderline tumor (E-BT), sertoliform endometrioid carcinoma (SEC), FIGO grade 1 endometrioid carcinoma (E-CA), and carcinoid tumor (CT). Extent of staining was based on the percentage of positive cells: 0, <5%; 1+, 6-25%; 2+, 26-50%; 3+, 51-75%; and 4+ 76-100%. Intensity of staining was scored as weak (W), moderate (M), or strong (S).

Results: WT1 was expressed in the nucleus in 96% SCT, 16% E-BT, 25% SEC, 13% E-CA, and 0% CT. Table 1.

Table 1. Expression of WT1 in ovarian tumors

Tumor	0*	1+*	2+*	3+*	4+*
SCT (n=26)	4%	0%	0%	8% (0W,2M,0S)	88% (0W,2M,21S)
E-BT (n=25)	84%	0%	12% (0W,2M,1S)	4% (0W,1M,0S)	0%
SEC (n=12)	75%	8% ((0W,1M,0S)	8% (0W,1M,0S)	8% (0W,1M,0S)	0%
E-CA (n=23)	87%	0%	9% (0W,2M,0S)	0%	4% (0W,0M,1S)
CT (n=22)	100%	0%	0%	0%	0%

Key: *, results listed as percentage of cases showing each immunoscore for extent of staining (number of cases showing each grade of intensity in parentheses)

Conclusions: Ovarian pure SCT should be added to the growing list of WT1-positive tumors. WT1 is frequently and diffusely expressed in ovarian pure SCT. This marker is useful for distinction from other tumors with sertoliform architecture and can be included in an immunohistochemical panel for this differential diagnosis.

1005 Endometrial Serous Carcinoma with a Tubuloglandular Pattern: An Underrecognized Form of Aggressive Endometrial Cancer

W Zheng, M Kelly, X Yi, O Fadare, PE Schwartz, Z Jiang, SX Liang. University of Arizona, Tucson, AZ; Yale University, New Haven, CT; Fudan University, China; Wilford Hall Hospital, Lackland AFB, TX; UMASS, Worcester, MA; SUNY, Stonybrook, NY.

Background: Endometrial serous carcinoma (ESC) may contain tubuloglandular foci, and we have encountered cases composed entirely of them. These examples of ESC with a tubuloglandular pattern (ESC-TG) are an underrecognized form and are probably frequently misclassified as a high grade endometrial endometrioid carcinoma (EEC). Since ESC and EEC have significantly different clinicopathologic profiles, their distinction is of paramount importance. In this study, we compared clinicopathologic data between conventional papillary ESC (ESC-P) and ESC-TG.

Design: Slides from 106 cases of endometrial carcinoma with a serous component were reviewed and grouped into the following categories: Pure ESC-P (n=51), pure ESC-TG (n=12), mixed ESC-TG and EEC (n=7), and ESC-X (n=36). For the mixed ESC-TG, we required that there be more than 25% of the ESC-TG component, which characteristically exhibited a tubuloglandular growth pattern and notably high-grade nuclei. The ESC-X group contained cases with an ESC component admixed with either clear cell, sarcomatous, high-grade EEC or poorly/undifferentiated regions. Immunohistochemistry for p53, estrogen (ER), and progesterone (PR) receptors, and IMP3 was performed. Survival and recurrence rates among the groups were analyzed.
Results: The clinicopathologic features of the ESC-TG group resembled those of ESC-P and ESC-X groups. There was no significant difference in the survival and recurrence rates among the 3 groups. The immunoprofile of the 3 groups are summarized below. Notably, IMP3 was markedly positive in the ESC-TG and ESC-P group and the serous component of the ESC-X group, suggesting that IMP3 may be a marker for serous carcinomas. The combination of positive IMP3 and p53 expression and lack of ER/PR expression best classified ESC-TG as a morphologic variant of ESC-P.
Conclusions: Our data support the concept that there is a group of endometrial cancers composed of tubular glands that are indeed serous carcinomas. ESC-TG display a low-grade, glandular architecture but are characterized by notably high-grade nuclei. ESC-TG and ESC-P have essentially identical clinicopathologic profiles.

	IMP3 (%)	p53 (%)	ER (%)	PR (%)	p value
ESC-TG	90	95	10	5	
ESC-P	92	94	10	8	0.8
ESC-X	77	81	14		0.7

1006 The RNA-Binding Protein IMP3: A Novel Cytoplasmic Marker for Endometrial Serous Carcinoma

W Zheng, X Yi, S Liang, M Kelly, P Schwartz, Z Jiang. University of Arizona, Tucson; Fudan University, Shanghai, China; State Univeristy of New York at Stony Brook; Yale University; University of Massachusetts Medical Center.
Background: IGF-II mRNA-binding protein 3 (IMP3) is a novel oncofetal RNA-binding protein highly expressed in renal and pancreatic carcinomas. Recently, Zhong *et al* found that the protein expression predicts the metastasis and poor survival in renal cell carcinomas. The aim of this study is to determine if the expression of IMP3 has any diagnostic usage for endometrial carcinomas.
Design: IMP3 expression by immunohistochemistry (IHC) was examined in 298 endometrial samples. These included benign endometrium (n=68), atypical hyperplasia (AH)/endometrial intraepithelial neoplasia (EIN) (n=35), endometrial glandular dysplasia (EmGD) (n=21), endometrial intraepithelial carcinomas (EIC) (n=18), endometrioid carcinomas (EEC) (n=70), mucinous carcinomas (MC) (n=8), serous carcinomas (ESC) (n=51), clear cell carcinomas (CCC) (n=12), and other malignancies (n=15). The benign endometrium included 12 atrophic, 18 proliferative, 14 secretary, 8 menstrual, and 16 gestational enometrium. The carcinomas were either pure form or mixed type with < 10% of a second component . A renal cell carcinoma with known IMP3 expression was used as a positive control. The criteria for positivity was cytoplasmic stains with moderate intensity in at least 50% of target cells.
Results: Among malignant cases, IMP3 expression was mainly found in ESC and its putative precursor lesions: EmGD- 3/21 (14%), EIC-16/18 (89%), and ESC-48/51 (94%) ($p < 0.001$). In contrast, IMP3 expression was significantly lower in non-serous malignancies with 0%, 7%, 0%, 25%, and 33% positive stains in AH/EIN, EEC, MC, CCC, and other malignancies, respectively. IMP3 staining was typically diffuse and strong in ESC, while patchy and moderate in non-serous malignancies. Among benign endometrium, only decidualized stroma (100%) was positive. Trophoblasts in the first trimester chorionic villi were also diffusely positive, which was consistent with previous findings.
Conclusions: We conclude that IMP3 is a new sensitive and specific marker for ESC including serous EIC. Therefore, immunostains with IMP3 antibodies may be of a diagnostic utility in evaluation of endometrial cancinomas, particularly when biopsy material is limited and a concern of ESC arises. Although the significance of IMP3 expression in decidualized endometrial stroma remains unclear, differential diagnosis between decidual changes and ESC is barely problematic.

Head & Neck

1007 Non-Keratinizing Carcinoma of the Sinonasal Tract Is a Clinicopathological and Molecular Entity Different from Keratinizing Squamous Cell Carcinoma

L Alos, M Santos, J Ordi, A Nadal, JL Blanch, I Alobid, B Lloveras, A Cardesa. Hospital Clinic, Barcelona, Spain; Hospital Princeps d'Espanya, Bellvitge, Barcelona, Spain.
Background: Sinonasal Non-keratinizing Carcinoma (NKC) of the sinonasal tract has been referred in the past by several terminologies as Schneiderian Carcinoma, Transitional Cell Carcinoma and Cylindrical Cell Carcinoma. The last WHO classification of Tumors of the Head and Neck, includes NKC as a variant of Squamous Cell Carcinoma. The aim of this study is to characterize and compare the clinicopathological features, HPV DNA prevalence and immunophenotypes of NKC and usual Keratinizing Squamous Cell Carcinoma (KSCC).
Design: Sixty-five Squamous Cell Carcinomas of the sinonasal tract were reviewed and the medical records were obtained from patients' files. Thirty-six tumors were selected to perform immunohistochemical studies for p16INK4 (Biocare Medical, Walnut Creek, CA. Clone JC8, dilution 1:100); Ki-67 (Immunotech, Marseille, France. Clone MIB-1, dilution 1:200); p53 (Novocastra Laboratories, Newcastle, UK. Clone BP53-12, dilution 1:50). PCR studies for detecting HPV DNA using both SPF10 and GP5+/6+ techniques were also applied in the same cases.
Results: Forty-eight tumors were KSCC (74%); and 17, NKC (26%). Both tumor types affected predominantly males, in a 3:1 ratio, in the seventh decade of life. NKC had a higher predilection affecting naso-ethmoid structures instead of maxillary sinuses, whereas KSCC ocurred in both sites equally. HPV16 was detected in 8/13 NKC (61%) and 4/23 KSCC (15%). p16 was positive in 8/13 NKC (61%) and 2 KSCC (8%). A high Ki67 positivity (>30%) was observed in 10/13 NKC (67%) and 8/23 KSCC (35%), whereas p53 was highly positive (>30%) in 7/13 NKC (54%) and 16/23 KSCC (70%). KSCC had higher recurrence and metastatic rates (82 and 20% of the cases respectively) than NKC (50 and 7% of the cases, respectively). The overall survival was shorter for KSCC than NKC ($p<0.0012$).
Conclusions: NKC is a distinctive neoplasm of the sinonasal tract with high prevalence of HPV infection. It has a characteristic immunophenotype, frequently expressing p16 and high Ki67 positivity. This tumor type has less tendency to recur and metastasize, and better prognosis than KSCC.

1008 Detection of Human Papillomavirus-16 in Fine Needle Aspirates: A Strategy for Localizing Site of Tumor Origin in Patients Presenting with Metastatic Squamous Cell Carcinoma

S Begum, WH Westra. The Johns Hopkins Medical Institutions, Baltimore, MD.
Background: Patients with head and neck squamous cell carcinoma (HNSC) often clinically present with metastases to regional lymph nodes. Fine needle aspiration (FNA) of neck masses is routinely used to establish the presence of metastatic carcinoma and, in turn, to initiate a subsequent workup to determine the site of tumor origin. Human papillomavirus 16 (HPV16) is an important etiologic agent for HNSCs that arise from the oropharynx but less so for tumors from non-oropharyngeal sites. HPV16 detection thus provides a strategy for localizing an important subset of HNSCs, but this approach has not been applied to FNA specimens.
Design: We performed in-situ hybridization for HPV16 on 77 consecutive aspirated neck masses diagnosed as metastatic squamous cell carcinoma. P16 immunohistochemistry was also performed because p16 overexpression may serve as a surrogate marker of HPV-associated HNSC.
Results: HPV16 was detected in 13 (17%) of the 77 aspirates. By site of origin, HPV16 was detected in 10 of 19 metastases from the oropharynx, but in none of 46 metastases from other sites (53% vs. 0%, $p < 0.0001$). HPV16 was not detected in 2 branchial cleft cysts misdiagnosed as metastatic squamous cell carcinoma, but it was detected in 3 of 10 metastases from occult primary tumors. P16 expression was associated with the presence of HPV16: 12 of 13 HPV16 positive metastases exhibited p16 expression, whereas only 4 of 62 HPV16 negative metastases were p16 positive (92% vs. 6%, $p < 0.0001$). P16 expression also correlated with site of tumor origin: 13 of 19 oropharyngeal metastases were p16 positive whereas only 1 of 46 non-oropharyngeal metastases was p16 positive (68% vs. 2%, $p<0.0001$).
Conclusions: HPV16 status can be determined in tumor cells aspirated from the necks of patients with metastatic HNSC. Its presence is a reliable indicator of origin from the oropharynx.

1009 Immunohistochemistry of Molecular Markers in Sinonasal Undifferentiated Carcinomas

AM Bellizzi, TD Bourne, SE Mills, EB Stelow. University of Virginia, Charlottesville, VA.
Background: Sinonasal undifferentiated carcinoma (SNUC) is an uncommon and highly aggressive neoplasm of the nasal cavity and paranasal sinuses. Treatment may consist of craniofacial resection and adjuvant chemoradiation. The goal of this study is to expand our knowledge of the immunophenotype of this lesion, focusing on markers potentially mechanistic in the disease process or of possible therapeutic importance.
Design: Our surgical pathology files were searched for cases diagnosed as SNUC. Poorly differentiated carcinomas (PDCs) of the sinonasal cavity and SNUC-like lesions of the larynx served as controls. Immunohistochemistry (IHC) was performed for the following markers: MLH-1, MSH-2, β-catenin, p16, p53, CD117, and EGFR. The immunohistochemical stains for MLH-1 and MSH-2 were scored as intact or lost. The other stains were scored semiquantitatively based on the percentage of tumor cells staining: <5%=0, 5-25%=1+, 26-50%=2+, 51-75%=3+, >75%=4+.
Results: Eleven SNUCs with residual material available for IHC were identified, as were 4 poorly differentiated carcinomas of the sinonasal tract and 2 SNUC-like lesions of the larynx. MLH-1 and MSH-2 were intact in all SNUCs in which concomitant evaluation was possible (10/10). Nuclear staining for β-catenin was not identified in any case. Nuclear and cytoplasmic staining for p16 was observed in 7 of 11 SNUCs (1+ in 5, 2+ in 2). p53 staining was observed in 10 of 11 (1+ in 5, 2+ in 3, 3+ in 1, and 4+ in 1). Four of 11 cases stained for CD117 (2+ in 1, 3+ in 3). SNUCs (and control cases) were uniformly negative for EGFR. MSH-2 was lost in 1 sinonasal PDC; MLH-1 and MSH-2 were intact in the remaining control cases. Control cases were negative for β-catenin nuclear positivity. Four of 4 and 0 of 2 sinonasal PDCs and SNUC-like laryngeal tumors were positive for p16. All control cases marked for p53. CD117 positivity was found in 2 of 4 and 1 of 2 of sinonasal PDCs and SNUC-like laryngeal tumors, respectively.
Conclusions: Aside from p53 immunoreactivity, the most common molecular abnormality idnetified in our SNUC cases was over-expression of p16. Although this finding is non-specific, it suggests that HPV may play a role in the development of at least some SNUCs. The idenitifcation of CD117 immunoreactivity, although potentially pointing to a treatment option, may instead suggest that some of these lesions are poorly differentiated neoplasms of the seromucinous glands. Future directions of study may include more in depth assessments of the role of HPV or CD117 with these lesions.