and evaluation of flawed specimens. The tools may be implemented through off-site preliminary assessment or in depth testing using on-site observations of skills and cognitive processes. Depending on the type of assessment, the tools contain between 10 and 50 cases representing cases of differing complexity, quality, and difficulty. We developed the modules by selecting exemplar cases from a pool of over 3,000 cases and validating expertise using blinded concordance of two internationally expert cytopathologists and clinical-histopathologic follow-up. We established the baseline performance of 10 pathologists (3 practicing pathologists and 7 trainees) on expert-based modules and performed root cause analysis to determine error source and areas of improvement for each pathologist. Performance modules for expert, competent, and novice practices were developed.

Results: In using expert-based performance modules, baseline scores (20%-70%) correlated with level of training. Practicing board-certified cytopathologists showed baseline levels of competency (50%), and no pathologist scored as an expert (>90%). Root cause analysis showed that diagnostic errors for practicing pathologists were more common in poor quality specimens and for specific lesion types (e.g., lobular carcinoma). Specific areas of weakness were identified for specific practicing pathologists (e.g., tubular carcinoma or fat necrosis). Pathology resident weaknesses correlated with year of training and generally were related to cognitive failures in synthesis rather than assessment of criteria.

Conclusions: We conclude that our competency assessment program for breast cytology is able to stratify pathologists and trainees by level of performance. We are able to identify errors and their causes for improvement purposes.

554 Systematic Prediagnostic Review of Endoscopy Reports Significantly Enhances Resident Performance during Slide Preview.

LYA Watts, M Alexander, H Wu, S Hammond, K Downey, Y Toribio, M Roehrl. Boston Medical Center, MA.

Background: Gastric biopsy specimens are frequently diagnostically challenging, particularly for pathologists-in-training. However, an advantage of GI pathology is the routine availability of a detailed gross impression provided by the concomitant endoscopy report. We hypothesized that systematic combined review of the endoscopy report together with the glass slide will improve diagnostic accuracy of residents.

Design: Classic biopsy cases of gastric pathology were selected, including normal, gastritis, polyps, and neoplasia. 50% of our resident population diagnosed this study set, once without any knowledge of the endoscopic findings and then again at least 2 weeks later together with the full text of the endoscopic reports. The accuracy of each resident's diagnoses was compared with the staff diagnosis and scored as acceptable, partially acceptable or unacceptable. The unacceptable category was in particular used when either the biopsy was completely negative and the resident had assigned any pathology or the biopsy showed pathology but was called within normal limits by the resident.

Results: Overall diagnostic accuracy was excellent with 80-85% of diagnoses falling into acceptable or partially acceptable categories, whether or not an endoscopy report was available. Importantly however, when considering the critical distinction between resident diagnoses that were unacceptable after preview and those that were at least partially acceptable, 60% of residents showed improvement after having read the endoscopy report and thus did not make a critical mistake. Resident performance improved most significantly with cases that exhibited subtle pathologic changes that would have been missed, but were diagnosed correctly when an endoscopy report describing a mild abnormality was available. >90% of residents enjoyed the preview experience and felt more confident when an endoscopy report was available compared to a glass slide with limited or no clinical information.

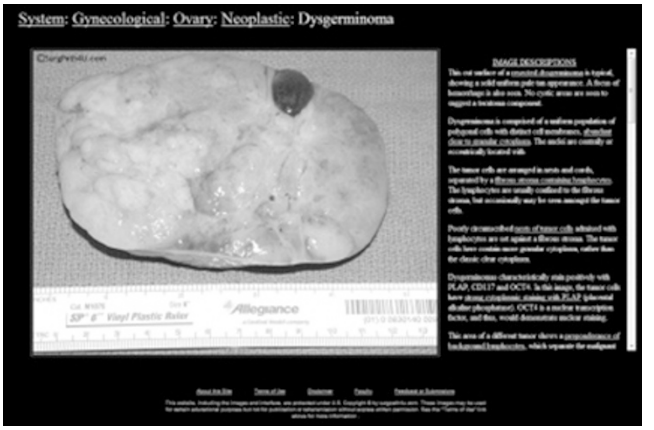
Conclusions: GI pathology demands that the pathologist be able to incorporate all endoscopic and other clinical findings related to the patient's biopsy to render optimal diagnoses. Routine incorporation of endoscopy reports into pathology resident education and independent slide preview enhanced diagnostic accuracy and boosted individual confidence for making diagnoses. Our results support the notion that residency education will be improved by routinely making endoscopy reports available during preview of GI biopsies in addition to the standard limited information provided on the requisition sheets.

555 Development of a Comprehensive Surgical Pathology Website for Teaching and Self-Assessment at the Resident and Clinical Practice Level.

C Wu, D Rogers, G Olson, L Cerilli. Univ of New Mexico, Albuquerque; Naval Hospital Pensacola, Pensacola, FL.

Background: Mastery of surgical pathology is daunting for pathology trainees and clinicians in other specialties. Traditional learning methods rely on cumbersome and expensive textbooks as well as glass slides with faculty teaching, which is not always available. Review of the currently available on-line pathology resources revealed no comprehensive and well designed site tailored to the resident level and above. We built an on-line learning tool at SurgPath4U.com to showcase pathology images and information in an intuitive format. We hypothesized that learning pathology using this website provides more efficient learning compared to currently available on-line sources and textbooks.

Design: 14 Ob/Gyn residents at UNM were randomly divided into two groups (traditional learning versus our website) within their year of training. All were given a 32 question pre-test followed by a list of study topics. The traditional group then studied using textbooks and the internet, without access to SurgPath4U.com. Our website group exclusively used SurgPath4U.com to view cases.



A post-test of identical questions to the pre-test was given after two hours. To eliminate bias, all test questions and images were generated by a pathologist not familiar with the content of SurgPath4U.com.

Results: The overall pre-test score 36.4%, and post-test score was 50.9%. When separated by the learning method, the traditional group scored 37.5% in the pre-test and 45.1% in the post-test, with an improvement of 7.6%. The website group scored 35.4% in the pre-test and 56.7% in the post-test, with an improvement of 21.3%. The increase in post-test scores in the website learning group was significant (p=0.027).

Conclusions: To facilitate pathology education in all disciplines of medicine, we developed SurgPath4U.com, an user-friendly pathology website. Analysis of pre- and post-test of Ob/Gyn residents comparing traditional learning methods versus web-based learning demonstrated that residents using SurgPath4U.com learned more effectively. We conclude that SurgPath4U.com is a dynamic and innovative way to learn pathology.

Endocrine

556 Biomarker Expression in Pancreatic Endocrine Tumors.

LJ Adhikari, JA Gilbert, RV Lloyd, MM Ames. Mayo Clinic, Rochester, MN; University of Wisconsin School of Medicine, Madison.

Background: Pancreatic endocrine tumors (PET) represent 1-2% of all pancreatic neoplasms. Complete surgical resection is the most successful treatment for patients with PETs; however, some patients present with advanced disease for which few treatment options exist. Somatostatin therapy can ameliorate clinical symptoms and perhaps induce tumor growth stabilization, but PETs have poor or short-lived rates of response to conventional chemotherapeutic agents [Gastroenterology 2008, 135:1469] and novel therapeutic strategies are needed.

Design: We assessed 67 PETs from 44 patients for immunohistochemical expression of biomarkers targeted by novel therapeutic drugs currently under development in other forms of cancer. A tissue microarray of 67 formalin-fixed, paraffin-embedded tissues with each tissue represented as a triplicate of 0.6mm cores were evaluated. Primary neoplasms from 41 of these patients were included in the data. The markers include insulin-like growth factor 1 receptor (IGF1R), transforming growth factor-ß receptor 1 (TGFBR1), heat shock protein 90 (Hsp90), somatostatin receptor subtypes 2A and 5 (SSTR2A and SSTR5), platelet-derived growth factor alpha (PDGFA), *O*6-methylguanine DNA methyltransferase (MGMT), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor 1 (VEGFR1), and mammalian target of rapamycin (mTOR).

Results: All of the assessable PETs stained positively for IGF1R, TGFBR1, Hsp90, SSTR5, SSTR2A, and PDGFRA, with 98% positivity with EGFR, VEGFR1, and mTOR. 24% of the PETs were negative for MGMT (predictive of a favorable response to temozolomide therapy [Clin Cancer Res 2009:15:338]), and 52% were weakly staining for MGMT. Proteins for which the largest number of PETs exhibited the strongest staining level (score of 3) were VEGFR1 (80% of PETS), and TGFBR1 (69%), PDGFRA (65%), SSTR2A (55%), SSTR5 (55%), and IGF1R (47%).

Conclusions: High immunohistochemical expression of VEGFR1, TGFBR1, PDGFRA, and IGFR1 is encouraging of additional research into the role played by these proteins in PET growth. Lack of MGMT immunohistochemical expression in some PETs suggests that temozolomide might be a useful therapeutic agent.

557 Immunohistochemical Staining of Thyroidectomy Specimens for PTEN Can Aid in the Identification of Patients with Cowden Syndrome.

JA Barletta, AM Bellizzi, JL Hornick. Brigham and Women's Hospital, Boston, MA.

Background: Cowden syndrome (CS) is an autosomal dominant disorder with germline mutation of *PTEN* characterized by the development of multiple hamartomas and carcinomas of the thyroid, breast and uterus. Recognition of CS is important so that cancer screening and genetic counseling can be initiated. Pathologic findings in thyroidectomy specimens suggestive but not specific for CS include multiple adenomatous nodules and follicular adenomas, with or without follicular carcinoma or papillary thyroid carcinoma. The aim of our study was to determine if immunohistochemical staining for PTEN could aid in the identification of CS in patients with these pathologic findings.

Design: We studied 24 thyroidectomy specimens from patients with a known history of CS or with pathologic findings that raised the possibility of CS. Immunohistochemistry for PTEN was performed on all cases (rabbit monoclonal antibody, clone 138G6,

Cell Signaling, 1:100 dilution). Assessment of PTEN expression was performed by a pathologist blinded to the clinical history and recorded as follows: intact expression in all lesional nodules, complete loss of expression in all lesional nodules, or focal loss of expression, with some nodules showing intact expression and others showing complete loss of expression.

Results: Ten cases showed loss of PTEN expression (five complete and five focal) and 14 cases showed intact expression. Of the 10 cases that showed loss of PTEN expression, seven patients were clinically confirmed to have CS (five showed complete loss of staining and two showed focal loss). Two additional patients had clinical findings strongly suggestive of CS, although *PTEN* mutational testing was not performed. One case had a clinical history that was not suggestive of CS and no further testing was pursued. Of the 14 cases that showed intact PTEN expression, none had CS by clinical history or genotyping. Two patients had *PTEN* testing that was negative, one had *PTEN* testing with equivocal results, nine had clinical examinations that were not suggestive of CS and no further testing was pursued, and two patients had no follow-up information available.

Conclusions: Immunohistochemical staining of thyroidectomy specimens for PTEN can aid in the identification of patients with CS. Loss of PTEN expression, either complete or focal, appears to be both sensitive and specific for CS, although the molecular mechanism underlying heterogeneous loss of PTEN expression is unknown. These findings warrant further evaluation of PTEN staining in thyroids from a larger cohort of patients with and without CS.

558 Utility of PAX8 in the Diagnosis of Anaplastic Thyroid Carcinoma.

JA Bishop, WH Westra. The Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Anaplastic thyroid carcinoma (ATC) can be difficult to diagnose as these tumors do not show evidence of thyroid differentiation morphologically or immunohistochemically. Depending on the histologic variant, ATC may be confused with high grade sarcoma or poorly differentiated squamous cell carcinoma. Paired box gene 8 (PAX8) is a transcription factor expressed in the nuclei of thyroid follicular epithelium. Expression of PAX8 is retained in thyroid cancers including some ATCs. Moreover, the restricted expression of PAX8 to the thyroid and only a few tissues outside of the thyroid (e.g. kidney and female genital tract) suggests that PAX8 staining could be diagnostically useful when dealing with sarcomatoid or squamoid tumors of the neck. The purpose of this study was to determine the frequency of PAX8 staining in ATC, and to evaluate PAX8 immunohistochemistry as a means of distinguishing the squamoid variant of ATC from squamous cell carcinoma of the head neck (HNSCC).

Design: PAX8 immunohistochemistry was performed on 26 ATCs and 101 HNSCCs. Staining results of the ATCs were correlated with histologic features and status of the BRAF gene.

Results: The dominant histologic patterns of the ATCs were squamoid (n=15), giant cell/pleomorphic (n=6) and spindled (n=5). Overall, PAX8 staining was present in 21 (81%) of the ATCs. By histologic variant, PAX8 was positive in 15 of 15 (100%) squamoid ATCs, 3 of 6 (50%) giant cell/pleomorphic ATCs, and 3 of 5 (60%) spindled ATCs. ATCs arising in association with papillary carcinomas were not more likely to express PAX8 than those arising in association with follicular carcinomas (88% vs 100%, p = 1). There was no correlation between PAX8 staining and activating mutations of the BRAF gene. All 101 HNSCCs were PAX8-negative.

Conclusions: PAX8 expression is retained in the majority of ATCs including the squamoid variant, but PAX8 is not expressed in HNSCC of mucosal origin. In effect, PAX8 staining is an excellent marker for carcinomas of follicular epithelial origin, including those carcinomas that are undifferentiated in most other respects. The tissue specificity of PAX8 expression may be useful in resolving the differential diagnosis of ATC such as in the distinction between the squamoid variant of ATC and HNSCC.

559 SDHB: A Marker of Malignancy in Familial and Sporadic Pheochromocytomas/Sympathethic Paragangliomas.

A Blank, AM Schmitt, E Korpershoek, FH van Nederveen, T Rudolph, N Weber, R Strebel, R de Krijger, P Komminoth, A Perren. Klinikum Rechts der Isar, Technische Universitaet Muenchen, Munich, Germany; Josephine Nefkens Institute, Erasmus MC, University Medical Center, Rotterdam, Netherlands; University Hospital Zurich, Switzerland; University of Bern, Switzerland; City Hospital Triemli, Zurich, Switzerland.

Background: To date there is no reliable histopathological marker of malignancy for pheochromocytomas/sympathetic paragangliomas (PCC/PGL). It is well known that PCC/PGL in the hereditary context of an *SDHB* germline mutation very often metastasize. The immunohistochemical loss of SDHB expression was recently shown to be a surrogate marker for the presence of an *SDH* germline mutation in PCC/PGL. SDHB loss is supposed to be tumorigenic via activation of hypoxia signals. Our aim was to clarify: 1. the potential use of SDHB immunohistochemistry as a marker of malignancy in PCC/PGL; and, 2. its association with classic hypoxia signalling.

Design: The protein expression of SDHB, Hif-1α and its targets CA-9 and GLUT-1 was examined by immunohistochemistry using a tissue micro array containing a series of familial and sporadic tumors of 115 patients. The obtained results were correlated with survival data which were available for 66 patients.

Results: In the tumor tissue of 12 of 99 patients a loss of SDHB protein expression was observed. In 5 of these 12 patients an *SDHB* germline mutation was present while in 4 patients no germline mutation was detected. In 3 patients mutational status remained unknown in parts. There was no association between loss of SDHB expression and increased classic hypoxia signalling as detected by HIF-1α, CA-9 or GLUT-1 immunohistochemistry. Loss of SDHB expression correlated significantly with a shortened disease specific survival.

Conclusions: Our findings do not support the current hypoxia hypothesis in malignant PCC/PGL. We propose SDHB protein loss as a marker of malignancy both in sporadic and in familial PCC/PGL.

560 Differential Expression of FGFRs Correlates with Outcomes in Pheochromocytomas and Paragangliomas.

CA Cassol, SL Asa. University Health Network, Toronto, ON, Canada.

Background: FGFRs are receptor tyrosine kinases (RTK) that have been implicated in various malignancies. A recent report from our institution of successful treatment of malignant pheochromocytoma (PCT)/paraganglioma (PGL) with a multitargeted RTK inhibitor (RTKi) provided clinical evidence for involvement of RTKs in the pathogenesis of these tumors. To date, a single study documents overexpression of FGFR1 and 2 in paragangliomas. We analyzed expression of several FGFRs in both tumor types.

Design: We identified 153 PCT/PGL from our institutional files of 2001-2009. Of those, 132 tumors from 115 patients provided enough tissue for Tissue Microarray (TMA) construction. Slides were stained with anti-FGFR2 (SC-122), -3 (SC-123) and -4 (SC-124) antibodies (Santa Cruz), scanned with ScanScope (Aperio) and analyzed using Spectrum Color Deconvolution Algorithm (Aperio). Parameters used for exploratory statistical analysis on SPSS 11.0 were percent total positive (PTP), a weighted score calculated by the program [1*(%Weak) + 2*(%Medium) + 3*(%Strong)], relevant clinical data and 3 outcomes (local recurrence, metastasis, death from disease).

Results: Of 115 patients, 71 were female and 39 were male. Mean age at diagnosis was 46.8 years (±12.3). 18.3 % had bilateral/multiple tumors and 7.8% had a family history of PCT/PGL. 7 PGL patients had metastases, 4 had local recurrences, and 2 died of disease. Of 132 tumors, 43 were PCTs, 82 PGLs and 7 PGL metastases. FGFR2 and 4 were overexpressed ($p<0.01$) and FGFR3 was underexpressed ($p<0.05$) in tumors compared to normal medulla. The presence of bilateral/multiple tumors was associated with local recurrence (p=0.021) and the 3 outcomes combined (p=0.055). An inverse correlation between tumor size and FGFR2 score (p=0.002,R=-.276) and PTP (p=0.002,R=-.275) was observed. Increased risk of metastasis was associated with increased tumor FGFR4 score (OR=1.17;95%CI=1.05-1.30;p=0.003) and PTP (OR=1.18;95%CI=1.06-1.31;p=0.003). Conversely, decreased risk of the 3 negative outcomes combined was associated with increased tumor FGFR3 score and PTP (both OR=0.96;95%CI=0.93-0.99;p=0.01).

Conclusions: FGFR2 and 4 are overexpressed in PCT/PGL, while FGFR3 is underexpressed. FGFR2 inversely correlates with tumor size, consistent with a proposed tumor suppressor role for this receptor. FGFR4 overexpression was associated with increased risk of metastases, providing evidence for a role for FGFR4 involvement in the progression of PCT/PGL. Whether a differential expression of these markers could be predictive of RTKi sensitivity in these tumors warrants futher studies.

561 Analysis of the FGFR4 Single Nucleotide Polymorphism in Pheochromocytomas and Paragangliomas.

CA Cassol, SL Asa. University Health Network, Toronto, ON, Canada.

Background: FGFR4 harbors a common SNP at codon 388, which results in Gly to Arg substitution at the transmembrane domain of the protein in aproximately 50% of the population. Recent studies have associated the FGFR4 Arg^{388} allele with tumor progression and bad prognosis in several malignancies. To date, no studies have examined the impact of this SNP in pheochromocytomas (PCT) and paragangliomas (PGL).

Design: We collected tissue for DNA extraction and tissue microarray (TMA) construction from 115 PCT/PGL patients identified in our institutional files of 2001-2009. Exon 9 of *fgfr4* was PCR amplified and RFLP digested with *Bst*N1 to distinguish 3 FGFR4 genotypes: wild type (Gly/Gly), heterozigous Gly^{388} (Gly/Arg) and homozygous Arg^{388} (Arg/Arg). TMA slides were stained with anti-FGFR4 antibody (SC-124) (Santa Cruz Biotech), scanned with ScanScope (Aperio) and analyzed using Spectrum Plus Color Deconvolution Algorithm (Aperio). Relevant clinical data and patient outcomes were recorded. Logistic regression was performed using SPSS 11.0 with statistical significance at $p<0.05$.

Results: We successfully genotyped 105 patients; 39 had PCT and 66 had PGL. Of these, 65 were female and 35 were male. Mean age at diagnosis was 46.7 years (±12.5). 17.1% of patients had bilateral or multiple tumors and 8.6% had a family history of PCT/PGL. Five patients had metastatic disease, 3 experienced local recurrence, and two died from metastatic disease, all with PGL. Two additional patients died of unrelated causes. FGFR4 genotyoping revealed 38 patients (36.2%) were Gly/Gly, 66 (62.9%) Gly/Arg and only one (1%) Arg/Arg. There was no significant difference in the distribution of these alleles between PCTs and PGLs. No correlation was found between FGFR4 genotype and FGFR4 expression, nor with individual or combined negative outcomes, age, gender, bilateral/multiple tumors, and family history.

Conclusions: The distribution of FGFR4 alleles in our pheochromocytoma/paraganglioma cohort differs from that of the normal population suggesting a possible predisposition of FGFR4R388 to this disorder. However, no significant correlation was found between genotype and clinical characteristics or outcomes. This may be due to the small number of patients in our study, particularly the low frequency of negative outcomes in our cohort, which hinders the strength of statistical correlations. Since these are relatively indolent neoplasms, a longer follow-up period might be necessary to uncover possible prognostic correlations with FGFR4 alleles.

562 Potential Role of MAPK Inhibitor in the Benzo(a)pyrene-Induced Anti-Apoptosis Against Cisplatin Treatment and Tumor Tumor Invasion of Papillary Thyroid Carcinoma Cells.

YS Chae, K-S Ahn, H Kim, NH Won. Korea University College of Medicine, Seoul, Korea.

Background: Constitutive activation of the MAPK pathway through genetic alterations, including RAS and B-type RAF (BRAF) mutations, is common in human cancers and is associated with cell malignant transformation and aggressiveness, implicating that targeted inhibition of the MAPK pathway may potentially be an effective therapy for human cancers.

Design: To investigate the role of B(a)P in the progression of papillary thyroid carcinoma, 8 primary cultured PTC cells were obtained from PTC patients and then examined the effects of B(a)P on activation of MAPK signaling using Western blot. B(a)P induced tumor invasion was examined using *in vitro* tumor invasion chamber and MMPs mRNA of invaded cells was then examined using real-time PCR.

Results: Blockage of MAPK cell signaling successfully not only inhibits cisplatin-induced apoptosis but also partially suppresses ability of PTC cell invasion. And induction of phospho-JNK and phospho-ERK-1/2 were noted when primary PTC cells were treated with 1 nM B(a)P. In in vitro tumor invasion assay, B(a)P-induced PTC cell invasion was reduced by either PD98059 or JNK II inhibitor. PD98059 effectively inhibited B(a)P-induced tumor invasion when compared to control. The transcription luciferase reporter system demonstrated that PD98059 effectively reduced AP-1 activity when compared to control. The levels of MMP-2 and MMP-9 mRNA expression were reduced slightly by treatment with MEK inhibitor.

Conclusions: We conclude a significant association of PTC cell invasion when PTC cells continuously was exposed to B(a)P. Our findings suggest that MEK inhibitor may function as a small molecule inhibitor of tumor invasion and may provide novel mechanistic insights into the potential therapy for human PTC.

563 AQP4 and Histone H1.5 Immunostaining Can Distinguish Benign Oncocytic Follicular Lesions from Oncocytic Follicular Carcinoma.

J Chepovetsky, DE Burstein, E Genden, M Rivera. Mount Sinai Medical Center, NYC, NY.

Background: The accurate pre-operative diagnosis of follicular patterned lesions of the thyroid on fine needle aspiration specimens remains elusive. Frequently, it is difficult to distinguish oncocytic follicular neoplasms from cellular hyperplastic oncocytic nodules or foci of nodular oncocytic metaplasia in lymphocytic thyroiditis. Aquaporin 4 (AQP4) is an integral membrane protein that conducts water through the cell membrane. It is expressed in cells of the kidney, CNS and thyroid. Histone H1 (H1.5) is a member of the histone family and functions as a "linker" molecule, playing a role in organizing nucleosomes into higher-order structures. In this study, we evaluate the differences in AQP4 and H1.5 expression in various encapsulated follicular neoplasms and non-neoplastic lesions of the thyroid in surgical pathology specimens.

Design: 85 cases from 2002 to 2009 were selected that were previously diagnosed as Oncocytic follicular adenoma (OA, n=11), Oncocytic follicular carcinoma (OC, n=15), nodular hyperplasia (NH, n=14), and lymphocytic thyroiditis (LT, n=11). All cases were reviewed to confirm the original diagnosis. Immunohistochemical analysis with rabbit produced antibodies to AQP4 (concentration: 0.53 mg/ml, dilution: 1:3000) and Histone H1.5 (concentration 0.5 µg/ml, dilution 1:800) was performed on a representative section from each case. Staining for AQP4 was considered positive if >30% of the cells were highlighted in a membranous pattern. A positive cut-off of >20% of cells showing distinct nuclear staining was set for H1.5.

Results: Normal thyroid is characteristically AQP-positive and H1.5 negative. All cases of NH and 82% (9/11) of LT cases were positive for AQP4. In cases of NH with extensive oncocytic metaplasia staining for AQP4 was particularly intense. H1.5 was negative in all cases of NH and LT. 100% of OA stained positive for AQP4 while only 9% (1/11) were positive for H1.5.. In contrast, 73 % (11/15) of OC were AQP-negative, while 53% (8/15) of HC were positive for H1.5

Conclusions: The combination of AQP4 and H1.5 immunostaining can distinguish many benign oncocytic follicular lesions from oncocytic follicular carcinoma. This combination of markers may play a role in the pre-operative assessment of oncocytic follicular lesions.

564 Characterization of "Dysplastic" Foci in Chronic Lymphocytic Thyroiditis.

MH Chui, O Mete, C Cassol, SL Asa. University Health Network, Toronto, ON, Canada.

Background: Epidemiological studies have shown an increased risk of papillary thyroid carcinoma (PTC) in patients with chronic lymphocytic thyroiditis (CLT). The follicular epithelium in CLT is often atypical, with nuclear features resembling PTC. There is considerable debate as to whether these cells are pre-malignant, and previous studies have been equivocal. In this study, we examined the expression of immunomarkers in reactive, hyperplastic, and malignant lesions in CLT and compared them with lesions classified as "dysplastic" based on pre-defined morphologic criteria.

Design: A tissue microarray was constructed from 70 cases diagnosed as PTC with background CLT. For each case, representative cores were taken in triplicate, where applicable, from areas of normal epithelium with bland nuclei (N), reactive atypia in follicular cells maintaining normal architecture (RA), reactive epithelium surrounding lymphoid aggregates (RA-L), nodular hyperplasia/adenoma with bland (NH-n) or atypical (NH-atyp) nuclei, microcarcinoma (PMC), PTC and dysplasia (Dys). The term dysplasia was reserved for foci of atypical cells <0.1 cm with complex architecture distinct from the surrounding parenchyma. Immunostains for HBME-1 and Galectin-3 (Gal3) were analyzed using automated image analysis software (ScanScope and Spectrum Plus, Aperio, Membrane and Color Deconvolution algorithms respectively).

Results: Staining patterns were clearly distinct between normal and carcinomas with no normal tissue showing immunoreactivity for either marker. CLT lesions had different immunohistochemical profiles as shown in the table below. While atypical cells were present in all cases, lesions qualifying as dysplastic were found in 25/70 cases.

Lesions	HBME-1 Positivity	Mean Score of HBME-1+ cases (0-3)	Gal3 Positivity	Mean Score of Gal3+ cases (0-300)
N	0% (0/60)	-	0% (0/60)	-
RA	4.5% (3/66)	1.0**	3.0% (2/67)	36*
RA-L	89% (62/70)	1.1**	36% (25/70)	41*
NH-n	0% (0/32)	-	0% (0/32)	-
NH-atyp	28% (9/32)	1.4**	3.1% (1/32)	52
PMC	91% (21/23)	2.6	74% (17/23)	120**
PTC	73% (51/70)	2.5	53% (37/70)	97**
Dys	92% (23/25)	2.4	72% (18/25)	60

Statistical significance between Dys and other lesions for HBME-1 and Gal3 staining intensity scores by Mann-Whitney test and Independent Samples t-test, respectively. *P<0.05, **P<0.01

Conclusions: Our data identify proteomic changes that reflect a spectrum of morphologic alterations in CLT and define a novel category of "dysplasia" that is intermediate between reactive change and malignancy in thyroid.

565 Claudin-1 and -7 Expression in Solid Cell Nests of the Thyroid: An Enigmatic Analogy with Papillary Carcinoma.

C Colato, A Fierabracci, A Dardano, F Monzani, M Chilosi, G Martignoni, F Menestrina, M Ferdeghini. University of Verona, Italy; Children's Hospital Bambino Gesù, IRCCS, Rome, Italy; University of Pisa, Italy.

Background: Solid cell nests (SCNs), a normal component of the human thyroid gland, are considered remnants of the ultimobranchial body. The immunohistochemical profile of SCNs is well characterized and, as they apparently harbor the minimal properties of a stem cell phenotype, can be regarded as a pool of stem cells of the adult thyroid. Moreover, both the immunohistochemical detection of p63 in SCNs and BRAF mutation in solid cell nest hyperplasia suggest a link between these embryonic remnants and papillary thyroid carcinoma (PTC). However the biological significance of SCNs remains disputable. Claudins (CLDNs), a family of tight junction proteins, play a role in adhesion, cell proliferation, and tumorigenesis. Recently, CLDN1 was found to be up-regulated in PTC both at the gene and protein level. CLDN7 is also expressed in the thyroid, both during embryonic development and in the adult, and its expression is modulated in thyroid cancer.

Design: To assess the immunohistochemical expression of CLDN1 and 7 in a collection of 20 SCNs, found incidentally in specimens resected for PTC, follicular adenomas and multinodular goiter as well as to compare their staining pattern with that of PTC.

Results: In all cases, SCNs displayed a strong, diffuse and linear membranous CLDN1 positivity, forming a honeycomb-like pattern. In contrast, the C cells were constantly negative and in normal thyroid tissue only scattered positive cells were observed. CLDN7 staining was similar to that of CLDN1 with strong or moderate intensity.

Conclusions: We report for the first time CLDN1 and 7 expression in SCNs. CLDN1 appears a highly sensitive tool in detecting SCNs, as described for p63 and Galectin-3. CLDN7 is constantly expressed in SCNs as in fetal and adult thyroid, suggesting a potential role in architectural stability and functioning of follicular cells. We confirm that CLDN1 is frequently up-regulated in PTC and may represent a novel marker for this tumor as well as Galectin-3. This finding suggests the hypothesis of a histogenetic link between SCNs and PTC. Finally, CLDN1 may also be useful in separating SCNs from C cells, as normal C cells do not express this membrane protein.

566 Expression of an Activated Mammalian Target of Rapamycin (mTOR) in Gastroenteropancreatic Neuroendocrine Tumors (GEPNETs).

D Dhall, S Bose. Cedars-Sinai Medical Center, Los Angeles.

Background: mTOR is an important regulator of cell proliferation, which is activated in various malignancies. A recent phase II trial has revealed the efficacy of mTOR inhibitor (Rapamycin and its derivative RAD001) against advanced low to intermediate grade neuroendocrine tumors. The purpose of this study was to evaluate the expression pattern of mTOR in GEPNETs and to correlate with Ki-67 proliferative index.

Design: Formalin-fixed, paraffin-embedded tissue sections from 27 well-differentiated neuroendocrine tumors, including 9 from pancreas, 11 from gastrointestinal tract, and 7 from metastatic sites (mostly liver), were immunohistochemically stained for phosphorylated mTOR (p-mTOR) using a rabbit monoclonal antibody (Cell Signaling). The percentage of tumor cells positively stained and staining intensity were recorded for each case. Less than 30% staining of tumor cells and weak staining were considered negative.

Results: Eighteen patients presented with a stage IV disease and 9 with either localized or regional metastasis. Twenty-two tumors (81%) showed moderate to strong diffuse staining for p-mTOR, including 6 (67%) pancreatic tumors, all 11 (100%) gastrointestinal tumors, and 5 (71%) metastatic tumors. Of 21 tumors in which Ki-67 proliferative index was available, 11 cases (79%) with a < 5% and 6 cases (85%) with a > 5% Ki-67 labeling index demonstrated positive staining for p-mTOR. The percentage of tumors positively stained for p-mTOR did not differ between cases with and without stage IV disease (78% and 89%, respectively).

Conclusions: 1) p-mTOR is overexpressed in most GEPNETs, irrespective of their site of origin, and whether they are primary or metastatic. 2) p-mTOR staining does not correlate with Ki-67 proliferative index. 3) p-mTOR expression is low in some GEPNETs, which may account for their lack of response to m-TOR inhibitors. 4) Additional studies are warranted to determine the role of p-mTOR in the selection of patients with targeted therapy.

567 The Use of Immunohistochemical Expression of SF-1 and EMA in Distinguishing Adrenocortical Tumors from Renal Neoplasms.

ML Enriquez, P Lal, A Ziober, L Wang, JE Tomaszewski, Z Bing. Hospital of the University of Pennsylvania, Philadelphia.

Background: Steroidogenic factor -1 (SF-1) is an orphan member of the nuclear hormone receptor superfamily, and is considered to play an important role in the differentiation of steroidogenic tissues. Previous studies have shown SF-1 to be specifically expressed in normal adrenal gland, adrenal adenomas and carcinomas, and have also shown negative immunoreactivity in renal cell carcinomas (type not specified). In this study, we further examined the expression pattern of SF-1 in adrenal tumors by immunohistochemical staining using tissue microarrays of adrenal glands and adrenal cortical tumors. Furthermore, in order to evaluate the potential of SF-1 in distinguishing adrenocortical tumors from renal neoplasms, we compared the expression of SF-1 and EMA in the adrenal cortical TMAs versus a renal tumor TMA.

Design: The adrenal tissue microarrays consisted of 19 cases of normal adrenal cortex, 22 cases of adrenal adenoma, and 20 cases of adrenocortical carcinoma. The renal tissue microarray consisted of 20 cases of each of following types of renal cell carcinoma: clear cell, papillary, and chromophobe. 20 cases of oncocytoma were also included. Immunohistochemistry for SF-1 and EMA was performed on the adrenal and renal TMAs. Nuclear staining for SF-1 in ≥10% of cells was considered positive, and intensity of staining was graded (1=weak, 2=moderate, 3=strong).

Results: SF-1 showed positive staining in all cases (100%) of normal adrenal cortex and adrenal adenoma, and in 18/20 (90%) cases of adrenocortical carcinoma. In renal tumors, SF-1 showed negative staining in all cases (100%) of oncocytoma, papillary and chromophobe renal cell carcinoma. 17/20 (85%) of clear cell renal cell carcinomas were negative for SF-1, while the three positive cases showed only weak staining in 10% of tumor cells.

EMA stained positively in 85%, 95%, 100% and 95% of clear cell, papillary, chromophobe renal cell carcinomas, and oncocytomas, respectively. EMA was completely negative in the adrenal TMAs.

Conclusions: SF-1 is a sensitive and specific marker for tumors of adrenal cortical origin. SF-1 and EMA can be used to differentiate adrenal tumors from renal tumors in difficult cases.

568 Comparison of an Expanded Immunohistochemical Panel To Distinguish High Grade Non-Merkel Cell Neuroendocrine Carcinomas from Merkel Cell Carcinoma.

A Faustin, C Annaiah, D Dhall, B Balzer. Cedars Sinai Medical Center, Los Angeles, CA.

Background: High grade neuroendocrine carcinoma (HGNEC), particularly when metastatic, can be a challenge to distinguish from primary or metastatic Merkel cell carcinoma (MCC). Although each diagnosis constitutes a high grade carcinoma, identifying the primary site still plays a role in therapeutic decision making. Currently there is no specific marker for either diagnosis, but a standard set of immunohistochemical (IHC) stains (CK20, Synaptophysin (SYN), Chromogranin (CHR), TTF-1) is often applied to each case. Their pattern of reactivity overlaps between MCC and non-MCC HGNEC, and frequently a definitive diagnosis is withheld in the absence of reliable clinical information. Fli-1, a Ewing Sarcoma marker; Tdt, a marker of immature B-cells; and C-kit have all been reported as variably positive in MCC; however, no study has tested these markers in non-MCC HGNEC to determine whether or not they have differentiating reactivity from MCC. Our study aims to address this question.

Design: A total of 10 cases of non-MCC HGNEC and 8 cases of MCC were retrieved from archival material and selected for study following confirmation of diagnoses by two practicing surgical pathologists. Sites of origin for HGNEC were bronchopulmonary (3), bladder (2), esophagus (2), liver (1), colon (1), cervix (1). All MCC were cutaneous and involved head and neck (4) and extremity (4). Sections from corresponding tissue blocks were immunohistochemically stained for pancytokeratin, TTF-1, SYN, CHR, CK20, C-kit, Fli-1, and TdT. Stains were scored as positive or negative with the following pattern qualifications: pancytokeratin, CHR, SYN, C-kit (cytoplasmic); TTF-1, Fli-1 and TdT (nuclear); CK20 (perinuclear dot-like).

Results: All MCC and non-MCC were appropriately positive for pancytokeratin, SYN, and CHR. The number of cases positive for the remaining stains is summarized in table 1.

Table 1

STAIN	non-MCC HGNEC (N=10)	MCC (N=8)
CK20	1	8
Fli-1	1	8
C-kit	6	7
TdT	0	8

Conclusions: In cases for which HGNEC has been made, the most sensitive marker in discriminating MCC from non-MCC is TdT (100% vs. 0). Fli-1 and CK20 (8/8 MCC and 1/10 non-MCC) are also highly discriminatory in this setting. Although C-kit has been reported as a positive marker in MCC, it is non-specific in this setting and overlaps significantly with non-MCC HGNEC. We propose that in cases for which the differential diagnosis includes MCC and non-MCC HGNEC an expanded panel including CK20, TdT, and Fli-1 can be useful adjuncts in making this distinction.

569 Evaluation of SDHB Immunohistochemistry in the Evaluation of Pheochromocytomas and Paragangliomas from Patients with and without Succinate Dehydrogenase B (SDHB) Mutations.

L Fishbein, K Nathanson, VA LiVolsi, PJ Zhang, KT Montone. University of Pennsylvania, Philadelphia.

Background: Pheochromocytomas and paragangliomas may arise in patients with germline mutations in succinate dehydrogenase (SDH) subunits B, C, and D as well as in patients with germline mutations in VHL, NF1, and ret. Recently, Gill et al (Human Pathol 41:805, 2010) reported loss or diminished expression of SDHB protein by immunohistochemistry in tumors from patients with germline mutations of SDH B, C, and D whereas those associated with other germline mutations showed strong cytoplasmic granular staining. We evaluated the utility of SDHB immunohistochemistry for evaluating a series of patients seen at our institution with genetically associated paraganglioma/pheochromocytomas.

Design: Eighteen paraganglioma/pheochromocytomas from 18 patients with known germline mutations of SDHB (6 patients), SDHD (4 patients), ret (4 patients) and NF1 (4 patients) were evaluated by immunohistochemistry using a commercially available SDHB antibody (Abcam 1:50). Staining was evaluated by light microscopy and signal was scored as negative (no staining), weak non-granular cytoplasmic positivity, weak granular cytoplasmic positivity and moderate to strong granular cytoplasmic positivity

Results: Moderate to strong granular cytoplasmic positivity was noted in all paragangliomas/pheochromcytomas from patients with ret, NF1 and SDHD germline mutations. In the tumors from patients with germline SDHB mutations, 5 tumors showed weak granular cytoplasmic positivity and 1 tumor showed weak non-granular cytoplasmic positivity.

Conclusions: By immunohistochemistry, we continued to observe weak, mostly granular cytoplasmic positivity for SDHB in tumors from patients with known SDHB germline mutations. In addition, strong granular reactivity was identified in tumors from patients with confirmed SDHD as well as NF-1 and ret mutations. While weak granular reactivity for SDHB may suggest the possibility of SDHB mutation, in our experience this immunostain does not replace testing for germline mutations and does not predict the presence of SDHD mutations.

570 TSH Receptor mRNA in Peripheral Blood in Conjunction with Fine Needle Aspiration for the Pre-Operative Identification of Papillary Thyroid Cancer.

NS Freed, M Milas, DJ Chute. Cleveland Clinic Foundation, OH.

Background: Thyrotropin stimulating hormone receptor (TSHR) mRNA in peripheral blood is a newly developed test that has shown some utility in the pre-operative detection of papillary thyroid carcinoma (PTC). We assessed the accuracy of the TSHR mRNA blood test to pre-operatively identify PTC in patients undergoing thyroidectomy and investigated the impact of adding TSHR mRNA results on the sensitivity and specificity of fine needle aspiration (FNA) in detecting PTC.

Design: We retrospectively examined patients who underwent a total thyroidectomy at one institution from 2007-2010 who also had a TSHR mRNA peripheral blood test and thyroid FNA pre-operatively. Cases were included if the thyroid gland was entirely submitted for histology regardless of diagnosis, or was partially submitted with a diagnosis of PTC. TSHR mRNA peripheral blood levels were performed using quantitative PCR, and were considered positive if greater than 1.0 ng/ug. Pre-operative thyroid FNAs within the previous year were categorized as positive, atypical, or negative for PTC. For the purpose of sensitivity and specificity calculations, FNA interpretations of positive and atypical were included together as a positive result.

Results: TSHR mRNA testing demonstrated a sensitivity of 50.3% and specificity of 86.2% for the detection of PTC as illustrated in Table 1.

Thyroids stratified by TSHR mRNA status and the presence or absence of PTC

Table 1		Diagnosis		
		PTC	No PTC	Total
TSHR	+	99	13	112
mRNA	-	98	81	179
	Total	197	94	291

p<0.0001

The result of TSHR mRNA testing with respect to the FNA result and the presence or absence of PTC is demonstrated in Table 2.

Thyroids stratified by TSHR mRNA status and FNA result with or without PTC

Table 2		PTC		No PTC		
		TSHR mRNA +	TSHR mRNA -	TSHR mRNA +	TSHR mRNA -	Total
	Positive	37	31	0	0	68
FNA	Atypical	51	46	8	51	156
	Negative	11	21	4	28	64
	Total	99	98	12	79	288

p<0.001

The sensitivity and specificity of FNA alone in determining PTC was 83.8% and 34.0% respectively. However, when FNA and TSHR mRNA were combined with either test positive, the sensitivity increased to 89.3%, but the specificity was 29.8%. When requiring both tests to be positive, the sensitivity dropped to 44.7%, but the specificity increased to 91.2%. Of note, in the subset of cases with an atypical FNA, the specificity increased to 52.6%, with a sensitivity of 86.4% using TSHR mRNA.

Conclusions: TSHR mRNA is a useful pre-operative test in conjunction with thyroid FNA for the identification of patients with suspected PTC.

571 Heat Shock Protein 90 (HSP90) Expression in Pancreatic Endocrine Neoplasms: A Potential Therapeutic Target and Predictor of Behavior.

AA Gonzalez-Longoria, H Remotti. Columbia University Medical Center, New York, NY.

Background: HSPs are molecular chaperones which facilitate tumor growth and resistance to radiation/chemotherapy. Hsp90 antagonists are being pursued in therapeutic trials. Scant data is available regarding HSP expression in Pancreatic Endocrine Neoplasms (PEN). We evaluated the expression of Hsp90 in PEN by immunohistochemistry.

Design: Representative tissue microarrays from paraffin blocks of 58 PEN representing 26 PEN localized low grade (PENL), 13 PEN localized with vascular invasion

(PENVI) and 19 malignant cases with metastasis to liver or lymph nodes (PENM) were immunostained with antibodies to Hsp 90. Expression of Hsp90 was validated on endometrium and internal pancreatic ductal and islet controls. The intensity of cytoplasmic and nuclear Hsp90 expression of tumor cells was compared with non-neoplastic pancreatic islet cells and semiquantitatively scored, as 0= less than or negative, 1= similar to, and 2= greater than non-neoplastic islet cells.

Results: The immunoreactivity was cytoplasmic and nuclear. The percentage of positive cases is tabulated. Among every case studied, cytoplasmic Hsp90 was diffusely expressed with a score of 1 and 2 in 100% of cases with PENL and PENVI and 95% (18 /19) cases of PENM. Nuclear Hsp90 showed more variation. The overall mean percent of tumor with positive nuclear staining was higher (score 1-2) in angioinvasive (PENVI) and metastatic (PENM) as compared to low-grade PEN: PENL 69%, PENVI 92% and PENM 89% (p=0.04) (see Table).

Table: Hsp90 Nuclear Stain Distribution

Tumor Type	Score 0 =less than normal islet or negative	Score 1 =similar to normal islet	Score 2=more than normal islet
PENL (26)	8 (31)	11 (42)	7 (27)
PENVI(13)	1 (8)	7 (54)	5 (38)
PENM (19)	2 (10)	7 (37)	10 (53)
Total = 58	11	25	22

Percentage in parenthesis

Conclusions: Hsp90 is expressed in PENs. Hsp90 showed increased nuclear expression in angioinvasive and metastatic PENs compared to localized PENs without angioinvasion. The expression of Hsp90 may reflect its role in PEN oncogenesis and serve as a potential therapeutic target.

572 Analysis of BRAF^{V600E} Mutational Status and the Biology of Papillary Thyroid Microcarcinoma.

Y Han, W Sacks, A Treml, X Fan, S Bose, J Lopategui, G Braunstein, D Frishberg. Cedars Sinai Medical Center, Los Angeles, CA.

Background: Papillary thyroid microcarcinomas (PTMCs) usually have an excellent clinical outcome, but recent studies have demonstrated that a significant number of PTMCs may show more aggressive behavior, with extrathyroidal extension and cervical lymph node metastasis. During the past few years, it has become evident that aberrant signaling through the RAS–RAF–MEK cascade is crucial for the development of thyroid cancer. BRAF mutation has been reported to be correlated with high aggressiveness, such as advanced disease stage and cervical lymph node metastasis in thyroid papillary carcinoma. However, the importance of BRAF mutation in prognostication of PTMCs is still unclear, and has not been studied in North American patients. BRAF^{V600E} mutation is the most common BRAF mutation that accounts for over 90% of all BRAF mutations. In this study, we analyzed the occurrence of BRAF^{V600E} mutations in PTMCs to elucidate the relationship between the occurrence of BRAF mutations and biologic behavior.

Design: This retrospective study was performed in 40 cases of PTMCs between the years 1999 to 2007. BRAF^{V600E} mutation was detected by real-time PCR for all cases on formalin fixed, paraffin embedded sections. Statistical significance was determined by Fisher's exact test and Student t-test.

Results: Among the 40 cases, 18 cases demonstrated lymph node metastases and 9 cases with extrathyroidal extension at time of surgery. Overall, 55.3 % of PTMCs harbored a BRAF^{V600E} mutation. BRAF^{V600E} was present in 87.5 % of cases with lymph node metastases, and 31.8% of cases without lymph node metastases ($P<0.01$). The prevalence of BRAF^{V600E} mutation was higher in stage T3/T4 PTMCs than in stage T1 ($P<0.05$). Among BRAF^{V600E}-positive cases 66.7% showed lymph node metastasis as compared with only 11.8% of wild-type BRAF PTMCs ($P<0.01$). Among all other clinicopathologic features that we analyzed, BRAF^{V600E} mutation was found to be uncorrelated with age, focality, vascular invasion, multinodular goiter, or lymphocytic thyroiditis. In 4 to 10 years of follow-up only 2 cases of PTMC showed evidence of clinical recurrence; both were BRAF^{V600E}-mutated.

Conclusions: BRAF mutated PTMCs exhibit signs of higher aggressiveness than PTMCs without this mutation. BRAF mutation could be a marker useful in identifying PTMCs that require a more intensive primary treatment, such as node exploration. The role of BRAF in long-term behavior of this usually indolent tumor is less clear, and studies with longer term follow up are needed.

573 Cell Survival in MEN2 Thyroid and Adrenal Medullary Neoplasms: Role of PI3 Kinase/AKT Pathways.

T Ioannidis, R Lam, L Lam, A Blanes, SJ Diaz-Cano. King's College Hospital, London, United Kingdom; University of Malaga School of Medicine, Spain.

Background: The cell survival in tumors is heterogeneous and includes PI3 kinase/AKT, JAK/SRC, and NFkB pathways. The contributions of these pathways in MEN related tumors and the differential expression in pheochromocytomas and medullary thyroid carcinomas remain unknown.

Design: We selected small (1-2cm) familial (MEN2A and MEN2B) pheochromocytomas (PCC, 29) and medullary thyroid carcinomas (MTC, 35), diagnosed and classified according to WHO criteria. We analyzed in a low-density selective cDNA array (LD-SelGEA): BCL2, CCND1, FN1, F13A, JUN, MMP7 (matrilysin), MYC (PI3 Kinase / AKT Pathway): BCL2, BCL2L1 (Jak / Src Pathway): BCL2A1 (Bfl-1/A1), BIRC2 (c-IAP2), BIRC3 (c-IAP1), NAIP (BIRC1), TERT (NFκB Pathway); and CXCL12, CXCR4 (basic regulators of stem cell phenotype). Total RNA was extracted, cleaned from normal and neoplastic tissues (RNeasy columns), first-strand cDNA synthesized using T7-(dT24)-oligomer and used as template for cRNA synthesis. The cRNA was fragmented, Cy3-/Cy5-labeled, and hybridized to LD-SelGEA noncompetitively, cross-validating the results (expression factor>2, significance<0.01). Variables were studied regarding the histological diagnosis and the familial syndrome (MEN2A/MEN2B). Significant variables were then tested on tissue sections by immunohistochemistry.

Results: Statistical significant differences (expression factor>2 at least) were observed for CCND1 (cyclin D1 – up-regulated in MTC), FN1 (fibronectin – up-regulated in MTC), CXCL12/CXCR4 (up-regulated in MTC), and F13A (coagulation factor XIII A – up-regulated in PCC). All other genes showed no statistically significant differences by histological subtypes and no significant differences were observed for each diagnostic group by the DNA genotype (MEN2A vs. MEN2B).

Conclusions: Cell survival is differentially promoted in MEN2 thyroid and adrenal medullary neoplasm: (a) MTC cell survival depends on the PI3 kinase/AKT pathways (cyclin D1, fibronectin) and basic regulators of stem cell phenotype (CXCL12/CXCR4). (b) PCC tumor cell promotion is mainly dependent on the vascular supply and the role coagulation factor XIII A subunit. The cell survival mechanism is independent of the familial genotype.

574 Expression of Adenosine Receptors in Thyroid Carcinoma.

T Kondo, T Nakazawa, T Kawasaki, K Mochizuki, D-F Niu, T Yamane, R Katoh. University of Yamanashi, Chuo, Japan.

Background: The adenosine receptors, comprising A1, A2a, A2b and A3, are G protein-coupled receptors with adenosine as endogenous ligand. A1 and A3 receptors inhibit adenylyl cyclase via coupling to Gi, while A2a and A2b receptors stimulate adenylyl cyclase activity via Gs. Recently overexpression of adenosine receptors have been identified in several human cancers.

Design: To explore the putative importance of adenosine receptors in thyroid carcinogenesis, we examined the expression of four isoforms of adenosine receptors in normal thyroids (25 cases), various thyroid tumors (adenomatous goiter, 18 cases; follicular adenomas, 10 cases; follicular carcinomas, 17 cases; papillary carcinomas, 27 cases; undifferentiated carcinomas, 21 cases) and 6 thyroid carcinoma cell lines (KTC-1, TPC-1, WRO, 8505C, UA-1 and UA-2). To further examine the function of ADORA1 in thyroid carcinomas, highly selective ADORA1 antagonist DPCPX was applied for MTT cell growth assay, matrigel invasion assay, and gelatin zymography for matrix metalloproteinase (MMP) -2 and -9 activities.

Results: Using RT-PCR and Western blotting, we found that adenosine receptor A1 (ADORA1) was consistently upregulated in papillary carcinomas and thyroid cancer cell lines compared with normal thyroids. There were no distinct differences in the mRNA expression of adenosine receptor A2a, A2b and A3 subtypes between normal and neoplastic thyroids. In immunohistochemistry, diffuse cytoplasmic immunopositivity of ADORA1 was observed in a majority of follicular carcinomas, papillary carcinomas and undifferentiated carcinomas, while negative or focal immunopositivity in normal thyroids. The pattern of ADORA1 immunostaining in adenomatous goiter and follicular adenomas varied from negative to diffusely positive among cases. Pharmacologic ADORA1 inhibition significantly impaired thyroid tumor cell growth and tumor cell invasion. In addition, DPCPX suppressed secretion of active forms of MMP-2 and -9 that were induced by selective ADORA1 agonist CPA in thyroid carcinoma cells.

Conclusions: In conclusion, we demonstrated enhanced expression of ADORA1 in human thyroid carcinoma tissues, and potential role of ADORA1 for tumor invasion, suggesting the adenosinergic system as a potential pharmacological target in thyroid cancer therapeutics.

575 A Genome-Wide Screening of Promoter DNA Methylation Reveals Differential Profiles of Follicular-Cell Derived Thyroid Neoplasms.

T Kondo, T Nakazawa, T Kawasaki, K Mochizuki, D-F Niu, T Yamane, R Katoh. University of Yamanashi, Chuo, Japan.

Background: Thyroid cancer show wide spectrum of differentiation from indolent well-differentiated carcinomas to lethal undifferentiated carcinoma. This spectrum of progression has been linked with a pattern of cumulative intragenic defects that correlates with tumor differentiation, aggressiveness, and metastatic potential. Recent advances have further disclosed the significance of epigenetic alterations in the development and progression of human cancers.

Design: In this study, we analyzed a global DNA methylation in human thyroid carcinomas (papillary carcinoma, 5cases; undifferentiated carcinoma, 2 cases) as compared to normal thyroid tissues (5 cases), and 6 thyroid cancer cell lines (TPC-1, KTC-1, WRO, 8505C, ASH-3 and KMH-2) using Illumina Beadchip (HumanMethylation27). Quantitive measurements of promoter DNA methylation were determined for 27,578 target CpG sites spanning a total of 14,495 genes. DNA methylation profiles were analyzed by Gene Cluster 3.0 and Java Treeview software.

Results: There were 866 genes that showed significantly increased or decreased promoter methylation in thyroid carcinomas and thyroid carcinoma cell lines. Using these genes for cluster analysis, we found differential epigenetic profiles among thyroid cancer. Three distinct patterns of aberrant methylation were identified. The first gene group showed stepwise hypermethylation among normal thyroid, papillary carcinoma and undifferentiated carcinoma. The second gene group demonstrated hypermethylation especially in undifferentiated carcinoma. The third gene group exhibited hypermethylation in normal thyroid, meanwhile hypomethylation in undifferentiated carcinoma. Methylation status of thyroid cancer cell lines was similar to undifferentiated carcinoma tissues.

Conclusions: We concluded that methylation status of certain subsets of genes were associated with histological types of thyroid cancer. Our data suggested the involvement of epigenetic events in the progression of thyroid carcinoma, and also provide a basis of molecular targets for epigenetic therapy in thyroid cancer.

576 Value of Islet 1 and PAX-8 in Identifying Metastatic Neuroendocrine Tumors of Pancreatic Origin.

J Koo, R Mertens, HL Wang, F Chung, D Dhall. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Neuroendocrine tumors (NETs) can often present as metastases to the liver before the primary tumor is discovered. While the transcription factors TTF-1 and Cdx2 have been shown to aid in the identification of NETs of lung and gastrointestinal (GI) origin, respectively, no such marker has been established for the detection of NETs of pancreatic origin. Recently, two transcription factors, Islet 1 and PAX-8, have been proposed as markers for pancreatic NETs. The purpose of this study was to evaluate the usefulness of Islet 1 and PAX-8 in distinguishing metastatic pancreatic NETs from those originating from other primary sites, as well as to examine the sensitivity and specificity of TTF-1, Cdx2, Islet 1 and PAX-8 for metastatic NETs from a variety of primary sites.

Design: A total of 69 liver metastases of NETs with known primary sites were studied. The primary sites were established based on prior, concurrent or followup surgical pathology (53) or well documented clinical history (16). Immunohistochemistry was performed using antibodies against Islet 1, PAX-8, TTF-1 and Cdx2. Nuclear staining of at least 5% of tumor cells was considered a positive result. Staining intensity was also scored as weak, moderate or strong.

Results: Immunohistochemistry results are summarized below:

Staining of Metastatic NET in Liver

Site of Primary	TTF-1	Cdx2		Islet 1	PAX-8
		Weak	Mod-Strong		
Pancreas (n=22)	1 (5%)	1 (5%)	0	16 (73%)	2 (9%)
Lung (n=7)	4 (57%)	0	0	1 (14%)	2 (29%)
Ileum (n=35)	0	8 (23%)	24 (69%)	0	0
Duodenum (n=1)	0	0	0	0	0
Colon (n=2)	0	0	1 (50%)	0	0
Rectum (n=2)	0	0	0	2 (100%)	0

For metastatic pancreatic NETs, Islet 1 has a sensitivity of 73% and specificity of 94%, while PAX-8 has a sensitivity of 9% and specificity of 96%. For metastatic GI NETs, Cdx2 has a sensitivity of 83% and specificity of 96%. For metastatic lung NETs, TTF-1 has a sensitivity of 57% and specificity of 98%. Islet 1 expression was found in 1 metastatic lung NET and 2 metastatic rectal NETs, and PAX-8 expression was seen in 2 metastatic lung NETs. Of the 3 metastatic lung NETs with aberrant Islet 1 or PAX-8 staining, all 3 also showed strong co-expression of TTF-1. Similarly, the 1 metastatic pancreatic NET with aberrant TTF-1 and Cdx2 staining also showed strong co-expression of Islet 1.

Conclusions: Islet 1 is more sensitive than PAX-8 for the detection of metastatic NETs of pancreatic origin. When used in combination with TTF-1 and Cdx2, Islet 1 is of help in determining the origin of a metastatic NET.

577 Molecular Analysis of Adrenal Cortical Carcinomas in Clinical and Histologic Context.

AE Kovach, Q Lam, D Dias-Santagata, PM Sadow. Massachusetts General Hospital, Boston; Harvard Medical School, Boston, MA.

Background: Adrenal cortical carcinomas (ACC) are rare, aggressive neoplasms that represent a small subset of all adrenal cortex-based masses. Iterations of the Weiss criteria remain the go-to standard for histologic parameters to help predict the biologic potential of an adrenal cortical neoplasm. More recently, due to consistently inconsistent data, we have been turning increasingly toward molecular criteria to supplement histologic findings.

Design: In this retrospective study approved by our internal review board (2009P002721), we identified 68 adrenal cortical carcinoma resections from 1976 to 2009; 35 cases were appropriate for further analysis. Clinical and morphologic review was performed, along with molecular studies on a subset of cases using a SNaPshot multiplexed PCR-based clinical assay developed in-house that tests for recurrent mutations in 13 known cancer genes.

Results: Patients included 24 women and 11 men, with a mean age at resection of 53 years (SD 15). Sixteen patients showed clinical signs related to supraphysiologic hormone production, including hypertension (9), concomitant Cushingoid features (4), or hypokalemia (3). Nineteen masses were left-sided, 1 bilateral, with a mean maximum dimension of 12.0 cm (SD 1.8). In 11 cases, the surgical margin was positive and/or metastases were apparent at the time of resection. Twelve patients died because of metastatic disease, including 5 of the 11 with positive surgical margins or metastases at presentation, with a mean time from resection to death of 1.9 yrs. Histologically, tumor necrosis was extensive in 24 cases and absent in 4. Lymphovascular invasion was identified on H&E sections in 14 cases, all but one of which also had extensive necrosis. Degree of necrosis paralleled degree of proliferation, with a mean mitotic rate of 20/50 HPF. Of a subset of 10 carcinomas characterized by rapid disease progression compared to other cases in the cohort, molecular analysis revealed 5 beta-catenin mutations and 1 mutation in APC.

Conclusions: Although universally associated with poor prognosis, ACCs with relatively favorable outcome had complete surgical resections and lack of metastases at presentation, independent of histologic parameters. Beta-catenin and APC mutations appear common in tumors with relatively unfavorable outcomes. Continuing studies include comparative molecular analysis in an expanded cohort including benign adrenal cortical adenomas and neoplasms where histologic parameters suggest equivocal biologic potential.

578 Serotonin-Producing EC-Cell Tumors of the Pancreas. Morphological, Immunohistochemical, Ultrastructural and FISH Study of 15 Cases and Comparison with Intestinal EC-Cell Tumors.

S La Rosa, A Perren, A Schmitt, L Albarello, B Bernasconi, G Finzi, C Capella. Ospedale di Circolo, Varese, Italy; University of Berne, Switzerland; San Raffaele Scientific Insitute, Milan, Italy; University of Insubria, Varese, Italy.

Background: Serotonin-producing tumors of the pancreas (5HT-PETs) are rare neoplasms composed of EC-cells which have been mainly described in the literature as case reports. Studies analyzing the clinico-pathological features of a consistent number of such pancreatic endocrine tumors, also investigating the similarities to and differences from intestinal EC-cell tumors, have never been reported.

Design: The morphological, immunohistochemical, ultrastructural and FISH features of 15 pancreatic and 20 intestinal serotonin-producing neoplasms were compared. In addition, a review the literature on 5HT-PETs has been performed to better understand the clinico-pathological features of this rare tumor type.

Results: Tumors of the present series were not associated with the carcinoid syndrome. The lack of substance P and acidic fibroblast growth factor immunoreactivity, the low immunohistochemical expression of CDX2, VMAT1, connective tissue growth factor and prostatic acid phosphatase, the low positivity for the diazonium reaction, the lack of S100-positive sustentacular cells, the strong expression of VMAT2, and peculiar ultrastructural features characterized 5HT-PETs and differentiated them from intestinal ones, although both categories showed similar chromosome 18 cytogenetic alterations mainly consisting in monosomic clones for chromosome 18. The review of the literature indicated that pancreatic 5HT-functioning tumors (associated with the carcinoid syndrome) arose in younger patients, were larger, more frequently malignant and more aggressive neoplasms than pancreatic 5HT-nonfunctioning ones.

Conclusions: The results suggest that 5HT-PETs show an immunohistochemical and ultrastructural profile more similar to that of gastric EC-cells, rather than intestinal EC_1-cells, and show several different morphological features from the related intestinal tumors, although both categories present similar cytogenetic alterations on chromosome 18. 5HT-PETs are more frequently endocrinologically nonfunctioning. The distinction between functioning and nonfunctioning neoplasms is of clinical relevance since the former are more frequently malignant and associated to a worse patient survival.

579 Immunohistochemical Subclassification of Thyroid Tumor with Prominent Hyalinizing Trabecular Pattern.

S Lee, JS Koo, SW Hong. Yonsei University College of Medicine, Seoul, Korea.

Background: In surgical pathology practice, thyroid tumors with hyalinizing trabecular pattern are occasionally noted. The purpose of this study was to investigate immunohistochemical characteristics of 12 cases of thyroid tumor with prominent hyalinizing trabecular pattern (TTHTP).

Design: A retrospective review of all patients with a diagnosis of thyroid tumor with hyalinizing trabecular pattern who underwent surgery at Severance Hospital from January 2000 to December 2008 was undertaken. The criteria of TTHTP in this study was defined as thyroid tumor showing more than 90% hyalinizing and/or trabecular pattern without definitely papillary or follicular architecture. The various immunohistochemical stains were applied to 12 cases of TTHTP, and the clinicopathologic features were evaluated.

Results: The mean tumor size was 2.1 cm (range; 0.5-6.0 cm). PTC was synchronously identified in 5 cases which showed contralateral PTC in 3 cases and bilateral PTC in 1 case. One patient demonstrated bone metastasis. All TTHTPs were positive for TTF-1 and thyroglobulin. Chromogranin A, synaptophysin and p63 were negative in all TTHTPs. The same six (50.0%) cases showed expression of CD56 and Ki-67. Out of these 6 cases, 4 cases satisfied the strict criteria of Carney. CK19 and galectin-3 was positive in 2 and 5 cases, respectively.

Conclusions: All TTHTPs meeting the criteria of Carney histologically were HTT, and TTHTP which did not satisfy the criteria of Carney could be subclassified according to the results of immunohistochemistry into HTT type [Ki-67 (+) and CD56 (+)], PTC type [CK19 or galectin-3 (+)/Ki-67 (-) and CD56 (-)], and null type [Ki-67 (-), CD56 (-), CK19 (-) and galectin-3 (-)].

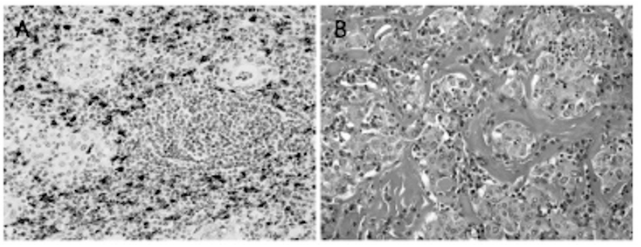
580 A Comparative Histopathological Evaluation between Subgroups of Hashimoto's Thyroiditis.

Y Li, Z Liu, T Ozaki, E Taniguchi, I Mori, K Kakudo. Wakayama Medical University, Japan.

Background: Hashimoto's thyroiditis (HT) is commonly considered as a well-defined clinicopathological entity. Its diagnosis and treatment have changed little over the last few decades. We herein describe a subsection of HT characterized histologically by dense lymphoplasmacytic infiltrate, stromal fibrosis, large numbers of IgG4-positive plasma cells, and serologically by elevated IgG4 titer. These cases seem to represent a distinct form of HT and have close relationship with IgG4-related sclerosing disease.

Design: 114 cases of surgical samples from patients with HT were involved in this study. Immunostaining of IgG4 and IgG was performed on paraffin sections and the histopathological characterisitics of these cases were evaluated.

Results: On the basis of immunohistochemistry of IgG4 and IgG4/IgG ratio, the 114 patients with HT were divided into two groups: IgG4 thyroiditis (33 cases) [figure1A] and non-IgG4 thyroiditis (81 cases).



Histopathologically, IgG4 thyroiditis [figure1B] showed higher grade of stromal fibrosis, lymphoplasmacytic infiltration and follicular cell degeneration than non-IgG4 thyroiditis. In addition, before operation, serum IgG4 concentrations were significantly higher in IgG4 thyroiditis than non-IgG4 thyroiditis. After operation, the serum IgG4 levels of patients with IgG4 thyroiditis decreased significantly. Furthermore, IgG4 thyroiditis and non-IgG4 thyroiditis present different clinical features, with IgG4 thyroiditis being more closely associated with rapid progress, subclinical hypothyroidism, higher levels of circulating antibodies, and more diffuse low echogenicity. No other organs were found to be involved by IgG4-related sclerosing disease in this patients' series.

Conclusions: Hashimoto's thyroiditis can be subclassified into two groups, in regard of IgG4-positive plasma cell population: IgG4 thyroiditis and non-IgG4 thyroiditis. IgG4-positive plasma cell infiltrate is a marker of fibrotic variant of Hashimoto's thyroiditis, which shows atrophy of follicles and stromal fibrosis, resulting in subclinical hypothyroidism more. New insights into HT with special reference of IgG4-positive plasma cells offer a novel way of viewing this well-defined disease.

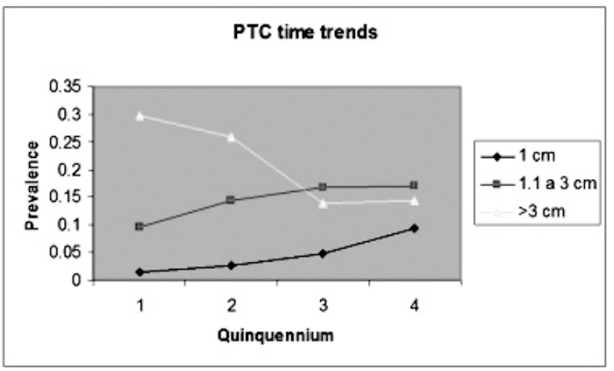
581 Trends in Thyroid Pathology. Steady Prevalence of PTC and Rise of Nodular Goiter in Thyroidectomies.

S Lino-Silva, E Medina-Lopez, D Acuna-Gonzalez, O Gonzalez-Trevino, A Gamboa-Dominguez. INCMN Salvador Zubiran, Mexico City, DF, Mexico.

Background: Changes in the prevalence of papillary thyroid carcinoma (PTC) have been reported in institutions and national cancer registries. Objective: to describe time trends in benign and malignant thyroid diseases in a national endocrine referral center.

Design: Systematic review and classification of consecutive specimens with slides/paraffin blocks in surgical pathology archives (January 1990 to December 2009). Institutional registries, size, type of surgery and number of inclusion blocks were recorded. Patients whose registries were granted before January 1990 without nodules, but treated after twelve months for a suspicious thyroid lesion, were included. These patients in a passive follow-up, permitted incidence density calculations. Cases were grouped by quinquennium.

Results: Institutional registers were conceded to 103,961 persons worthy of attention, and 1,269 were submitted to thyroidectomies (1.2%). One hundred twenty four patients, none treated for thyroid diseases before 1990, developed thyroid nodules after 1991. The incidence density for goiter was 0.05 person/year and for PTC 0.04 person/year in that group. In all series woman to man ratio was 9:1 with a mean age of 45 years. Total or near total thyroidectomies were performed in 60% patients and benign diseases were diagnosed in 732 (52%) cases. Thyroid surgeries increased since 2005 with a rise in goiter prevalence (0.25, 0.31, 0.35, 0.38), without significant increase in PTC prevalence (0.41, 0.43, 0.35, 0.40) in thyroidectomies. When PTC prevalence was analyzed by size a 520% rise in microscopic carcinomas was evident (0.015, 0.027, 0.047, 0.093), and a 76% increase for those between 1.1 and 3cm (0.097, 0.143, 0.167, 0.171), but not for tumors >3cm (0.297, 0.258, 0.138, 0.143).



Conclusions: Goiter as the only finding in thyroid specimens increased 52% in the last 20 years. In contrast to previous reports, PTC prevalence was steady. The higher prevalence of lesions <3cm in thyroidectomies, is a reflection of screen-detected papillary carcinomas (high definition ultrasonography and FNA biopsy). The incidence density of 0.05 and 0.04 person/year for goitre and PTC respectively, is in soundness with data from prospective cohort studies.

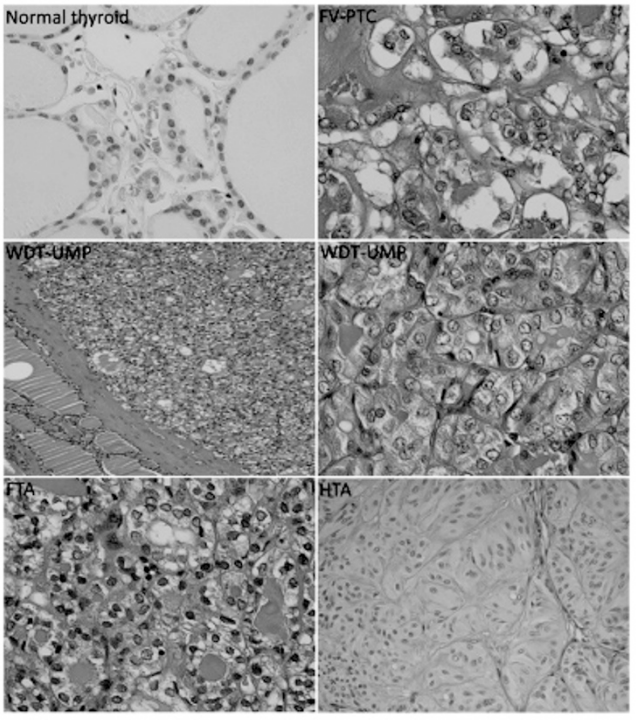
582 Encapsulated Follicular Thyroid Tumor with Equivocal Nuclear Changes, So-Called Well Differentiated Tumor of Uncertain Malignant Potential, a Morphological, Immunohistochemical and Molecular Appraisal.

Z Liu, Y Li, T Ozaki, I Mori, E Taniguchi, K Kakudo. Wakayama Medical University, Japan.

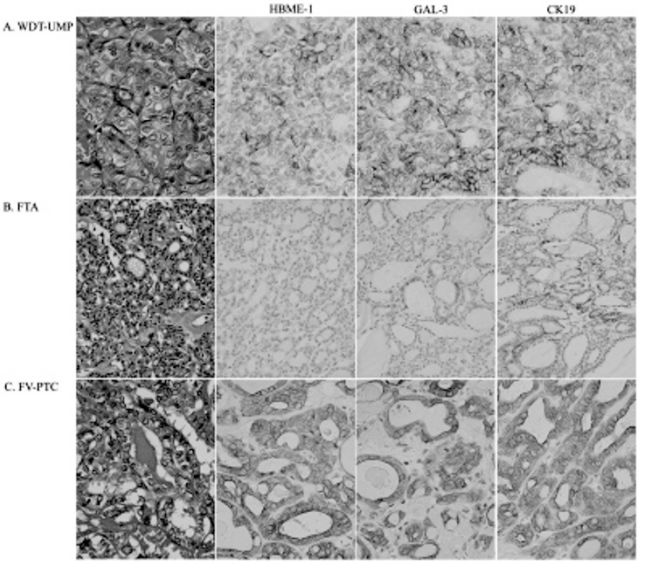
Background: Papillary thyroid carcinoma type nuclear changes (PTC-N) are the most reliable morphology in the diagnosis of PTC. However, there is a continuous debate regarding the classification of thyroid follicular lesions with equivocal PTC-N, and well differentiated tumor of uncertain malignant potential (WDT-UMP) was recently introduced by Williams to cover this problematic spectrum of thyroid tumors.

Design: A total of 30 cases of WDT-UMPs were examined. As a control, follicular adenoma (FTA, n = 16), follicular carcinoma (FTC, n = 8), hyalinizing trabecular adenoma (HTA, n = 5) and PTC (n = 48) were included. Immunohistochemical staining of HBME-1, cytokeratin 19 and galectin-3 were examined. $BRAF^{V600E}$ mutation and RET/PTC-1 rearrangement were investigated for all the WDT-UMPs.

Results: WDT-UMP had different nuclear features with the controls.



HBME-1, cytokeratin 19 and galectin-3 were positive in 12 (40%), 10 (33.3%) and 11 (36.7%) cases of WDT-UMP respectively.



According to the positivity of those markers, significant differences were obtained between WDT-UMP and PTC encapsulated common type ($P = 0.030$, 0.009 and 0.004), infiltrative follicular variant ($P = 0.020$, 0.008 and 0.026) and infiltrative common type ($P = 0.022$, 0.024 and 0.005) respectively, but no difference was demonstrated between WDT-UMP and FTA or FTC. Absence of $BRAF^{V600E}$ mutation but occurrence of RET/PTC1 rearrangement was found in 2 (6.7%) cases of WDT-UMP. None of the 20 patients with WDT-UMP developed recurrence with an average follow-up of 80 months.

Conclusions: These findings indicate that WDT-UMP has a favorable outcome and is distinct from PTC in morphological, immunohistochemical and molecular characteristics. We propose that WDT-UMP should be classified as 'well differentiated tumor with uncertain behavior'.

583 Morphoproteomics Demonstrates Activation of mTORC2 in Aggressive Histologic Variants of Papillary Thyroid Carcinoma.

J Liu, RE Brown. University of Texas Health Science Center at Houston Medical School.

Background: Papillary thyroid carcinoma (PTC) is the most common thyroid cancer and, in general, has an excellent prognosis with appropriate treatment. However, a small subgroup of patients has aggressive or radioactive-iodine-resistant PTCs of which the tumorigenesis is not quite understood and the criteria for the histologic subclassification are still controversial. Morphoproteomics is a method using immunohistochemistry to identify the expression of signal transduction pathways. In this study, we use morphoproteomics to investigate the activation of mammalian target of rapamycin (mTOR) signaling pathway in PTCs.

Design: Archival paraffin-embedded, tissue of PTC was obtained from 31 patients including 15 conventional type, 9 follicular variant, 4 tall cell variant, 1 columnar cell variant, 1 diffuse sclerosing variant, and 1 cribriform variant. Immunohistochemical stains were performed for the following three phosphorylated (p) analytes: p-mTOR (Ser 2448), p-Akt (Ser 473), and p-p70S6K (Thr 389). Chromogenic signals and subcellular compartmentalization (nuclear, cytoplasmic, and plasmalemmal) were evaluated.

Results: Immunoreactivities of p-mTOR, p-Akt, and p-p70S6K were observed in all PTCs. In addition to the expression of p-mTOR in cytoplasmic and/or plasmalemmal locations, the nuclear translocation of p-mTOR with concomitant nuclear expression of p-Akt was identified in all tall cell variant, columnar cell variant, and diffuse sclerosing variant PTCs. A strong nuclear expression of p-p70S6K was present in all PTCs.

Conclusions: The expressions of p-mTOR in cytoplasmic and/or plasmalemmal locations with concomitant nuclear expression of p-p70S6K in all PTCs indicate the activation of mTOR complex 1 (mTORC1). The nuclear translocation of p-mTOR evidences the activation of mTOR complex 2 (mTORC2) per literature and is identified only in the known aggressive histologic variants including tall cell, columnar cell, and diffuse sclerosing variants of PTC in this study. These results show the constitutive activation of mTOR signaling pathway in PTCs and provide a new insight of biologic basis for the aggressive histologic variants of PTC. The expression of mTORC2 in these variants may serve as a diagnostic marker and a therapeutic target.

584 Should Reflexive BRAF Testing Be Performed on Atypical/ Borderline Thyroid Nodules?

JD Marotti, JA Lefferts, VA Memoli, AC Golding, GJ Tsongalis, V Padmanabhan. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: Diagnosis of classical papillary thyroid carcinoma (PTC) is based on characteristic nuclear features and is usually not a problem. However, follicular lesions with borderline nuclear features of PTC (thyroid tumors of uncertain malignant potential, atypical adenomas) create diagnostic difficulty and clinical uncertainty. Atypical adenomas are diagnosed based on cytologic atypia within otherwise encapsulated, noninvasive lesions that lack sufficient cytological features of encapsulated, noninvasive follicular variants of PTC. Though all variants of PTC share the same nuclear features, thresholds required to recognize them differ among pathologists. This remains subjective with considerable variability in diagnoses. The frequency of BRAF V600E mutations has been shown to range from 12% in follicular variants, to 60% in classical PTCs. Atypical adenomas have not been well studied for the presence of the BRAF gene mutation.

Design: We retrospectively identified and reviewed all thyroid surgical pathology cases that contained "atypical" within the final pathology report during a five-year period (2005-2010). Nodules described as having nuclear atypia insufficient for a diagnosis of thyroid carcinoma were selected. Clinical, pathologic, demographic, and prior FNA information were recorded. Blocks were selected that best represented atypical areas. DNA was extracted from macro-dissected or whole paraffin-embedded sections, and BRAF testing was performed using two allele-specific real-time PCR designed to detect wild-type BRAF and the BRAF V600E mutation.

Results: Twenty cases were identified out of 895 consecutive thyroid specimens (0.02 %). Of these, 16 cases (17 nodules) were available for both histologic review and BRAF testing. Six PTC and 5 multinodular hyperplasia cases were also tested as controls. BRAF mutations were not identified in any of the borderline nodules or in cases of multinodular hyperplasia. Three of six PTC (50%) contained BRAF mutations.

Conclusions: In our population, performing BRAF testing on atypical thyroid nodules with histologic features insufficient for diagnosis of PTC did not yield additional information. Currently, histology remains the gold standard for diagnosis of PTC, and reflexive BRAF testing for borderline cases on thyroidectomy/ lobectomy specimens may be of limited use.

585 An Analysis of the Revised CAP Protocol for Reporting Papillary Thyroid Carcinoma (PTC).

MB McCready, H Mani, N Williams, HS Crist. PSHMC, Hershey, PA.

Background: The CAP protocol for reporting thyroid tumors was revised in 2009. We analyzed the effect of these changes in PTC reporting by general surgical pathologists in a large academic center.

Design: All PTCs diagnosed from Jul 2009 to Jul 2010 were retrieved and information from CAP synoptic reports tabulated. Next, slides of tumors were coded for blinding and independently classified by 3 head-neck pathologists and 1 pathology fellow, followed by consensus review. Results were analyzed.

Results: Of 51 PTCs diagnosed by general surgical pathologists in the period under study, 18 were reported using the revised and 33 by the prior (2006) CAP protocol. Follicular variant PTC (FVPTC) had been overdiagnosed using the prior protocol (12/33) as compared to the revised protocol (3/18). On review, 3/12 (25%) in the former group, but none in the latter, were reclassified (2 classical, 1 solid). Of 17 microcarcinomas identified on review, only 2/9 were reported by the prior vs. 5/8 by the revised protocol. Thus the diagnosis of FVPTC and microcarcinoma was more precise with the revised protocol. Second tumors, when present, were mentioned in all reports. However 3/9 second tumors in the prior reports lacked a specific variant classification, while all second tumors in the later reports had been subclassified. The presence of lymphovascular invasion was specified in both the prior and revised synoptic reports. However prior to the revision, the presence or absence of extrathyroidal extension was not specifically reported.

All cases were reviewed independently and by consensus. All 3 head-neck pathologists agreed initially on subclassification in 76% of cases, 2 of 3 in 96% cases, and there was complete disagreement in 2% cases. The greatest agreement was with classical, followed by FVPTC and columnar cell variants. Interobserver variation was greatest in designation of tall cell and solid variants. Compared to consensus diagnosis, the pathology fellow had correctly subclassified 89% of cases, but had misclassified 2 cases of classical PTC as oncocytic and 2 FVPTC as classical.

Conclusions: The revised CAP synoptic report resulted in more precise diagnosis of FVPTC and microcarcinomas. Improved diagnosis of FVPTC may be due to the requirement of specifically evaluating architecture in the revised protocol. The revised protocol also resulted in specific documentation of second tumors and extrathyroidal extension. Diagnosis of variants other than follicular and classical PTC is difficult. Inclusion of well-defined criteria and definitions within the CAP protocol notes may be helpful for both general surgical and head-neck pathologists.

586 Relationship of Mitogen-Activated Protein Kinase and Stromal Reaction in Sporadic Thyroid Malignancies: Role of MAP3K8 in Papillary Carcinomas.

J Moorhead, R Lam, L Lam, A Blanes, SJ Diaz-Cano. King's College Hospital, London, United Kingdom; University of Malaga School of Medicine, Spain.

Background: The relationship between kinetic features, mitogen-activated protein (MAP) kinase and the types of stromal reaction are not known in common thyroid malignancies, both follicular and parafollicular.

Design: We selected sporadic thyroid carcinomas including papillary (PTC, 29), follicular carcinomas (FTC, 15 minimally-invasive and 15 widely-invasive), adenomas (FTA, 19), medullary (MTC, 18) and anaplastic carcinomas (ATC, 10) (WHO criteria) to analyze in a low-density selective cDNA array (LD-SelGEA) for proliferation-apoptosis pathways (MKI67, ERK1, CASP3, MAP3K8, AKT1, CHUK, MAP2K4, MAP3K14, NFKB1, TRAF2), and stromal-inflammatory reaction types (CDH11, SDC1, SPARC, MMP11, CSF1, CD68, CD163, and FCGR2). Total RNA was extracted, cleaned from normal and neoplastic tissues (RNeasy columns), first-strand cDNA synthesized using T7-(dT24)-oligomer and used as template for cRNA synthesis. The cRNA was fragmented, Cy3-/Cy5-labeled, and hybridized to LD-SelGEA noncompetitively, cross-validating the results (expression factor>2, significance<0.01). Variables were studied regarding the histological diagnosis and common molecular profile: BRAF and RAS mutation, RET/PTC and PAX8/PPAR fusion gene, and TP53 LOH/ mutation and combinations. Significant variables were then tested on tissue sections by immunohistochemistry.

Results: Statistical significant differences (expression factor>2 at least) were observed for MKI67 (down-regulation in MTC, along with ERK1), MAPK3K8 (up-regulated in PTC and ATC), and CDH11 (up-regulated in PTC). Down-regulated genes throughout the series regardless of the diagnosis included: TRAF2, MMP11, CSF1, CD68, and FCGR2. Genes with no significant differences comprised: CASP3, CHUK, MAP2K4, NFKB1, SDC1, SPARC, CD163. No significant differences were observed for each diagnostic group by the DNA genotype (point mutations, fusion genes and LOH).

Conclusions: MAP3K8 upregulation in PTC results in activation of MAP kinase and JNK kinase pathways that promote both (a) T lymphocyte activation contributing to the presence of the lymphocytic infiltrate and (b) a typical stromal reaction of desmoid-type fibromatosis. No CSF1 macrophage response characterizes thyroid malignancies, and the types of stromal reactions are genotype independent.

587 FGFR3 Transcript and Protein Down-Regulation Is a Negative Prognostic Factor and Correlates with Promoter Methylation in Pancreatic Islet Cell Tumors.

M Nassiri, S Ramos, M Jorda. Indiana University School of Medicine, Indianapolis; Univerisity of Miami, FL.

Background: Prognostic factors for pancreatic islet cell tumors are few. Recently fibroblast growth factor receptor pathway role in endocrine neoplasm has been investigated and new drugs that affect this pathway have been introduced. We studied the expression of fibroblast growth factor receptor-3 (FGFR-3) and its correlation with clinical behavior of pancreatic islet cell tumors.

Design: Thirty-five cases of islet cell tumors were evaluated for hormone expression (gastrin, insulin, glucagon, ACTH, somatostatin, pancreatic polypeptide, calcitonin, serotonin and vasoactive intestinal polypeptide) and proliferation index (PCNA). FGFR-3 was studied by immunohistochemistry and quantitative real-time PCR. FGFR3 promoter region methylation was studied by MSP-PCR.

Results: Cytoplasmic expression of FGFR-3 was associated with lower proliferation index; 94% of cases with low PCNA score were positive for FGFR-3 vs. 35% of cases with high score ($p<0.001$). Less differentiated tumors (as evidenced by lack of hormone's expression) showed low or no expression FGFR-3 ($p= 0.02$), and loss of FGFR-3 expression was associated with metastasis ($p= 0.02$). FGFR3 transcript level correlated

with its protein expression and promoter methylation status, as lower transcript levels were associated with promoter methylation and lower protein expression.
Conclusions: Decreased cytoplasmic expression of FGFR-3 is associated with less differentiated tumors; higher proliferation index and poor outcome in islet cell tumors and correlates well with decreased FGFR3 transcript level and promoter methylation.

588 Immunohistochmical Detection of SSTR2a in Gastro-Entero-Pancreatic Neuroendocrine Tumors (GEPNET) and Response to Somatostatin Analogue (SA).

RY Osamura, H Hirabayashi, M Matsuda, T Itoh. IUHW Mita Hospital, Minato-ku, Tokyo, Japan.
Background: It has been reported that somatostatin analogue(SA) is expected to be effective for the gastroentero-pancreatic neuroendocrine tumors(GEPNETs) when they express somatostatin recetor(SSTR)2a by immunohistochemistry(IHC). This study is aimed at to elucidate the incidence of SSTR2a-positive GEPNETs and to correlate the SSTR2a positivity and response to SA.
Design: Total 133 cases of GEPNETs(62 pancreas, 12 duodenum, 11 rectum,6 stomach, 17 others and 25 liver metastasis) were subjected to IHC. In brief, formalin-fixed paraffin embedded(FFPE) sections were reacted with anti-SSTR2a(1:1000, Gramsch Laboratories Germany). The staining was graded 0, 1+,2+ and 3+ according to Volante et al.(Modern Pathol 2007). Clinical follow-up was obtained in total 25 cases of SSTR2a 2+ and 3+ cases who were treated with SA, alone or in combination with chemotherapy. The response of the SA therapy was classified into (1)tumor shrinkage,(2)suppression of hormone level,(3)stable disease(SD),(4) increase in tumor size,(5) unknown.
Results: The results of IHC is as follows. Negative-30 cases(22.5%) ,1+ 30 cases(22.5%), 2+ 13 cases(9.8%) and 3+ 60 cases(45.2%.). The overall positive rate(1+2+ 3+), (2+ 3+) was 73.8% or 55% Response to SA in 25 cases was as follows: (1)tumor shrinkage 9 cases:35%,(2) respectively decreased hormone level 2cases:8%,(3) SD 8 cases:32%, (4)increased tumor size 4 cases(16%),(5)unknown 2 cases(8%). 75% of SSTR2a 2+ and 3+ immunoreactivity responded to SA therapy. The increase in size after SA was noted in pancreatic neuroendocrine carcinoma: NEC(1 case),rectal NEC(2 cases) and gastric NEC(1 case).
Conclusions: High proportion of GEPNETs was positive for SSTR2a. 75% of SSTR2a 2+ and 3+ tumors responded to SA therapy. The small proportion of resistant tumors may require other type of targeted therapy such as mTOR inhibitor. This report verifies the SSTR2a immunostaining for the therapeutic benefits of GEPNETs.

589 Paradoxal Loss of PDGF-CC Expression in Pheochromocytomas and Paragangliomas.

A Pinto, V Sujoy, U Eriksson, M Neville, V Nose. Jackson Memorial Hospial/University of Miami, Miami, FL; Ludwig Institute for Cancer Research, Karolinska Institute, Stockholm, Sweden.
Background: Physiologically, platelet-derived growth factors (PDGFs) have been demonstrated to play a role in the angiogenesis and embryonic development of kidney, brain, cardiovascular and respiratory systems. Pathologically, these factors have been implicated in the genesis of a number of tumor types. They belong to a growth factor family that exert their cellular effects through PDGF-α and PDGF-β protein tyrosine kinase receptors. More recently, a new member of the family of PDGFs (CC) has been reported to have similar effects as PDGF AA and BB.
It was previously shown that PDGFR is overly expressed in the normal adrenal gland cells. However, its role in this organ environment and if it plays a role on adrenal tumorigenesis is not yet well known. Initial studies are showing that drugs that inhibit PDGFs may be therapeutically effective for treatment of paragangliomas and pheochromocytomas. Our objective was to define the relationship between a specific subtype of PDGF (CC) and distinct types of adrenal lesions, to investigate if there is an overexpression or loss in any hyperplasia or neoplasia.
Design: We studied the reactivity to PDGF-CC by immunohistochemistry in normal adrenal glands and in forty cases of adrenal cortical hyperplasia and neoplasia, pheochromocytomas (including sporadic and familial syndromes- MEN, VHL, SDHB),
paragangliomas, neuromas, ganglioneuromas and ganglioneuroblastomas.
Results: We found that normal adrenals expressed PDGF-CC reactivity in both cortex (ranging from 1+ to 2+) and medulla (3+). Sustentacular and endothelial cells also showed intense positivity to the antibody. However, 100% of the pheochromocytomas and paragangliomas completely lost their expression of PDGF-CC. Other medullary tumors and cortical lesions had a variable expression profile (ranging from 1+ to 2+).
Conclusions: Although some initial clinical trials are showing effectiveness of PDGFRs inhibitors in the treatment of pheochromocytomas and paragangliomas, the analysis of PDGFR-CC expression on adrenal cells showed a paradoxically loss of this marker and tumor progression. Additional studies are necessary to demonstrate if this phenomenon has any relation with tumorigenesis, and also to reveal if this receptor selectiveness would play any role in future therapeutic interventions.

590 TRAP-1 Is a New Surrogate Marker for *SDH* Mutation in Pheochromocytoma/Paraganglioma and a Potential Target for Chemotherapy.

JF Powers, SM Fliedner, I Leav, DC Altieri, K Pacak, AS Tischler. Tufts Medical Center, Boston, MA; NIH/NICHD, Bethesda, MD; University of Massachusetts Medical Center, Worcester; Wistar Institute, Philadelphia, PA.
Background: TNF receptor-associated protein 1 (TRAP-1) is an anti-apoptotic protein related to HSP-90 that accumulates in mitochondria of a variety of tumor cells but not of normal cells. Gamitrinibs are a new class of drugs that target TRAP-1 and induce tumor cell death (Kang et al, J Clin Invest 119:454-64, 2009). The mitochondrial enzyme succinate dehydrogenase (SDH) is the critical link between the Krebs cycle and the mitochondrial electron transport chain. Germline mutations of the *SDHA, B, C* and *D* genes are causes of hereditary pheochromocytoma/paraganglioma (PCC/PGL) syndromes. PCC/PGL associated with *SDHB* mutations are particularly likely to metastasize, and there are currently no effective treatments after metastases have occurred. It is therefore important to distinguish patients harboring *SDH* mutations from those with sporadic tumors.
Design: We hypothesized that PCC/PGL harboring *SDH* mutations might be particularly prone to accumulate TRAP-1 because of their intrinsic mitochondrial defect. Eighteen genetically characterized PCC/PGL (10 *SDHB* or *SDHD*, 5 sporadic, 3 *VHL*) were analysed for TRAP-1 protein expression by immunohistochemistry (IHC). Staining was scored 0-3 based on percentage of stained cells and staining intensity. Immunoblots (IB) were also performed on 9 of the tumors. Gamitrinib cytotoxicity was tested using Gamitrinib-triphenylphosphonium in cell cultures of a mouse pheochromocytoma (MPC) cell line expressing high levels of TRAP-1 and in primary cultures of a separate set of 5 human PCC/PGL that showed variable TRAP-1 expression in IBs.
Results: TRAP-1 immunostaining was positive in all but one *SDH*-mutated tumor and was completely negative in all sporadic tumors. One *VHL* tumor also showed positive staining. IB confirmed the association of TRAP-1 with *SDH* mutation. In the mouse cell culture model gamitrinib (10uM) caused 60-65% cell death at 1 week of treatment and 90-95% cell death at 2 weeks. Responses of the human tumors ranged from no effect to ~ 60% cell death at 2 weeks.
Conclusions: IHC for TRAP-1 is promising as a new tool to triage patients who present with apparently sporadic PCC/PGL to be genetically tested for *SDH* mutations. In addition, some PCC/PGL expressing high levels of TRAP-1 might respond to treatment with gamitrinibs. The latter possibility requires further pre-clinical testing. This research was supported in part by a grant from the PheoPara Alliance (to AST).

591 Prognostic Value of Cervical Lymph Node Metastases in Papillary Thyroid Carcinoma.

M Rivera, I Ganly, N Katabi, W Fu, A Shaha, JP Shah, J Fagin, M Tuttle, R Ghossein. Memorial Sloan-Kettering Cancer Center, NYC, NY; Memorial Sloan-Kettering Cancer Center, NY, NY.
Background: The impact of cervical lymph node (LN) metastases (M) on survival in papillary thyroid carcinoma (PTC) is controversial. Our aim is to assess the prognostic value of multiple histologic parameters associated with LNM in PTC in order to better stratify patients for therapy.
Design: All PTC patients with LNM at presentation identified between 1980 and 2000 were analyzed. A meticulous histopathologic examination of the primary tumor and associated LNM was undertaken. The histopathologic data was correlated with outcome.
Results: 246 patients satisfied the inclusion criteria with a median age of 36 years and a median primary tumor size of 1.9 cm.There were 136 (55.5%) classical PTC, 23 (9.5%) follicular variant PTC, 44 (18%) tall cell PTC, 35 (14%) microcarcinoma PTC and 8 (3%) PTC of other subtype. Extra-thyroid extension was found in 156 (64%) of cases and positive margins in 46 (19%).The median number of metastatic LN was 6. The median size of the largest metastatic LN was1.3 cm. Extra-nodal extension was present in 75 (32%) of cases. Median follow up was 10.8 years. In the whole study population, the presence of >3 metastatic nodes was along with older age and extensive extra-thyroid extension an independent predictor of decreased recurrence free survival (RFS) (p=0.03). The number of metastatic nodes (>3) remained an independent prognostic factor for RFS in young individuals (<45 years) lowering RFS from 97% to 88% at 10 years (p=0.05). In patients < 45 years, none of 45 cases with 1-2 metastatic LN recurred including 26 patients without radioactive iodine (RAI) therapy with a median follow up of 12.99 years. In patients 45 years and older, the presence of >5 metastatic LN was an independent predictor of neck RFS (p=0.04) decreasing RFS from 94% to 75% at 10 years. In this older population, patients with extra-nodal extension had a lower disease specific survival (90% at 10 years) than those without (100% at 10 years, p=0.008).
Conclusions: 1) The number of metastatic LN is an independent predictor of recurrence in all patients and identifies a subset of young patients with excellent prognosis who do not need RAI therapy. 2) Extra-nodal extension predicts DSS in older individuals 3) The number of metastatic LN and the presence of extra-nodal extension should be included in the pathology report of PTC in order to help guide therapy.

592 Cyclin D1 Expression in Benign and Malignant Differentiated Tumors of the Thyroid Gland.

TP Seybt (1), P Ramalingam, J Huang (2), SW Looney (2), MD Reid. Georgia Health Sciences University, Augusta; Emory University Hospital, Atlanta, GA.
Background: The gold standard for diagnosis of thyroid tumors is histology, which is often challenging and subjective. Morphologic overlap between benign and malignant tumors also occurs frequently. We examined cyclin D1 expression in benign and malignant differentiated thyroid tumors to determine its diagnostic utility and correlation with tumor type and node (LN) status.
Design: Twenty-nine (29) follicular adenomas (FA), 23 follicular carcinomas (FCA) and 43 papillary thyroid carcinomas (PTC) (including 22 with and 21 without nodal metastases), were stained with cyclin D1. PTCs included 27 classical (PTCC) and 16 follicular variants (PTCFV). Staining intensity was negative (0), weak (1+), moderate (2+) or strong (3+). Stain distribution was negative (0%); 1+ (<25%); 2+ (25-75%) or 3+ (>75%) of tumor cells. Cochran-Mantel-Haenszel method tested for significant associations of cyclin D1 staining, distribution and intensity with tumor type (FA, FCA, PTC), tumor type after subtyping PTCs (FA, FCA, PTCC and PTCFV), and PTC LN status.

Results: A statistically significant association was found between tumor type and cyclin D1 staining, distribution, and intensity. There were fewer cyclin D1-positive (+) FAs than PTCs (52% vs 88% respectively; p<0.001). Stain distribution was greater in PTC than FA (p=0.032). More PTCs were (+) than FCAs (88% vs 61% respectively; p=0.013). FA did not differ significantly from FCA in staining or intensity. There were fewer cyclin D1 (+) FAs than PTCC (52% vs 89% respectively; p=0.003) and PTCFV (52% vs 88% respectively; p=0.023). FCA also differed significantly from PTCC in cyclin D1 staining (61% vs 89% respectively; p=0.044) and intensity (p=0.024). FA had significantly less intense staining than PTCC (p=0.004). No significant associations were found between PTC LN status and any cyclin D1 characteristic.
Conclusions: Frequency and intensity of cyclin D1 expression was increased in differentiated malignant tumors relative to benign ones, and was progressively amplified from FAs to carcinomas (PTC and FCA). Distribution and intensity was significantly higher in (all) PTCs than FA and FCA, especially FA. The heterogeneity in distribution and intensity of staining in all thyroid tumor types disqualifies cylcin D1 as a primary diagnostic marker. However, it may be helpful in distinguishing FA from PTC, especially PTCFV. Cyclin D1 expression by benign and malignant thyroid tumors suggests a possible role in tumor development and progression, which should be investigated further.

593 CDX2 Is Rarely Expressed in Columnar Cell Variant of Papillary Thyroid Carcinoma: A Study of Ten Cases.

V Sujoy, A Pinto, CM Kovacs, V Nose. Jackson Memorial Hospital/University of Miami, Miami, FL.
Background: Columnar cell variant is a recognized rare variant of papillary thyroid carcinoma (PTC) associated with an uncertain clinical course. This variant has been regarded as a more aggressive form in comparison to the more common classical and follicular variants. These tumors have morphological resemblance with colonic adenocarcinoma. CDX2, a transcription factor of the caudal homeobox family, plays a key role in intestinal development and differentiation and it is widely used as a marker to detect adenocarcinoma of intestinal and colonic origin. CDX2 has been rarely reported in PTC. Only a single report of three cases of columnar cell variant of PTC has suggested that CDX2 should be considered a novel marker for diagnosis of this entity.
Design: We studied ten cases of columnar cell variant of papillary thyroid carcinoma. The histological, architectural, and cytological features fulfilled the diagnostic criteria of the columnar cell variant of papillary thyroid carcinoma as defined by the current WHO classification including neoplastic follicular cells with basally pseudostratified, hyperchromatic nuclei with eosinophilic-to-clear cytoplasm and supranuclear and/or subnuclear cytoplasmic vacuoles. Ten patients (6M:4F) ranging from 32 to 90 years of age (mean 58.3 years) presented with tumors classified as indolent (4 cases) or aggressive (6 cases); 3 with $BRAF^{V600E}$ mutation. All cases were β-Catenin negative. The Ki-67 proliferative index was up to 50%. All cases were TTF-1 positive.
Using paraffin embedded blocks, immunohistochemistry for CDX2 (mouse monoclonal to CDX2 (CDX2-88), 1:50 dilution; Biogenex Laboratories) was performed to evaluate the reactivity of this antibody to this variant of PTC.
Results: Nuclear positivity for CDX2 was detected in one out of the ten cases studied (10%), the other nine cases did not express CDX2.
Conclusions: Only one of our cases showed nuclear positivity for CDX2, therefore our study failed to confirm this as a marker for columnar cell variant of papillary thyroid carcinoma. The absence of CDX2 in the majority of the cases does not support the theory of CDX2 playing a role in the intestinal phenotype of these tumors.
The use of CDX2 as an indicator of this rare variant of thyroid cancer should be considered with caution based on our study.

Gastrointestinal

594 Effects of Tissue Fixatives on Immunohistochemical Expression of MSI Makers in Colon Adenocarcinoma.

PA Adegboyega. LSU Health Sciences Center, Shreveport, LA.
Background: Colorectal adenocarcinomas with microsatellite instability (MSI) do not respond well to Fluorouracil-based chemotherapy; and do have treatment outcome that differs from that seen in microsatellite stable tumors. In clinical settings, immunohistochemical staining for makers of MSI [Mismatch repair (MMR) gene products] is used to screen for the presence of MSI; and has been shown to have comparable sensitivity and specificity with MSI detection by PCR. However, the effects of tissue fixatives on the immunohistochemical expression of MMR gene products are not known. This study explores the effects of two routinely used tissue fixatives [Dissect Aid and 10% Neutral Buffered Formalin (NBF)] on the immunohistochemical expression of three MMR gene products (MLH1, MSH2 and MSH6).
Design: Study materials consisted of 7 colectomy specimens received for tumor diagnosis and staging. Samples of normal colon and tumor from each specimen were fixed in NBF and Dissect Aid solutions. Matched samples from each fixative were submitted for routine processing and paraffin embedding after fixation for the following day(s): 1, 7, 14, 28, 42, 56, 84 and 112. Immunohistochemistry for MLH1, MSH2 and MSH6 was performed on representative sections of each block. Immunoreactivity scoring was done in a blinded fashion using a semi-quantitative score of 0, 1, 2, 3, and 4. The code for the fixatives was broken after scoring was done.
Results: MLH1 immunoreactivity scores for samples processed in fixative A was at least one score less than that observed for corresponding samples in fixative B; and almost undetectable by the end of week 4 for samples processed in fixative A but remaining strong throughout the study for samples processed in fixative B. Both benign and tumor samples stained strongly positive for MSH2 and MSH6 in all tissue samples processed in fixative B and the strong staining reaction was maintained throughout the study. In comparison, negative staining reaction was observed in both tumor and benign mucosa for all samples processed with fixative A – with as early as 24 hours fixation.
Conclusions: For all three MSI markers investigated, fixative B (10% Neutral Buffered Formalin solution) is the preferred fixative for immunohistochemical assay. Fixative A (Dissect Aid) may produce erroneous negative immunostaining results for MSI markers, even with as little as 24 hours fixation. These findings highlight the need for protocol modifications to separate the mesentery from the colon for fixation of colon in NBF and mesentery in Dissect Aid for optimal processing of both specimen components.

595 HER2 Assessment in Gastric Cancer Surgical Specimens: Proposal of a Work-Flow for Practical Routine Use.

S Asioli, F Maletta, L Verdun di Cantogno, MA Satolli, M Schena, C Pecchioni, C Botta, G D'Angelo, D Recupero, G Ingravallo, E Maiorano, A Sapino. University of Turin, Italy; University of Bari, Italy.
Background: In gastric cancer (GC) the expression of HER2 is known as a marker of prognosis and recently it has been confirmed as a predictive marker of response to Trastuzumab.
Design: GC specimens of 100 patients were collected. Representative samples from both primary tumors (100 samples) and lymph node metastases (24 samples), were selected. In each case, 4B5 (Ventana), CB11 (kit Oracle Menarini), HercepTest (Dako) antibodies were tested in immunohistochemistry (IHC) and scored as proposed. *HER2* gene status was studied by double probe fluorescence in situ hybridization (FISH) in all cases. Concordance among IHC scoring results of the 3 antibodies and between FISH results and IHC (0/1+ and 2+/3+), independently from the percentage of positive cells, were evaluated using the Cohen-Fleiss' kappa statistic (K). The number of specimens needed to be tested in cases with <10% of HER2 overexpression was assessed. Finally, influence of gain of CEP17 (copies number >3) on the results of FISH ratio was considered.
Results: The 3 antibodies showed a K of 84% (p<0,05). The overall concordance of FISH/IHC was >80% (p<0,05) in the primary tumor and was >85% (p<0,05) when correlated with lymph node metastases for the 3 antibodies. Nine cases showed 2+/3+ in <10% of cells which corresponded to a IHC score value of 0. In 8 of these cases the percentage increased to >10% adding 2 more sections from different tissue blocks of the primary tumor. In our case series, the gain of CEP17 did not influence the final score ratio of FISH analysis.
Conclusions: The HER2 analysis of surgical specimens of GC has to consider the tumor heterogeneity. When the IHC score is 0/1+ on 1 tissue block, we recommend to test 2 more tissue blocks, particularly in the cases were the negative score is related to the low percentage of positive cells (<10%). Our work flow protocol avoids working over-load and solves equivocal cases.

596 TTF-1 and Napsin-A Frequently Positive in Esophageal and Pulmonary Adenocarcinomas: Dispelling Myths and Offering New Insights.

KS Aulakh, C Chisholm, VO Speights. Texas A&M Health Science Center – Scott & White Hospital, Temple.
Background: Because the esophagus is situated in close proximity to the bronchial tree and lungs, invasive adenocarcinomas of these approximated anatomic structures can directly invade the other, creating a diagnostic challenge to determine which is the primary tumor. Tumors which metastasize to the esophagus include the thyroid, stomach, breast, ovary, and most commonly, the lung. Thyroid Transcription Factor-1 (TTF-1) protein is a reliable marker of pulmonary adenocarcinoma, being positive in up to 75% of these tumors. Despite its ubiquitous use in the evaluation of primary pulmonary tumors and distant metastases, its expression in primary esophageal adenocarcinoma has never been thoroughly investigated. We have found that the literature is entrenched with the presumption that esophageal adenocarcinomas lack TTF-1 expression, and subsequently, any TTF-1 positivity should preclude a diagnosis of primary esophageal adenocarcinoma. Other common and uncommon stains such as 34βE12, N-Cadherin, p63, Napsin-A, and IMP3 have also not been used in larger studies comparing these two entities.
Design: We applied these 6 stains (TTF-1, 34βE12, N-Cadherin, p63, Napsin-A, and IMP3) to primary esophageal and pulmonary adenocarcinomas that have been resected or biopsied and evaluate for immunohistochemical positivity. Immunohistochemical staining was scored semiquantitatively. The percentage of stained cells was graded from 0 to 4+: 0, no staining; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, 76%-100%.
Results:

Percent of Positive Cases

Adenocarcinoma Type	TTF-1	Napsin-A	N-Cadherin	P63	34βE12	IMP3
Pulmonary	92%	92%	42%	50%	96%	54%
Esophageal	88%	79%	0%	17%	92%	96%

Conclusions: According to our study, TTF-1 is frequently expressed in primary esophageal adenocarcinomas, contrary to presumptions that have been propogated in multiple literature sources broaching the subject. TTF-1, 34βE12, and Napsin-A fail to discriminate between esophageal and pulmonary adenocarcinomas due to frequent positivity in both tumor types. IMP3 is frequently positive in esophageal adenocarcinomas. Failure to stain with IMP3 should virtually exclude a diagnosis of esophageal adenocarcinoma. N-Cadherin did not stain any esophageal cases. Positive N-cadherin should also virtually exclude a diagnosis of esophageal adenocarcinoma.