440 Lipodermatosclerosis: A Histological Study of 25 Cases

SN Walsh, A Cheng, J Petersen, DJ Santa Cruz. WCP Laboratories, Inc, St. Louis, MO; Washington University/Barnes-Jewish Hospital, St. Louis, MO.

Background: Lipodermatosclerosis, also known as sclerosing panniculitis, is a degenerative disease that affects both lower legs. This chronic condition classically affects white females in their sixties.

Design: We collected 25 cases prospectively from our daily practice between September 1998 and December 2005. Patient demographics, lesional characteristics, and clinical information were gathered from submitted specimens and treating dermatologists. All biopsies were stained with H&E, von Kossa and Verhoeff-van Gieson (VVG).

Results: Patient age ranged from 33 to 84 years with an mean age of 62.6 years. There was a strong female predominance with a female to male ratio of approximately 12 to 1. All lesions were present on the lower extremities, at varying sites between the knee and ankle. Lesion duration ranged from 2 months to 2 years with a mean of 9.5 months. Clinically, the lesions were described as erythematous, tender, indurated plaques or nodules. The characteristic histological findings were seen almost exclusively in the subcutaneous tissue, involving primarily the lobules but also the septa. Adipose changes included micro and macrocyst formation, necrotic adipocytes, lipomembranous change and lipogranulomas with xanthomatous macrophages. The lesions were largely devoid of inflammatory changes. Medium vessel calcification was seen in 13 cases. A conspicuous change was the presence of accumulation of basophilic elastic fibers located deep in the septa, present in all of the cases. These fibers had a moth-eaten appearance and resembled the elastic fibers of pseudoxanthoma elasticum. These fibers were positive with both the von Kossa stain and VVG in 21 of the 25 cases.

Conclusions: The constellation of these histological changes is diagnostic of lipodermatosclerosis. While the pseudoxanthoma elasticum –like changes are very characteristic of this condition, they can be rarely seen in other disorders including thalassemia and calciphylaxis.

441 Lymphatic Invasion Revealed by Multispectral Imaging Is Common in Primary Melanomas and Associates with Prognosis

X Xu, PA Gimotty, D Guerry, P VanBelle, H Liang, K Montone, T Pasha, M Ming, G Acs, M Feldman, S Barth, R Hammond, R Elenetsis, PJ Zhang, DE Elder. University of Pennsylvania, Philadelphia, PA.

Background: Lymphatic invasion by tumor cells has been noted infrequently in primary melanomas. Metastasis to regional lymph nodes is more frequent and is associated with poor patient outcomes. We hypothesized that use of a specific immunohistochemical (IHC) marker of lymphatic vessels in primary lesions would increase the frequency of detection of lymphatic invasion and that lymphatic invasion would correlate with regional nodal metastasis.

Design: We studied the primary lesions of a sample of 106 patients from a retrospective cohort of 489 patients with melanomas diagnosed between 1972 and 1991 who had at least 10 years of follow up. We performed IHC stains for podoplanin (a marker for lymphatic vessels) and S-100 (a marker for melanoma cells). Tumoral lymphatic invasion was identified and confirmed by multispectral imaging (MSI) analysis; and tumoral lymphatic density was counted. We computed the rates of detection of lymphatic invasion and presence of intratumoral lymph vessels and used the log-rank test to evaluate differences between the Kaplan-Meier survival curves for time to regional nodal failure.

Results: Using IHC we found that intratumoral lymph vessels were present in 90 (85%) of the 106 primary melanomas. Intratumoral lymphatic invasion was detected by routine microscopy in 5 (4.7%) of the 106 cases and by IHC staining augmented by MSI in 37 cases (35%) ($p<0.001$). Tumoral lymphatic invasion was significantly associated with time to regional nodal metastasis, first metastasis and melanoma-specific death.

Conclusions: Tumoral lymphatic invasion is an under-observed phenomenon in primary melanomas that can be better detected by IHC staining. The presence of intratumoral lymphatic invasion, a step in tumor progression beyond lymphangiogenesis, may be a clinically useful predictor of regionally metastatic disease.

442 Pax-2 Expression in Cutaneous Adnexal Neoplasms

LD Young, MT Sharp, KM Hiatt. University of Mississippi Medical Center, Jackson, MI; University of Arkansas for Medical Sciences, Little Rock, AR.

Background: Pax-2 is a transcription factor that functions in the regulation of epithelial-mesenchymal interactions in developing tissues and organs including skeleton, sense organs, limb muscle, endocrine pancreas, kidney, and brain. In these systems, its expression is down-regulated in terminally differentiated cells. The potential role of Pax-2 in epithelial-mesenchymal signaling in cutaneous adnexal neoplasms has not been previously explored. The regulatory role of Pax-2 in epithelial-mesenchymal interactions during development of other organs suggests a similar role in skin. Based on previous work showing mesenchymal expression in cutaneous adnexal development, it follows that adnexal neoplasms will show Pax-2 expression in a pattern analgous to that seen in renal neoplasms.

Design: Nuclear expression of Pax-2 was evaluated in 31 paraffin-embedded, formalin-fixed adnexal neoplasms: tricholemmoma (6), benign adnexal neoplasm (8), clear cell acanthoma (4), microcystic adnexal carcinoma (4), malignant adnexal neoplasm (2), sebaceous carcinoma (3), proliferating tricholemmal cyst (2), tricholemmal carcinoma (1) and syringoma (1). Metastatic renal cell carcinoma, with known pax-2 expression was used as a positive control.

Results: Scattered nuclei in 9 out of 31 lesions, both benign and malignant, showed strong to weak nuclear Pax-2 expression. This expression was seen in benign adnexal neoplasms (3), sebaceous carcinoma (2), tricholemmoma (1), proliferating tricholemmal cyst (1), clear cell acanthoma (1) and microcystic adnexal neoplasm (1).

Conclusions: Despite mesenchymal induction of overlying epithelium during embryologic development of cutaneous adnaxae and Pax-2 expression in the adnexal mesenchyme in fetal skin, analogous to that seen in renal tubular development, Pax-2 expression does not appear to be upregulated in cutaneous adnexal neoplasms as is seen in renal cell carcinoma. Although parallels in developmental processes and Pax-2 expression exist between renal tubular and cutaneous adnexal formation, this parallel does not carry through to upregulation of Pax-2 in the development cutaneous adnexal neoplasms.

Endocrine

443 Histologic Variants of Papillary and Follicular Carcinomas Associated with Anaplastic Spindle and Giant Cell Carcinomas of the Thyroid: An Analysis of Rhabdoid and Thyroglobulin Inclusions

J Albores-Saavedra, M Hernandez, S Sanchez-Sosa, KW Simpson, A Angeles, DE Henson. LSUHSC, Shreveport, LA; General Hospital, Mexico City, Mexico; University Hospital, Puebla, Mexico; Instituto Nacional de Ciencias Medicas y Nutricion, Mexico City, Mexico; George Washington University Cancer Center, Washington, DC.

Background: Anaplastic spindle and giant cell carcinomas (SGCC) of the thyroid arise from papillary and follicular carcinomas. However, the incidence of the histologic variants of papillary and follicular carcinomas associated with SGCC is unknown. Rhabdoid inclusions have been reported in SGCC and in poorly differentiated follicular carcinoma (PDC) and thyroglobulin inclusions in follicular neoplasms, but the incidences of these inclusions in these tumors are unknown.

Design: A total of 292 thyroid neoplasms were used for this study. One hundred nine were SGCC, 120 papillary carcinomas (PC) (all variants included), 23 differentiated follicular carcinomas (DFC) (6 with insular pattern), 6 PDFC and 34 follicular adenomas (FA). H&E stained sections were available for review in all cases. A specific search for rhabdoid and thyroglobulin inclusions was made in every case. Additional sections were obtained from thyroid neoplasms with cytoplasmic inclusions for immunohistochemical studies using the following antibodies: vimentin, desmin, pancytokeratin, thyroglobulin and calcitonin.

Results: The following differentiated thyroid carcinomas coexisted with SGCC: 51 (46.8%) PC (34 conventional type, 14 tall cell variant and 3 follicular variant), 6 (5.5%) DFC, 1 with insular pattern (0.9%) and 3 oncocytic carcinomas (2.8%). Eleven SGCC (10%) and 2 (33%) PDFC showed rhabdoid features but lacked thyroglobulin inclusions. Thyroglobulin inclusions were found in 10 FA(29%), 8 (17%) follicular variant of PC and 7 (30.4%) DFC. There were no rhabdoid inclusions in any of these differentiated thyroid tumors.

Conclusions: SGCC results from anaplastic transformation of papillary or follicular carcinoma although the mechanisms that underlie this transformation remain unknown. The finding that only 1 SGCC was associated with follicular carcinoma with insular pattern contradicts the opinion that this tumor occupies an intermediate position between differentiated and SGCC. Rhabdoid features are markers of PDFC and SGCC, which are associated with aggressive behavior. Thyroglobulin inclusions reflect functional differentiation, and are markers of FA and DFC with follicular phenotype.

444 Papillary Thyroid Carcinoma, Columnar Cell Variant: A Clinicopathologic and Molecular Study

J-H Chen, GS Pinkus, WC Faquin, RV Lloyd, V Nosé. Brigham and Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Mayo Clinic Rochester, Rochester, MN.

Background: Papillary thyroid carcinoma is usually an indolent neoplasm with good biological behavior. However, the columnar cell variant of papillary thyroid carcinoma has a variable clinical course. Encapsulated or well-circumscribed tumors are associated with a favorable prognosis, while others are locally aggressive with early dissemination. The purpose of this study is to correlate the clinicopathologic features of the columnar cell variant with BRAF gene mutations and prognostic markers.

Design: Thyroid specimens included 8 surgical and 1autopsy specimens. The clinical findings and pathology material were reviewed. All specimens met the current WHO classification criteria for this entity. Routine and immunohistochemical stains for cyclin D1, bcl-2, and Ki-67 (MIB-1) were performed. PCR and gene sequencing of BRAF were performed on paraffin embedded tissue.

Results: 9 patients ranged in age from 32 to 90 years and included 5 males (median 60, mean 65) and 4 females (median 38, mean 47). Clinical follow-up was available for 6 of 8 surgical patients. The cases were classified as clinically indolent or aggressive. Indolent tumors included 4 patients (1 male, 3 female; median age 38 years) with asymptomatic or painless masses which were encapsulated or well-circumscribed (1.3 to 4 cm). 2 patients with clinical follow-up had no evidence of residual disease, and 1 patient had an incidental tumor discovered at post-mortem. The remaining 5 patients (4 male, 1 female; median age 60 years) had diffusely infiltrative tumors (4 to 11.5 cm) with extrathyroidal extension, tracheal invasion, and/or metastases. Of this group, 1 patient is alive with disease (25 months follow-up) and 3 died of disease 17 to 45 months after the diagnosis. A $BRAF^{V600E}$ (single letter amino acid) missense mutation was present in 3 of 9 cases, including 2 aggressive neoplasms and the incidental tumor. Cyclin D1 expression was up-regulated in all cases. In both indolent and aggressive tumors, bcl-2 expression was variably decreased and the MIB-1 proliferative index ranged from <5% to 30%.

Conclusions: The $BRAF^{V600E}$ missense mutation was present in 3 of 9 cases, all of which were from older male patients. Interestingly, no detectable BRAF mutation was seen in younger female patients with indolent tumors. There was no significant correlation between indolent and aggressive tumors and expression of cyclin D1, bcl-2, and the MIB-1 proliferative index.

445 Molecular Markers of Follicular Variant Papillary Thyroid Carcinoma (FVPTC) and Follicular Adenoma

K Denning, P Smyth, S Cahill, S Finn, E Conlon, JH Li, R Flavin, S Guenther, A Ferlinz, JJ O'Leary, O Sheils. Trinity College Dublin, Dublin, Ireland; Applied Biosystems, Foster City, CA.

Background: FVPTC frequently poses difficulties from a diagnostic perspective in the laboratory. Previous work by our group using microarray technology revealed several genes to be associated with FVPTC. The purpose of this study was to examine the expression of a number of these genes in thyroid lesions and to determine if these targets could have utility as adjunctive markers in the diagnosis of FVPTC in particular.

Design: 14 FVPTC, 31 classic PTC, 17 FA and 10 FTC (n=72) H&E slides and corresponding blocks were collected from the archives of St James Hospital, Dublin (2000 to 2005). Slides were reviewed by a board certified pathologist, areas of interest were marked and Laser Capture Microdissection (LCM) was performed to procure homogenous cell populations and ensure only follicular epithelial cells were collected. RNA was extracted and cDNA was generated. LCM typically results in low yields of RNA, and consequently a novel multiplex preamplification technique was utilised (Applied Biosystems, Foster City, Ca). TaqMan® PCR analysis was then carried out for HLA-DMA, HLA-DBQ1, CD74, CSNK1, IRF3, KRAS2, LYN, MTIK, MTIX, RAB23, TGFB1, TOP2A, with CDKN1B and GAPDH as endogenous controls.

Results: HLA-DMA, HLA-DQB1, MTIX and RAB23 were found to be differentially expressed ($p<0.05$) when comparing FA and FVPTC. Comparison of FA and FTC groups showed significant differential expression for MTIK, MTIX and RAB23 ($p<0.05$).

Conclusions: This panel of targets discriminate between FA, FVPTC and FTC by their expression repertoires. They may have utility for broader use in the setting of fine needle aspiration (FNA) cytology and could improve the definitive diagnosis of certain categories of thyroid malignancy.

446 Differential Expression of Transcription Factors (TTF-1, PDX1, SOX2, CDX2) in Well Differentiated Neuroendocrine Tumors

M Dvorakova, J Kofler, S Kuan. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Lung, gastrointestinal tract and pancreas are common locations of well differentiated neuroendocrine tumors (WDNETs). Locating the primary site of metastatic WDNETs is difficult because of their similar morphology. In this study, we investigated if the following 4 region-specific transcription factors show organ-specific expression in WDNETs from different sites. Thyroid transcription factor 1 (TTF-1) is expressed in lung and thyroid. The expression of CDX2, a homeobox gene product, starts in proximal intestine and decreases posteriorly. PDX1, a pancreatic duodenal transcription factor, is observed in stomach, duodenum, and pancreatic β cells, whereas SOX2, a HMG-box gastric transcription factor, is found in gastric foveolar cells.

Design: A total of 55 WDNETs from the foregut (14 gastric, 5 duodenal), midgut (11 ileal, 3 appendiceal) and hindgut (7 rectosigmoidal) as well as pancreas (7 insulinomas) and lung (n=8) were reviewed. Formalin-fixed paraffin-embedded sections were immunohistochemically stained with antibodies to TTF-1, PDX1, SOX2, and CDX2. Homogeneous nuclear staining in more than 10% of tumor cells was considered positive.

Results: TTF-1 was only expressed in pulmonary, but not any gastrointestinal WDNETs. CDX2 was found predominantly in WDNETs from midgut (ileum, appendix) and PDX1 in those of foregut (stomach, duodenum, and pancreas). SOX2 had a more resricted expression than PDX1, mainly in gastric WDNETs. Both PDX1 and SOX2 showed variable expression in the precursor lesions of gastric WDNETs, including ECL-cell hyperplasia, microcarcinoids, and associated intestinal metaplasia. None of the markers was expressed in hindgut (rectosigmoidal) carcinoids.

Results

Site	TTF-1(+)	PDX1(+)	SOX2(+)	CDX2(+)
Lung (n=8)	5 (62.5%)	1 (12.5%)	0	0
Stomach (n=14)	0	10 (71.4%)	14 (100%)	0
Pancreatic insulinoma (n=7)	0	6 (85.7%)	0	0
Duodenum (n=5)	0	3 (60%)	0	4 (80%)
Ileum (n=11)	0	1 (9.1%)	0	11 (100%)
Appendix (n=3)	0	0	0	2 (66.7%)
Rectum (n=7)	0	0	0	0

Conclusions: Our results show that a panel of region-specific transcription factors may be useful to localize the primary site of WDNETs. Organ associated expression of region-specific transcription factors in WDNETs strongly suggests that WDNETs from different organs arise from their own precursor cells, rather than from a common universal precursor such as Amine Precursor Uptake and Decarboxylation (APUD) cells.

447 Thyroid Cancer Biomarker Discovery Using Two-Dimensional Liquid Chromatography-Tandem Mass Spectrometry (2D-LC-MS/MS)

SE Fischer, V Kulasingam, EP Diamandis, S Ezzat, SL Asa. University Health Network, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.

Background: Thyroid cancer makes up more than 90% of all endocrine cancers. Fine-needle aspiration cytology is the single most informative investigative tool, however, it has limitations and up to 40% of biopsies are either indeterminate or insufficient for conventional microscopic diagnosis. Molecular tools offer an opportunity to improve the diagnostic accuracy of this test by detecting ret/PTC rearrangements and BRAF mutations. Other molecular markers include galectin-3, CD44v6, oncofetal fibronectin, telomerase, calcitonin and carcinoembryonic antigen. However, to date, none of these markers has been shown to accurately distinguish benign from malignant, or papillary from follicular thyroid cancer. New strategies that facilitate proteomic analysis have recently been introduced for biomarker discovery but these have not yet been applied to thyroid cancer. We hypothesize that tumor cells secrete fingerprint proteins that may allow early cancer detection and sub-classification, and may predict tumor behavior and treatment response. The aim of this study is to identify differentially secreted and membrane-bound proteins in the conditioned media (CM) of human thyroid cancer cell lines using extensive fractionation by liquid chromatography (LC) by mass spectrometry (MS).

Design: TPC-1, MRO and ARO, human thyroid cancer cell lines, were incubated in serum-free medium. The conditioned medium (CM) was processed by dialysis, lyophilization, and trypsin digestion. The trypsin digested sample was fractionated by strong cation exchange LC. The eluted peptides were analyzed by tandem MS. The resulting mass spectra were searched using the MASCOT search engine on the NCBInr database.

Results: We identified 135 proteins – of these, 80 peptides were unique to the TPC-1 line while 55 others were exclusively released by MRO and ARO cells. Interestingly, none could differentiate ARO from MRO cells. The identified peptides included cell surface receptors and adhesion molecules to growth factors that may be implicated in growth and invasion. Further studies are under way to validate the most promising candidates as thyroid cancer biomarkers.

Conclusions: The use of MS combined with extensive fractionation by LC is a useful tool for cancer biomarker discovery by resolving a major part of the cell proteome.

448 Distinctive Genetic Alterations in Papillary Thyroid Carcinoma in Patients with and without Hashimoto's Thyroiditis

VR Flati, RS Saad, M Acon Laws, SD Finkelstein, JF Silverman, X Lin, YL Liu. Allegheny General Hospital, Pittsburgh, PA; RedPath Integrated Pathology, Inc, Pittsburgh, PA.

Background: Carcinogenesis involves sequential acquisition and accumulation of genetic alterations. Papillary carcinoma (PC) of the thyroid develops as a consequence of molecular changes in normal follicular cells. This progression requires years and possibly decades, and is accompanied by a number of recently characterized genetic alterations including RET/PTC and B-RAF mutations. It is well known that patients with Hashimoto's thyroiditis have an increased risk of developing PC. However, there have been no comparative studies of the genetic alterations of PC in patients with and without Hashimoto's thyroiditis. In this study, we investigated the genetic changes of papillary carcinoma in patients with and without Hashimoto's thyroiditis.

Design: Computer search identified 26 cases of PC including 13 cases with background of Hashimoto's thyroiditis. After review of each case, one slide representing the tumor was selected. Two target areas were microdissected and DNA was extracted. The genetic alterations were quantitatively determined to detect loss of heterozygosity (LOH) in 18 microsatellite repeat markers (1p,3p24,3p12,7p,9p,10q,17p,17q,18q,22q) and point mutation in B-RAF by polymerase chain reaction (PCR) with labeled oligonucleotides followed by automated capillary electrophoresis. Statistical analysis was performed using the SPSS statistical program.

Results: Statistically significant genetic alterations (LOH) observed in PC without Hashimoto's thyroiditis included 1p (69%), 10q (38%) and 22q (46%). Statistically significant genetic alterations seen in papillary carcinoma with Hashimoto's thyroiditis included 3p (46%). In addition, LOH at 3p12 and 7pDS is only seen in patients with Hashimoto's thyroiditis. In contrast, LOH at 1p (D1S1193, 46%) and 22q (46%) observed in patients without Hashimoto's thyroiditis is rarely seen in patients with Hashimoto's thyroiditis (7.6%). No statistically significant LOH involving 7p, 9p, 17p, 18q and 21q was present between the two groups. Although B-RAF mutation is more frequent in patients with Hashimoto's thyroiditis (30% vs 15%), it was not statistically significant.

Conclusions: Our results indicate that distinctive genetic alterations might be involved in the evolution of PC that develops in patients with and without Hashimoto's thyroiditis. Distinctive molecular abnormalities of PC in patients with Hashimoto's thyroiditis include 3p12 and 7pDS.

449 NF-kβ Activation Is Associated with Somatic and Germline RET Mutations in Medullary Thyroid Carcinoma

P Gallel, J Pallares, X Dolcet, D Llobet, M Encinas, V Palomar, D Mauricio, X Matias-Guiu. Hospital Universitari Arnau de Vilanova/University of Lleida, Lleida, Spain.

Background: The NF-kβ family of transcription factors regulates a wide variety of cellular processes including cell growth, differentiation, and apoptosis. NF-kβ has been shown to be activated through several signaling pathways that involve growth factor receptors. RET is frequently mutated in medullary thyroid carcinoma (MTC).

Design: A tissue microarray was constructed from paraffin-embedded blocks of 48 MTC (13 familial, 35 sporadic), previously evaluated for germline and somatic RET mutations. Immunohistochemical evaluation included members of the NF-kβ (p50, p65, p52, c-Rel, RelB) family, as well as putative targets of NF-kβ such as Flip, BclxL, and Cyclin D1. The TT cell line, which carries a RET mutation, was subjected to down-regulation of RET by si-RNA, and NF-kβ activity was subsequently evaluated by luciferase assays.

Results: Nuclear immunostaining for members of NF-kβ was frequent in MTC (p50, 19%; p65, 68%; p52, 86.6%; c-Rel, 75%; RelB, 36%). MTC with germline or somatic RET mutations (29 cases) showed NF-kβ nuclear translocation (particularly of p65, $p=0.035$) more frequently than MTC without RET mutations (19 cases). Immunostaining for putative targets of NF-kβ showed a significant statistical association between p65 and BcxL ($p=0.024$). Down-regulation of RET in the TT cell line produced a significant decrease in NF-kβ activation.

Conclusions: The results suggest that the NF-kβ is frequently activated in MTC. The results also support the hypothesis that RET activation by somatic or germline mutations may be responsible for NF-kβ activation in MTC.

450 Implications of the Changing Threshold for the Diagnosis of the Follicular Variant of Papillary Thyroid Carcinoma

K Guggisberg, S Widder, J Pasieka, M Khalil. University of Calgary and Calgary Lab Services, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada.

Background: The follicular variant of papillary thyroid carcinoma (FVPTC) is one of the most frequently encountered subtypes of papillary thyroid carcinoma (PTC). Its diagnosis is dependent primarily on the demonstration of the nuclear features of PTC in a follicular patterned lesion. Although the diagnostic criteria are well illustrated in the literature, there is a significant interobserver variation in applying these criteria even among the experts. The unexpected malignant behaviour of previously diagnosed "benign follicular lesions" at our institution, prompted us to undertake this retrospective review.

Design: All adult patients diagnosed with follicular adenoma (FA)/adenomatoid nodule, follicular carcinoma (FC) and FVPTC on resection specimens between 1993 and 2003 were identified from the files of one endocrine surgeon. The archived, H&E stained histology slides of the specimens were reviewed by 2 authors. Tumors were assessed for architecture and the presence or absence of any of the following features: capsule, capsular invasion, vascular invasion, nuclear features of PTC, psammoma bodies, extra-thyroidal extension and lymph node metastases.

Results: A total of 186 cases met the inclusion criteria. Eight cases were eliminated due to poor slide quality and unavailable tissue blocks. The original diagnoses of the cases were 43 FVPTCs (24%), 9 FCs (5%), 1 conventional PTC (<1%), 112 FAs (63%) & 13 adenomatoid nodules (7%). Review diagnoses were 68 FVPTCs (38%), 8 FCs (4%), 3 conventional PTC (2%), 64 FAs (36%) & 35 adenomatoid nodules (20%). The total number of changed diagnoses was 68 cases (38%). Clinically significant changes include 30 cases (17%) initially having a benign diagnosis that were revised to a malignant diagnosis, and 8(4%) cases initially having a malignant diagnosis that were changed to a benign entity.

Conclusions: The results of our study support previous assertions that there has been a temporal shift in the diagnostic frequency of FVPTC and confirm the presence of significant observer variation in making this diagnosis. The clinical implications of these issues raise ethical challenges both to pathologists and to clinicians and emphasize the need for more objective diagnostic methods.

451 Incidence and Survival of Thyroid Cancer with Follicular Phenotype – Papillary, Follicular, and Anaplastic

DE Henson, E Glazer, K Simpson, AM Schwartz, J Albores-Saavedra. The George Washington University, Washington, DC; LSUHSC, Shreveport, LA.

Background: From 2000-2003, thyroid cancer became the 7th leading cancer in women in the US. From 1973 to 2003, the incidence more than doubled. In a population analysis, we evaluated reasons for and the possible consequences of this increase in thyroid carcinoma.

Design: Data were obtained from NCI's Surveillance, Epidemiology, and End Results Program (SEER) from 1973-2003. In this period, 57,766 cases of thyroid cancer with follicular phenotype were recorded. For case selection, we used ICD-O codes for papillary, follicular and anaplastic histologic types. Relative survival was calculated from1988-2003.

Results: Since 1973, there were 48,982 cases of papillary carcinoma recorded in SEER, 7,791 of follicular type and 993 of anaplastic. Papillary carcinoma alone accounts for the increase of thyroid cancer in the last 30 years. The incidence of follicular carcinoma has not changed, but anaplastic has significantly decreased. Papillary carcinoma is more common in women than in men in both black and white races. In 2003, papillary carcinomas accounted for 87% of all cases reported, follicular 10%, medullary 2%, and anaplastic 1%. In black and white women, the rate of papillary carcinoma rapidly increases to age 50, then decreases. In contrast, follicular does not decrease, but continues to increase. The follicular variant of papillary carcinoma has contributed about 45% of the increase in papillary carcinoma. Papillary carcinoma has significantly increased in young adults. From 1973-1976, the rate was 2.1 cases/100,000 in women younger than 30 years, but for 2000-2003, it was 3.7 per 100,000. Anaplastic carcinoma occurs only in older age groups. For papillary carcinoma, females under 30 years have a 5-year survival of 99.7% and for follicular carcinoma, survival is 99.4%. The overall 5 year survival in all age groups was 98.8% for papillary, 96.1% for follicular, and 8.8% for anaplastic.

Conclusions: The increase of thyroid cancer is due exclusively to papillary carcinoma. The inclusion of the follicular variant in the papillary group in the 1980s has contributed to this increase. Because of the increase in young adults, excellent survival, and lack of new etiologic agents, the over-diagnosis of papillary carcinoma should be considered. Since anaplastic carcinoma results from dedifferentiation of papillary and follicular carcinomas, its decrease may be due to adequate treatment of these two tumors.

452 Expression of the Metastasis-Associated Gene 1 (MTA1) Is Associated with Malignant Behavior in Pancreatic Endocrine Tumors

MD Hofer, MC Chang, KA Lamb, MA Rubin, V Nose. Brigham and Women's Hospital, Boston, MA.

Background: Pancreatic endocrine tumors (PETs) are rare tumors with unpredictable clinical behavior. No histologic features or immunohistochemical markers reliably predict malignant progression. The molecular basis of molecular progression PETs remains unknown. The metastasis-associated gene 1 (MTA1) is thought to play a role in transcription repression and estrogen receptor interaction and is overexpressed in several human cancers, including endocrine neoplasms. Purpose of this study was to analyze the expression of MTA1 in PETs for its possible role in malignant progression.

Design: Twenty-seven cases of PETs were identified from our archive. Mean age at presentation was 57.2 years (range 28-86), 15/27 (55%) patients were male, and mean size was 4.52 cm (0.1-18 cm). The clinical follow-up data were examined and tumors classified according to the 2004 World Health Organization (WHO) criteria as benign behavior (WHO 1.1), uncertain behavior (WHO 1.2), well differentiated endocrine carcinoma (WHO 2), and poorly differentiated endocrine carcinoma (WHO 3). Histopathologic and immunohistochemical stains were evaluated and MTA1 expression scored semiquantitatively as absent, weak, moderate, or strong. Statistical analysis was performed using Kruskal-Wallis nonparametric analysis of variance with a significance level of 0.05.

Results: Six of 27 PETs (25%) were WHO 1.1, 4/27 (14%) were WHO 1.2, 10/27 (36%) were WHO 2, and 7/27 (25%) were WHO 3. Twenty (71%) tumors had equal to or less than 3 mitoses per 10 high-power fields. Two cases (7%) showed absent MTA1 expression, 3 cases (11%) had weak, 3 cases moderate, and 19 cases (70%) strong expression. MTA1 expression was significantly higher in malignant tumors with a mean staining intensity of 3.8 compared to 2.9 in benign tumors ($p=0.046$). Expression levels were significantly associated with WHO class ($p=0.028$), as well as size of tumor ($p=0.029$), and mitotic rate ($p=0.035$). MTA1 expression was associated with local invasion with borderline significance (0.062).

Conclusions: We demonstrate that MTA1 expression is significantly associated with malignant behavior in PETs. This may suggest a potential role for MTA1 in the malignant progression and metastasis of PETs. We are further evaluating the role of MTA1 as marker to predict clinical outcome in patients with PETs.

453 Over-Expression of Cellular Prion Protein Is Unique in Pancreatic Endocrine Tumors

SJ Kim, J Xu, C Li, MS Sy, W Xin. Case Western Reserve University, Cleveland, OH; University Hospitals of Cleveland, Cleveland, OH.

Background: The normal cellular prion protein (PrP^c) is found on many cell types, including pancreatic islet cells. It is a membrane protein with a glycosyl-phosphatidylinositol anchor. The normal physiologic functions of PrP^c are not fully understood. The functions of PrP^c may be cell type dependent, and include cellular adhesion, signaling, as well as pro- and anti- apoptotic activities. Pancreatic endocrine tumors (PETs) arise from pancreatic islet cells and belong to the neuroendocrine tumor (NT) family. In this study, we explored PrP^c expression in PET and its diagnostic value.

Design: Twelve cases of primary PETs, 7 gastrointestinal (GI) tract and 2 pulmonary NTs, as well as 10 metastatic neuroendocrine carcinomas (3 from pancreatic primaries, 7 from GI tract primaries) were studied. Immunohistochemical staining was performed using a high affinity monoclonal antibody, Mab 8H4, which is specific for an epitope at the C-terminal domain of PrP^c.

Results: Normal acinar cells showed spotty cytoplasmic PrP^c staining, while islet cells showed diffuse cytoplasmic staining. PrP^c expression was not detected in normal ductal epithelial cells. PrP^c was expressed in 75% (9/12) of PETs, and not expressed in any of the GI (0/7) and lung (0/2) NTs. Of the 10 metastatic neuroendocrine carcinomas, all 3 tumors from pancreatic primaries were positive for PrP^c expression, while the 7 tumors from GI primaries were negative for PrP^c expression.

Diagnosis	No. of Cases	PrPc Positive
PET	12	9 (75%)
Lung NT	2	0
GI NT	7	0
Metastatic NT, pancreas primary	3	3 (100%)
Metastatic NT, GI primary	7	0

Conclusions: Our data indicates that PrP^c is over-expressed in the majority of pancreatic endocrine tumors, and not expressed in NTs from the lungs or GI tract. Interestingly, PrP^c is over-expressed in metastatic PETs, but not in metastatic NTs from the GI tract. PrP^c can be used as an organ specific marker of pancreatic endocrine tumors. As PrP^c is an apoptosis regulator and matrix receptor, it may play an important role in the tumorogenesis of pancreatic endocrine tumors.

454 Fibroblast Growth Factor Receptors (FGFRs) 1 and 2 as Modulators of Thyroid Cancer Progression

T Kondo, S Ezzat, SL Asa. University Health Network and University of Toronto, Toronto, ON, Canada; Mount Sinai Hospital and University of Toronto, Toronto, ON, Canada.

Background: The fibroblast growth factors (FGFs) play fundamental roles in development and tumorigenesis. FGF signaling is mediated through four dedicated receptors. The prototypic FGFR consists of three extracellular immunoglobulin (Ig)-like domains, a transmembrane domain, and an intracellular tyrosine kinase domain. Alternative splicing occurs within the extracellular Ig-like domain of FGFRs 1-3, resulting in b and c receptor isoforms that exhibit distinct ligand binding specificities. The biological functions of these isoforms, however, remain to be elucidated in thyroid cancers.

Design: In this study we focused on the expression of the two principal members of the FGFR family (1 and 2) that transduce most FGF signals in thyroid tissues. We used siRNA-mediated downregulation and CMV promoter-mediated over-expression of FGFRs to determine the impact of FGFR1 and 2 on thyroid cell proliferation. In addition, epigenetic regulation of FGFR expression was analyzed by methylation specific PCR and DNA demethylation.

Results: FGFR1 and FGFR2b are reciprocally expressed in transformed and normal thyroid cells respectively. FGFR1 knockdown in thyroid carcinoma cells restrains MAPK (Erk1/2) and AKT activation and results in significant reduction of tumourous growth and invasion. In contrast, Re-expression of FGFR2b interrupts BRAF activation and consequently MAPK. Moreover, FGFR2b restrains cancer cell growth in vitro and in vivo. DNA methylation appears to be an epigenetic control mechanism involved in FGFR2b silencing in thyroid carcinoma cells.

Conclusions: The current study highlights the significance of a unique FGF receptor isoform profile in thyroid cancer cells. In contrast to the proliferative signal triggered by FGFR1, FGFR2b displays an anti-tumor effect with interruption of BRAF/MAPK activation. These data unmask the important balance mediated by FGFR1 and FGFR2b signals in modulating thyroid cancer progression.

455 Clonality of Papillary Thyroid Cancer. Clinical Implications

E Kuhn, L Teller, S Piana, J Rosai, MJ Merino. CCAPO, Milan, Italy; NCI, Bethesda; CDI, Milan, Italy.

Background: Papillary carcinoma is the most common type of thyroid cancer in the USA with approximately 20,000 news cases diagnosed each year. The morphology of these tumors is well known, but certain characteristics remain controversial. Multifocal involvement of the gland is common and its incidence has ranged from 18 to up 85%. Whether these lesions represent lymphangitic intrathyroidal spread or separate tumors is still in undecided. We report our findings regarding the clonal origins of papillary thyroid cancers.

Design: Eleven female patients ranging in age from 29-69 years (m 49.3) underwent total thyroidectomy. All patients had bilateral disease and two had associated lymph node metastasis. The size of the tumors varied from 2.5 cm to 0.1cm. Morphologically the tumors were of Classic Papillary Type (7) and Follicular variant (4). We investigated patterns of X-chromosome inactivation of multiple distinct foci of tumor using the human androgen receptor (Humara) gene. Formalin fixed paraffin embedded sections were obtained and tumor and normal cells were manually microdissected. After processing, DNA was then subjected to PCR and analyzed on the ABIPRISM 310 genetic analyzer.

Results: A total of 27 tumor nodules were microdissected; 2 tumor nodules in 7 cases, 3 tumors in 3 cases and 4 tumors in one case. DNA obtained was of good quality in 9 cases. Three cases showed completely different patterns of chromosome inactivation between the tumors consistent with different clonal cell origins. In three cases, there was clonality of two ipsilateral tumors, but different clonal patterns with the tumor present in the opposite lobe, indicative of different ipsilateral primaries. Another case show similar clonal patterns in the tumors consistent therefore, with clonal origin. One case was homozygous for the HUMARA allele and was considered non-informative. DNA from one case was of good quality but only in one tumor.

Conclusions: Our study confirms that separate tumors in the thyroid may have different clonal origins and represent different primaries especially if the tumors are present in different lobes. However, clonal lesions can also occur particularly in ipsilateral tumors. The growth of new independent tumors represented by multifocal involvement of the gland, confirms that total thyroidectomy is an adequate modality of treatment for patients with papillary thyroid cancer, to avoid the development of new tumor masses.

456 Abstract Withdrawn

457 Calcium Sensing Receptor Immunohistochemistry: An Alternative to Oil Red O for Assessment of Hyperfunctional Status in Parathyroids?

JB McHugh, JH Yim, RR Seethala. University of Pittsburgh, Pittsburgh, PA.

Background: Oil Red O (ORO), a stain for intracytoplasmic lipid, is a popular ancillary technique for assessment of parathyroid functional status. However, ORO is equivocal in a small percentage of cases, and is not possible on paraffin embedded tissues. Calcium Sensing Receptor (CaSR), a G-protein coupled receptor that modulates parathyroid hormone secretion in parathyroid tissue has been shown to be decreased in several states of hyperparathyroidism, most notably proliferating adenomas and carcinomas, making it an appealing substitute for ORO when the latter is equivocal or not possible. We report our experience with CaSR immunoexpression in comparison to ORO in the assessment of various disease states.

Design: 85 parathyroid sections were stained with a polyclonal rabbit anti-CaSR (Abcam Inc, Cambridge, MA) and evaluated for membranous staining by two observers. 7 adenomas/atypical adenomas had rims of normal tissue which were evaluated separately as well. A score was derived from the product of staining intensity (3 point scale) and percentage of positive cells. The final score was the average between the two observers. Statistical analyses were performed with SPSS software. CaSR was also compared to ORO when available.

Results: Mean scores and standard deviation for each category are summarized in table 1. Significant differences were noted by one way ANOVA ($p < 0.001$). By post hoc analysis, adenomas, atypical adenomas, carcinomas and hyperplasias each had significantly lower mean CaSR expression than normal parathyroid ($p < 0.001$). Interobserver agreement in recognition of CaSR underexpression was moderate (kappa = 0.457). ORO and CaSR were discordant in 7/42 (17%) cases. Discordant cases consisted of hypercellular glands in which CaSR was appropriately decreased but ORO was normal.

Conclusions: CaSR is decreased in all hyperparathyroid disease states with respect to normal and can thus be used to assess functional status of parathyroids, which is particularly useful when only paraffin embedded tissue is available. In some cases, CaSR may more accurately represent the functional status of a parathyroid than ORO.

CaSR Immunoexpression Scores

Category	N	Mean Score	Standard Deviation
Normal*	13	243.8	37.2
Adenomas	8	130.3	48.9
Atypical adenomas	14	93.9	33.6
Carcinomas	9	101.1	33.2
Hyperplasias	48	157.5	47.0

*includes 7 rims of normal parathyroid tissue from adenomas

458 S100A4 Expression Is Associated with Lymph Node Metastases in Papillary Microcarcinoma

HS Min, SH Park, SY Park. Seoul National University College of Medicine, Seoul, Korea; Seoul National Univeristy Bundang Hospital, Seongnam-si, Gyeonggi-do.

Background: Papillary microcarcinomas (PMCs) of thyroid are common incidental findings. Although most of them behave in an indolent manner and remain quiescent, some behave aggressively and metastasize early, giving rise to clinically significant disease. However, there have been few studies concerning predictive factors of lymph node metastasis in PMCs.

Design: We analyzed the expression of S100A4, cyclin D1, p27, MUC1, Cox-2 and the clinicopathologic features of tumor including patient's age, tumor size (> 5mm vs. ≤ 5 mm), extrathyroidal extension, multicentricity, histologic variant, sclerosis and encapsulation, in a series of 150 PMCs in relation to lymph node metastasis.

Results: On univariate analysis, extrathyroidal extension, multicentricity, tumor size more than 5 mm and expression of S100A4 predicted lymph node metastasis, whereas patient's age, expression of cyclin D1, p27, MUC1, and Cox-2 did not. On multivariate analysis, extrathyroidal extension and expression of S100A4 proved independent predictive factors of lymph node metastasis.

Conclusions: We concluded that S100A4 immunohistochemistry may be a valuable tool in predicting metastatic potential in PMCs.

459 MiRNA Expression Profiles of Thyroid Tumors: Clues for Tumor Classification, Biology, and Diagnosis

MN Nikiforova, A Riddle, M Medvedovic, DL Diorio, YE Nikiforov. University of Pittsburgh, Pittsburgh, PA; Cincinnati Children's Hospital, Cincinnati, OH; University of Cincinnati, Cincinnati, OH.

Background: Recently discovered small non-coding RNA molecules, known as microRNAs (miRNAs), function as negative regulators of coding gene expression. Expression of specific miRNAs varies between normal and cancer tissues and between different types of cancer.

Design: Expression pattern of 158 human miRNAs was determined in 32 thyroid samples including 3 normal thyroid tissues (NTs), 9 papillary carcinomas (PCs), 5 follicular carcinomas (FCs) of either conventional or oncocytic (Hurthle cell) type (OCs), 4 follicular adenomas (FAs), 4 poorly differentiated and anaplastic carcinomas, and 2 medullary carcinomas (MCs) using RT-PCR TaqMan MicroRNA Human Panel on ABI 7500 (Applied Biosystems). The Bayesian Infinite Mixture Model in the gimmR package was used to perform hierarchical clustering of samples.

Results: 148 (94%) miRNAs were found expressed in thyroid tissue. In thyroid tumors, 20% of miRNAs were upregulated and 31% downregulated with fold change >2.0 as compared with NTs. Cluster analysis of 158 miRNA demonstrated different miRNAs expression signatures for MCs, HCs and the rest of follicular cell-derived tumors. Unique expression profiles were found in PCs and FCs after filtering out miRNAs whose expression significantly deviated from NTs. Overexpression of several miRNAs correlated with specific genetic mutations (BRAF, RET/PTC, RAS, PAX8-PPARg) found in PCs and FCs. Several miRNAs were found to be highly (9-41-fold) and consistently overexpressed in PCs and FCs, but not in NTs and FAs. The top 4 miRNAs with the overall highest overexpression were mir-146b, mir-187, mir-221, and mir-222.

Conclusions: Our results indicate that a large number of miRNAs are differentially expressed in thyroid tumors as compared to normal tissue. A unique expression profile of OCs suggests that they may represent a distinct type of thyroid tumors. Expression of some miRNAs correlated with specific tumor-initiating mutations, suggesting that miRNAs may play a role in tumorigenesis. Several miRNAs may serve as novel diagnostic markers for thyroid cancer in fine needle aspiration (FNA) and surgically removed thyroid samples.

460 MEN1 Associated Pancreatic Endocrine Tumors Can Arise in Islets, but Not through an Obligatory Step of Islet Hyperplasia

A Perren, M Anlauf, T Henopp, T Rudolph, A Schmitt, A Raffel, O Gimm, E Weihe, WT Knoefel, H Dralle, PU Heitz, P Komminoth, G Kloeppel. Institute of Surgical Pathology, Zuerich, Switzerland; Kiel, Germany; Duesseldorf, Germany; Halle, Germany; Marburg, Germany.

Background: The occurrence of multiple small pancreatic endocrine tumors in patients suffering from multiple endocrine neoplasia type 1 (MEN 1) represents a unique possibility to study 'early' neoplasms and their potential precursor lesions. To date it is unknown whether small islet-like endocrine cell clusters and islets with an increased number of glucagon cells found in MEN1 patients are neoplastic or rather hyperplastic. It is also unclear whether microadenomas develop from normal or hyperplastic islets, ducts or other compartments of the pancreas.

Design: Using a technique combining fluorescence *in situ*-hybridization of the *MEN1* locus and the centromeric region of chromosome 11q with hormone immunostaining we examined pancreatic resection specimens from 4 MEN1 patients. We focused our investigations on LOH patterns in (1) typical microadenomas; (2) monohormonal endocrine cell clusters (MECC); (3) endocrine and exocrine structures entrapped in microadenomas and (4) morphologically normal islets as well as islets with an increased number of glucagon cells.

Results: Loss of one *MEN1* allele was found in all 27 microadenomas and in 19 of 20 (95%) MECC. It was absent in normal islets and islets with an increased number of glucagon cells and ductal or acinar structures. Endocrine cells localized within microadenomas of divergent immunophenotype showed retention of heterozygosity. Seven MECCs were detected in normal appearing islets, one in an islet with increased number of alpha cells and one MECC was localized in the duct epithelium.

Conclusions: Our results indicate that in MEN1 patients MECCs are monoclonal and thus represent minute microadenomas. The frequent presence of single nonneoplastic insulin positive cells in microadenomas and the occurrence of microadenomas in islets suggest an islet origin of some microadenomas. Islet hyperplasia does not seem to be an obligatory stage in pancreatic MEN1-associated tumor development.

461 RET Expression Is Increased by Neuron-Like Differentiation of Pheochromocytoma and Normal Adult Chromaffin Cells

JF Powers, KL Picard, AS Tischler. Tufts New England Medical Center, Boston, MA.

Background: Receptor tyrosine kinase RET is expressed at low levels in normal adrenal medulla and overexpressed in subsets of pheochromocytomas. Expression and activation of mutated RET is responsible for the development of pheochromocytomas in MEN2A and 2B, but the wild-type protein is also often overexpressed in non-hereditary tumors. In a recent immunohistochemical study we noted that pheochromocytoma cells expressing the highest levels of immunoreactive RET often had neuron-like morphological features, suggesting that the variable overexpression of RET in pheochromocytomas might in part be an epiphenomenon related to the known phenotypic plasticity of these tumors (JF Powers et al, Endocr Pathol 14:351-362, 2003).

Design: Primary cultures of human pheochromocytoma cells, normal human chromaffin cells and normal rat chromaffin cells were maintained in control medium or in the presence of nerve growth factor (NGF) for 2 weeks to study the effects of NGF-induced neuron-like differentiation on RET expression. The PC12 rat pheochromocytoma cell line line was studied in parallel experiments to determine the time course of induction, the effects of signaling pathway inhibitors on induction and the biological effect of induced Ret on cell survival and apoptosis after NGF washout in serum-free medium.

Results: Immunoblots demonstrated NGF-stimulated Ret increases in normal and neoplastic chromaffin cells of both humans and rats. PC12 cells, which express low levels of Ret in control medium, showed marked increases as early as 1 day after NGF exposure, before the onset of neurite outgrowth. The increases were abrogated by inhibitors of RNA and protein synthesis (actinomycin D and cycloheximide), indicating induction rather than stabilization of protein or mRNA, and were partially blocked by inhibitors of MAP kinase and protein kinase C (U0126 and Gö6976). Although the NGF receptor TrkA and Ret are known to utilize partly overlapping signaling pathways, the Ret-activating ligand GDNF could not substitute for NGF in promoting survival, measured by cell counts or metabolic assay, or in preventing apoptosis measured by caspase-dependent PARP cleavage.

Conclusions: Ret expression is increased in pheochromocytoma and normal chromaffin cells by activation of signaling pathways that promote neuron-like differentiation. Whether up-regulation of wild-type Ret plays a role in the pathobiology of pheochromocytomas or is merely a lineage marker for cells able to respond to those signals remains unknown. (supported by NIH grants CA48017 and NS37685).

462 NFκB and HIF-1α Pathways Are Down-Regulated in Follicular Thyroid Neoplasms but Not in Hyperplastic Nodules

S Rawlins, A Blanes, SJ Diaz-Cano. King's College Hospital, London, England, United Kingdom; University of Malaga School of Medicine, Malaga, Spain.

Background: This study aims to evaluate topographically both vascular and inflammatory signaling pathways by tumor phenotype in follicular thyroid carcinomas (FTC).

Design: We selected adenomatous hyperplastic nodules (FTHN, 18), adenomas (FTA, 19), carcinomas (FTC, 15 minimally-invasive and 15 widely-invasive), and anaplastic carcinomas (ATC, 10) (WHO criteria) to analyze by topographic compartments (internal/peripheral): NFκ-p50, NFκ-p65, HIF1α immunostaining, and low-density selective cDNA array (LD-SelGEA; p50, p65, IL6, TNFα, cyclin D1, IκB, rel B, HIF-1α, TGFα, VEGF, IFG2, PDGF, EGFR). 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 molecular profile: RAS mutation (8 FTA, 7 minimally-invasive FTC, 5 widely-invasive FTC, 4 ATC), PAX8/PPARγ fusion gene (2 FTA, 1 minimally-invasive FTC, 7 widely-invasive FTC), and TP53 LOH/mutation (4 ATC) and combinations (3 widely-invasive FTC, 2 ATC).

Results: Across all neoplasms, expression was down-regulated with respect to the controls and higher in the internal compartments, NFκB-p50/p65 ($p<001$) and HIF-1α ($p=0.049$) immunoexpressions increasing with increasing neoplastic grade accordingly (in particular for FTC with coexistent genetic alterations, RAS-PAX8/PPARγ in widely invasive and RAS-TP53 in ATC). These results were supported by down-regulation of p50, p65, IL6, TNFα, rel B, HIF-1α, TGFβ, VEGF, IFG2, and up-regulation of IκB in LD-SelGEA, as well as the absent of tumor infiltrating lymphocytes in 55/59 (93%) neoplasms. FTHN showed an exceptional level of HIF1α expression in the internal compartment (~25x the control value).

Conclusions: Down-regulation of NFκB pathways (p65 and p50) and HIF-1α pathways contributes to the poor intratumoral inflammatory response in follicular thyroid neoplasms, and plays a side role in tumor progression. Hyperplastic nodule growth is directly related with HIF1α upregulation and central hypoxia; this marker can be diagnostically useful for the distinction nodule/neoplasm.

463 CK19 Can Improve the Prognostic Value of the WHO Classification of Pancreatic Endocrine Tumors

AM Schmitt, M Anlauf, S Schmid, F Riniker, J Bauersfeld, A Barghorn, PU Heitz, H Moch, P Komminoth, A Perren. Institute for Surgical Pathology, Zurich, Switzerland; Institute for Pathology, Kantonsspital Baden, Baden, Switzerland; Institute for Clinical Pathology, Kiel, Germany.

Background: Predicting biological behaviour remains an issue in pancreatic endocrine tumors (PET). The 2004 WHO classification is based on clinico-pathological criteria and is a reliable tool to predict tumor free (DFS) and tumor specific survival (DSS). Immunohistochemical detection of cytokeratin 19 (CK19) has been proposed as another predictor of survival. Our aim was 1. to examine the prognostic power of CK19 in an independent patient collective and, 2. if a prognostic power could be confirmed, to find out if CK19 is a prognostic factor independent from the 2004 WHO classification.

Design: 145 PET samples from the archives of the Departments of Pathology Zurich and Basel were assembled on a tissue micro-array (TMA). CK19 expression was examined by immunohistochemistry. Tumors were scored as positive when at least some endocrine cells stained positive. The results were correlated with follow-up data available for 121 patients (median follow-up time 72 months, mean follow-up time 89.1 months) by the Kaplan-Meier method. The prognostic independence from the WHO 2004 classification was examined by multivariate analysis using the Cox Proportional Hazard Model.

Results: 69 of 145 PET were positive for CK19. CK 19 positivity correlated strongly with shortened DFS and DSS in univariate analysis ($p<0.001$). In multivariate analysis of the 2004 WHO classification and CK19 positivity the latter retained statistical significance with regard to DFS and DSS.

Conclusions: We could confirm the prognostic significance of CK19 positivity in our series of 121 PET patients. Furthermore, CK19 immunohistochemistry remained significant in multivariate analysis with the WHO classification. CK19 was able to further subdivide the WHO risk groups regarding DFS and DSS. We therefore suggest CK19 immunohistochemistry as an independent prognostic marker in PET which refines the prognostic power of the 2004 WHO classification.

464 Follicular Lesions of the Thyroid with Partial Features of Papillary Thyroid Carcinoma: Are They Borderline Tumors?

T Scognamiglio, CC Lubitz, J Kao, TJ Fahey III, YT Chen. Weill Medical College of Cornell University, New York, NY.

Background: Although the distinction between follicular lesions [follicular adenoma (FA) and follicular variant papillary thyroid carcinoma (PTCFV)] can easily be made in most cases, encapsulated follicular lesions with partial nuclear features of papillary thyroid carcinoma (FLPTC) are occasionally encountered, raising the possibility of biologically borderline lesions. In this study we sought to define this subset of lesions and their relationship to FA and papillary thyroid carcinoma (PTC).

Design: Eleven cases of FLPTC were identified. Immunohistochemistry (IHC) for cytokeratin 19 (CK19), galectin-3 (GAL3), and HBME1 was performed and 2-3+ staining in >10% of cells was scored positive. RAS and BRAF mutations were analyzed by DNA sequencing following microdissection. DNA microarray was done on these 11 cases, 10 PTC (including 2 PTCFV), and 8 benign follicular lesions (4FA, 4 hyperplastic nodules). Unsupervised clustering was performed using a list of 25 differentially expressed genes previously defined from a training set of 26 benign lesions (FA and hyperplastic nodules) vs. 24 PTC (including 13 PTCFV).

Results: One case was positive for all 3 antibodies, 2 were positive for 2 (2 GAL3/HBME1) and 8 were positive for only one (1 CK19, 5 HBME1 and 2 GAL3). Mutational analysis showed 1 NRAS61 and 1 HRAS12 mutation but no BRAF mutation. Unsupervised clustering of the microarray data accurately assigned all 8 benign follicular lesions and 10 PTC/PTCFV cases into their correct clusters. In contrast, 6 of the 11 study cases formed a third cluster, with 3 of the remaining cases clustering with the benign lesions and 3 with PTC. Moreover, the 6 cases that clustered with the benign or PTC groups were found to be closer to the "intermediate" cluster than the other cases of benign follicular lesions or PTC/PTCFV. No correlation was found between the IHC pattern, RAS mutations, and the expression profiles of these cases.

Conclusions: FLPTC were similar to FA in that they showed occasional RAS mutations and no BRAF mutation. However, the IHC findings were intermediate of typical FA and PTC. In parallel, these cases had gene expression profiles intermediate of FA and PTC, with some cases closer to FA and others to PTC. Our findings suggest that this subset of tumors are likely true biological intermediates between FA and PTC. As the clinical behavior of these "borderline" lesions is currently unknown, usage of the proposed term "well differentiated tumor of uncertain malignant potential" might be justified.

465 Fibroblast Growth Factor Receptors In Pancreatic Endocrine Tumors

S Serra, R Chetty, S Ezzat, SL Asa. University Health Network and University of Toronto, Toronto, ON, Canada; Mount Sinai Hospital and University of Toronto, Toronto, ON, Canada.

Background: Fibroblast Growth Factor Receptors (FGFRs) are a family of transmembrane receptors involved in mitogenesis, angiogenesis and carcinogenesis. Down-regulation of FGFR2 is associated with malignant progression. FGFR1 is expressed in differentiated cells and in benign and malignant tumors, whereas both FGFR1 and 3 are absent in poorly differentiated carcinoma cell lines. FGFR4 has a role in cancer progression and is expressed in aggressive neoplasms, in anaplastic and rapidly proliferative cell lines. The aim of this study was to examine the expression of FGFRs in pancreatic endocrine tumors (PETs) and to correlate expression profiles with clinico-pathological parameters.

Design: Fifty cases of PET were retrieved from the archives of the Department of Pathology, UHN, and utilized for tissue microarray construction. Tumor size, presence/absence of necrosis, invasiveness/demarcation, lymphovascular invasion, lymph node and liver metastasis were recorded. Mitotic count and MIB1 index were assessed. The TMA blocks were stained with an extensive panel of endocrine markers as well as with specific antisera to FGFR1, 2, 3 and 4.

Results: 24 patients were male and 26 female, ranging in age from 23 to 80 years. Seven patients had MEN1 syndrome and 1 had von Hippel-Lindau disease. The tumors size ranged from 0.8 cm to 8.7 cm. Tumors were divided into four groups according to their stage: 22 were localized to the pancreas, 12 showed lymphovascular involvement, 10 had lymph node and 6 hepatic metastases. FGFRs showed mainly cytoplasmic immunoreactivity of variable intensity and less frequently membranous or nuclear

positivity. FGFR1 and 2 were localized in all 50 cases studied. FGFR3 was positive in 90% (20/22) of the PETs localized to the pancreas, 83% of those with lymphovascular invasion, 60% and 50% of tumors with lymph node and hepatic metastasis, respectively. FGFR4 immunoreactivity was present in 23% of PETs localized to the pancreas, in 16% of those with lymphovascular invasion, and in 50% of cases with liver metastasis. None of the cases with lymph node spread expressed FGFR4.
Conclusions: FGFR1 and 2 are expressed in PETs irrespective of tumor size and stage, indicating that they are not likely to be involved in PET progression. FGFR3 appears to be lost gradually during PET progression. FGFR4 is expressed mainly, but not exclusively, in metastatic tumors and may represent a marker of more aggressive PETs.

466 MicroRNA Profiling of Papillary Thyroid Carcinoma Cell Lines

P Smyth, S Cahill, K Denning, J Li, SP Finn, S Guenther, R Henfrey, JJ O'Leary, O Sheils. Trinity College Dublin, Dublin, Ireland; Applied Biosystems, Foster City, CA.
Background: Activating mutations in BRAF and ret/PTC rearrangements are frequent genetic changes in papillary thyroid carcinoma (PTC). MicroRNAs (miRNAs) are a group of non-coding single stranded RNAs measuring approximately 22nt in length that have been found to control cell growth, differentiation and apoptosis. miRNAs negatively regulate their target genes by translational repression. Components of the miRNA machinery have been implicated in tumorigenesis. Furthermore, miRNA expression profiling correlates with various cancers, with these genes thought to act as both tumour suppressors and oncogenes. This study was undertaken to identify a miRNA signature for PTCs harbouring RET rearrangements and BRAF mutations and to elucidate the role of miRNAs in the development of PTC.
Design: Differential expression of 157 human miRNAs were analysed using a panel of four PTC cell lines, harbouring both native and transfected ret/PTC-1 rearrangements and BRAF (V600E mutations) and a normal thyroid cell line. Analysis was carried out using a stem-looped RT followed by TaqMan® PCR.
Results: 38 miRNA targets had >2-fold change in V600E mutated cell lines, the most significantly being mir-200a, mir-200b, mir-141, mir-127, mir-130a and mir-144. 35 miRNA targets had >2-fold change in ret/PTC-1 cell lines. Those showing significantly higher fold change differences included mir-128a, mir-128b, mir-135b, mir-200a, mir-154*, mir-181a, mir-302b and mir302c.
Conclusions: It has been suggested that miRNA signatures may be more effective than profiles of protein coding genes in distinguishing tumours from benign lesions. This study is an in vitro model for the biological and regulatory processes that occur in human thyroid diseases due to ret/PTC and BRAF mutation. Finally, future exploration on a larger cohort of samples will hopefully help aid in the identification of miRNA biomarkers. As miRNAs are stable, abundant and easily detectable they represent ideal candidates for effective diagnostic biomarkers.

467 Clinical Significance of Crooke's Cell Change in Pituitary Corticotroph Cell Adenomas

M Takei, H Kajiya, N Egashira, S Takekoshi, Y Ishii, S Tahara, S Teramoto, RY Osamura. Tokai University of School of Medicine, Isehara, Kanagawa, Japan; Nippon Medical School, Bunkyo-ku, Tokyo, Japan.
Background: Crooke's cells have been described as perinuclear "ring-like" accumulation of keratin filaments in corticotophes. Recentry, corticotroph adenomas with prominent Crooke's change have been designated as Crooke cell adenoma(CCA). (WHO classification 2004) Some reports have claimed CCAs are more aggressive compared with typical corticotoroph adenomas. In this study, we examined the relationship between the Crooke' cell change and clinical features in pituitary corticotroph adenomas.
Design: Among total 912 cases pituitary adenomas which underwent surgery at Nippon Medical School total 26 cases of pituitary corticotroph cell adenomas (14cases: Cushing disease microadenoma M/F=3/11, 5cases: Cushing disease macroadenoma M/F=2/3, 7cases: silent corticotoroph adenoma(SCA) M/F=4/3) were subjected to the current study. In the study, we defined macroadenoma as the tumors with minimum tumor diameter exceeds 20mm or more . Minimum tumor diameter was also grater than 20mm in SCAs. In order to evaluate Cushing's chage, these tumors were subjected to H&E staining and to immunohistochemical staining for CAM5.2 on the 4% paraformaldehyde fixd paraffin sections. The cells which were stained as eosinophilic and hyalinized perinuclear cytoplasm and with positive CAM5.2 were interpreted as Crooke cells. The staining was graded strongly positive(>50% of tumor cells), positive (<50%) and negative.The cases with strongly positive were interpreted as as CCA.
Results: Eight cases(63%) of Cushing disease exhibited Crooke cells. In this disease, all macroadenomas contained Crooke cells. One macroadenoma was interpreted as CCA. This particular case required four times of operation during seven and a half years follow-up. SCA cases were classified into basophilic type(3 cases) or chromophilic type(4cases). One SCA case with regrowth in was also interpreted as CCA.The Crooke cells tended to appear in the tumor with large tumor size. In particular, two reccurent cases and one case with tumor regrowth were CCA . We did not find CCAs in any microadenomas.
Conclusions: Crooke cell adenomas(CCAs) were observed in macroadenomas and not in microadenomas. One multiple recurrent case which needed repeated surgery was also CCA. The results of our study suggest the importance of distinction between typical corticotroph adenoma and CCA in surgical pathology. Recognition of Crooke cells requires immunohistochemical staining for keratin(CAM5.2).

468 Clinical and Morophological Characteristics of Functioning Pituitary Microadenomas and Macroadenomas: Analysis of Large Series

M Takei, H Kajiya, N Egashira, S Takekoshi, S Tahara, A Teramoto, RY Osamura. Tokai University School of Medicine, Isehara, Kanagawa, Japan; Nippon Medical School, Bunkyo-ku, Tokyo, Japan.
Background: Many pituitary tumors undergo transsphenoidal surgery under the diagnosis of microadenomas(microAs) or macroadenomas(macroAs). This study is aimed at to elucidated the clinical and morphological characteristics of microAs and macroAs.The differences of clinical and pathological nature of these two types of the adenomas was also attempted.
Design: Total 281 cases of pituitary adenomas which were operated at Nippon Medical School were classified into microAs(<1cm) or macroAs(>1cm). Immunohistocheical study was done on formalin fixed parfaffin embeddes section for various hormones, subunits and keratin(CAM5.2). The detection was done by indirect immunoperoxidase method.
Results: 184 cases was macroAs and 97cases were microAs. Among 97 cases of microAs, 42 cases(43.3%), 28cases(28.9%), and 26cases(26.8%) were ACTHomas,PRLomas, and GHomas. The proportion of macroAs are as follows ; GHomas-108/134(80.1%),PRLomas-55/83(66.3%),ACTHomas-10/52(19.2%),TSHomas-11/12(91.7%). Frequent multihormonal GHomas showed the combination of GH+PRL+αSU in 14/51(51.9%) and in 47/97(48.5%) in microAs and in macroAs respectively. There was no significant difference in phenotypes was noted between microAs and macroAs in GHomas. Typical dot-like keratin inclusions(fibrous body) were noted only in three of eight cases of macroGHomas. Cytoplasmic keratin staining was more frequently observed in microACTHomas. 42 of 52 cases(80.7%) of Cushing's adenomas were microAs. All eleven silient corticotroph cell adenomas were macroAs. Perinuclear keratin filaments has been designated as Crooke's change. The adenomas with diffuse Crooke's change(Crooke's cell adenomas:CCAs) were only observed in macroAs. These findings indicate that the microAs and macroAs show discernible difference in their function and morphological characteristic. It is important to be aware of these different features between microAs and macroAs for the appropriate pathological analysis and patient care. Two forms of keratin deposition(fibrous body and Crooke's chage) were more frequently seen in macroAs and suggested their anti-cell death effects including apoptosis.
Conclusions: Our study suggests that microAs and macroAs include different proportion of functional and morphological characteristics. Differentiating microAs and macroAs is important in clinical practice, as some macroAs(with prominent Crooke's change) behave worse.

469 Expression Profiles of micro-RNA in Paraffin Embedded Tissue from Benign and Malignant Thyroid Lesions

MT Tetzlaff, A Lu, G Xu, D Baldwin, SR Masters, VA LiVolsi, ZW Baloch. University of Pennsylvania Medical Center, Philadelphia, PA.
Background: Thyroid cancer, the most common endocrine malignancy, is typified by a number of classical genomic insults. Recent studies have found molecular signatures that could be exploited as an adjunct to morphologic parameters as a diagnostic algorithm for thyroid malignancy. MicroRNAs (miRNAs) are endogenously synthesized non-coding RNAs and function as regulators of gene expression at the post-transcriptional level. They have been implicated in tumorigenesis, and the identification of unique miRNA profiles in different tumors underscores their utility in more refined efforts of tumor diagnosis, classification and therapy. In this study, we used microarrays to identify aberrantly expressed miRNAs in classical papillary thyroid cancer (PTC).
Design: Paraffin-embedded surgical samples from 10 cases of multi-nodular goiter, 5 of classical papillary thyroid cancer (c-PTC) and 5 cases of follicular variant of papillary thyroid cancer (fv-PTC) were assessed for miRNA expression. The expression patterns of ~750 characterized and predicted human and mouse miRNAs were evaluated. The relative differences in signal strength were assessed by unpaired T-testing ($p<0.030$) and Statistical Analysis of Microarrays (SAM) (to correct for multiple hypothesis testing), imposing a 90% false detection rate (FDR)=0.
Results: Expression of 7 miRNAs was significantly reduced in c-PTC as compared with goiter: hsa-let-7b was reduced 6.5-fold (*p=0.013*); hsa-mir-103 was reduced 7.4-fold (*p=0.009*); hsa-mir-24 was reduced 7.8-fold (*p=0.011*); hsa-mir-23 was reduced 13.5-fold (*p=0.015*); hsa-mir-16 was reduced 11.2-fold (*p=0.013*) and hsa-mir-26a was reduced 12-fold (*p=0.030*). An uncharacterized miRNA (*pred00127*) was reduced 7.4-fold (*p=0.004*). No significant difference were noted between goiter and fv-PTC. However, several significant differences emerged when comparing c-PTC to fv-PTC: hsa-mir143 (*p=0.013*) was ~48-fold and hsa-mir-422b (*p=0.008*) was 12.2 fold reduced.
Conclusions: MiRNA profiling experiments can be successfully performed in formalin-fixed paraffin embedded thyroid tissue. We identified miRNAs unique to c-PTC versus goiter: notably, the let-7 family of miRNAs which have been implicated as repressors of RAS and mir-26 which has been previously shown to be decreased in PTC compared to benign thyroid. This study displays a molecular heterogeneity between classical PTC and fv-PTC.