were analyzed by unsupervised hierarchical cluster analysis. The clustering results were further tested statistically and for expression of proapoptotic molecules.
Results: (1) The cases were clustered into four categories, each with a characteristic expression pattern of the antigens. (2) The clusters were further analyzed by pairwise nonparametric test for their differences in antigen expression. Significance analysis of microarray indicated that MART1, MIB1 and BIRC5 were significant in separating the clusters, while the other antigens and clinical variables were not. (3) Importantly, on further testing of expression of key pro-apoptotic molecules, the clusters differed in expression of the mitochondrial protein SMAC/Diablo, an antagonist for the inhibitor of apoptosis proteins.
Conclusions: Hierarchical clustering analysis, even of small tissue microarray dataset, could help reveal hidden patterns not recognizable by conventional clinicopathological and statistical analysis. Clustering generates models testable for their biological significance. We emphasize the exploratory and hypothesis-generating function of clustering.

Endocrine

409 ISL1 Expression in Human Pancreas and in Pancreatic Endocrine Tumors

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Background: The LIM homeodomain protein Isl1 is expressed in developing pancreas in rodents. It is essential for the generation of pancreatic endocrine cells: targeted disruption of the *isl1* gene results in an early arrest of embryonic development with abnormality of the pancreas anlage and complete absence of endocrine cells. Isl1 expression in normal and neoplastic human pancreas has not yet been evaluated.
Design: To evaluate Isl1 protein and mRNA expression in developing and adult human pancreas and in pancreatic and extrapancreatic endocrine tumors. Methods. Normal samples of fetal (4) , pediatric (3) and adult (10), paraffin embedded pancreatic tissues were evaluated for Isl1 expression with immunohistochemistry utilizing two different monoclonal antibodies. A large series of pancreatic endocrine tumors -PET-, including benign (50), borderline (29), well differentiated (30) and poorly differentiated carcinomas (10), pancreatic ductal adenocarcinomas (20), lung (30) and gastrointestinal (60) endocrine tumors were immunostained. Selected samples of normal (4) and neoplastic cases (10) were evaluated for Isl1 mRNA expression with a RT-PCR technique on frozen material.
Results: Isl1 was expressed in the nuclei of pancreatic endocrine cells from the beginning of endocrine differentiation in the fetus till in the adult life. Isl1 reactivity was confined to the endocrine pancreatic compartment. All insulin and glucagon positive cells coexpressed Isl1. Both antibodies gave the same reaction pattern. No Isl1 immunoreactivity was observed in ductal adenocarcinomas. Nuclear Isl1 immunoreactivity was present in 87% of PET cases. Poorly differentiated pancreatic endocrine carcinomas displayed a decrease or absence of Isl1 immunoreactivity; no correlation with prognosis was observed. Ileal carcinoid cases were negative for Isl1, as well as most other low grade pulmonary and GI tract endocrine tumors; intense Isl1 staining was present in lung and GI small cell carcinomas. RT-PCR analysis paralleled ICH results.
Conclusions: Isl1 is expressed in developing and adult human endocrine pancreas, at variance with rodent pancreas where its expression is limited to the embryonic life. Isl1 expression is preserved in most pancreatic endocrine tumors but is absent in most lung and GI tract endocrine tumors, with the exception of small cell carcinomas, with potential diagnostic applications.

410 Precursor Lesions in Patients with Multiple Endocrine Neoplasia Type 1-Associated Duodenal Gastrinomas

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Background: The identification of precursor lesions has a great impact on the understanding of tumorigenesis. Precursor lesions of endocrine tumors are known to occur in the setting of the MEN1 syndrome. It was the aim of our study to test the hypothesis that MEN1-associated duodenal gastrinomas originate from diffuse preneoplastic gastrin cell changes. Precursor lesions may precede the development of duodenal gastrinomas, since, in contrast to sporadic gastrinomas, these tumors are usually multiple.
Design: The distribution of endocrine cells in the nontumorous duodenal tissue was analyzed qualitatively and quantitatively in 25 patients operated on for a duodenal gastrinoma. The MEN1 status was assessed clinically and by PCR based mutational analysis.
Results: Fourteen of 25 gastrinoma patients had proliferative, hyperlastic lesions consisting of gastrin cells in the nontumorous duodenal mucosa similar to the gastric ECL cell lesions observed in chronic atrophic gastritis. All ZES patients with proven MEN1 had such proliferative gastrin cell lesions, and all ZES patients without precursor lesions were MEN1 negative.
Conclusions: Duodenal gastrinomas in MEN1, but not sporadic duodenal gastrinomas, are associated with proliferative gastrin cell changes within the nontumorous mucosa. It is likely that these lesions precede the development of MEN1-associated duodenal gastrinomas.

411 Differential Topographic Kinetics in the Progression of Follicular Thyroid Neoplasms

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Background: The kinetic differences (proliferation and apoptosis) and their correlation with telomere length and telomerase expression by topographic compartments have not been studied in follicular thyroid proliferative lesions to date.
Design: We selected 82 follicular thyroid proliferative lesions (9 hyperplastic nodules, 22 adenomas, 14 minimally-invasive carcinomas, 24 widely-invasive carcinomas and 13 anaplastic carcinomas, classified according to WHO criteria). Representative samples were evaluated and selected for Ki-67 and telomerase immunostaining, In situ end labeling (ISEL) of DNA fragments (to detect apoptosis using Klenow fragment of DNA polymerase), and FISH-PNA of telomere in peripheral and internal tumor compartments. Appropriate controls were run in each sample. The results were statistically compared using analysis of variance and Student t-test, and considered significant if $P<0.05$.
Results: The Ki-67/ISEL indices revealed the predominant kinetic advantage in the internal compartments of benign follicular thyroid proliferative lesions and in the peripheral compartments of malignant follicular thyroid proliferative lesions due to statistically significant decrease of ISEL indices at internal compartment of benign follicular thyroid proliferative lesions (5.3±7.8% vs. 1.3±2.4%; P=0.0213) and at peripheral compartments in carcinomas (1.4±1.7% vs. 2.3±5.5%; P=0.0474), respectively. Telomerase expression was significantly higher in the internal compartments ($p<0.001$) and in malignant lesions ($p<0.001$), which only correlated with PNA-FISH detectable telomeres in internal compartments. PNA-FISH detectable telomeres in more than 20% of peripheral tumor cells was observed in high-grade lesions (widely-invasive and anaplastic carcinomas) only.
Conclusions: 1. Inverse and opposite proliferation/apoptosis correlations characterized follicular thyroid proliferative lesions, the kinetic advantage predominating in the internal compartment of benign lesions and in the peripheral ones of malignant lesions. 2. The direct telomere-telomerase correlation characterizes expansive internal tumor compartments, while the telomere preservation (even in the absence of detectable telomerase expression) at peripheral compartments kinetically defines high-grade lesions.

412 Adrenal Myelolipomas Show Non-Random X-Chromosome Inactivation in Hematopoietic Elements and Fat: Support for a Clonal Origin of Myelolipomas

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Background: The question of whether or not the hematopoietic cells of myelolipoma are truly 'normal' has not been evaluated extensively. In at least one study, a clonal cytogenetic abnormality was identified in a myelolipoma. We examined histologic, immunohistochemical features and comparisons of X-chromosome inactivation patterns in 19 myelolipomas.
Design: Formalin-fixed, paraffin-embedded archival tissue from 19 myelolipomas (16 adrenal; 3 – presacral) was stained with H&E and for CD138, CD34, CD117, CD42a, Hgb, myeloperoxidase, collagen IV, nerve growth factor receptor and reticulin. Histologic evaluation included: overall cellularity of hematopoietic tissue, myeloid to erythroid ratio, and numbers of megakaryocytes. X-chromosome inactivation analysis was performed on hematopoietic tissue, fat and adjacent adrenal tissue (normal control) from 11 female patients by PCR.
Results: Myelolipomas showed wide variation in cellularity (5% to 90%) with no relationship between cellularity and the patient's age. All of the myelolipomas demonstrated normal trilineage hematopoiesis and cellular morphology, with only rare early myeloid precursors were shown to be present in these lesions (CD117, CD34 staining). Most (14/19) had numerous megakaryocytes. The majority had a stromal composition and vascular patterns that were different from those of normal bone marrow. 18/19 cases showed reticulin fibrosis, 14/19 cases showed an increased nerve growth factor receptor and reticulin staining, suggesting increased stromal elements. X-chromosome inactivation studies demonstrated non-random X-chromosome inactivation in 8/11 myelolipomas from female patients, suggesting that both the fat and hematopoietic elements are a single, clonal population.
Conclusions: Non-random X-chromosome inactivation in fat and hematopoietic cells suggests that myelolipomas are clonal proliferations rather than hamartomas. These lesions have significant morphologic and stromal differences from the normal bone marrow.

413 Expression of Notch3 Protein in "High Altitude Paragangliomas"

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Background: Notch proteins are transmembrane receptors that participate in cell fate decisions during development and may play a role in differentiaton of neuroblastomas and other tumors. In a previous pilot study of genetically characterized pheochromocytomas (PC) and extra-adrenal paragangliomas (PGL) we found diffuse Notch3 immunoreactivity in 2 apparently sporadic adrenal PC and in 1 of 2 extra-adrenal PGL with a succinate dehydrogenase (SDH) mutation. PC occurring in von Hippel-Lindau (VHL) disease showed focal staining, while other syndromic tumors were negative. Both VHL and SDH tumors express markers of hypoxic signaling. The previous findings therefore suggested an association of Notch3 with an uncharacterized subset of PC/PGL and a potential association with hypoxia.

Design: Ten PGL were studied from Mexican patients who had lived at altitudes above 2000m. Nine tumors were carotid body PGL and one was retroperitoneal. The tumors were screened for mutations of SDHB and SDHD associated with syndromic PGL. Paraffin sections of the tumors were stained immunohistochemically for Notch3 after microwave antigen retrieval, using two different polyclonal antibodies against the C-terminal region. Five PGL from patients in Boston (3 carotid,1 vagal, 1 subclavian) were also studied by IHC. Neuroblastoma tissue served as a positive control and irrelevant IgG as a negative control.
Results: Sequence analysis established that all of the high altitude PGL contained wild-type SDHD and SDHB. Chief cells of all high altitude PGL showed diffuse nuclear immunoreactivity for Notch3. Two of 3 CB PGL from Boston patients were also positive, while the vagal tumor was focally positive and the jugular tumor was negative.
Conclusions: High altitude PGL from patients in the Andes have not previously been genetically characterized. We have shown that these tumors do not harbor known mutations associated with syndromic PGL. They do show diffuse immunoreactivity for Notch3 that may not be attributable solely to tumor location. It remains to be determined whether this results from hypoxia or from as yet unidentified genetic changes affecting Notch pathways. Supported by NIH grant CA48017 (AST).

414 Loss of PTEN Expression Is Associated with Malignant and Borderline Paraganglioma

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Background: Paraganglioma/pheochromocytoma (PG) are uncommon tumors. Currently, only metastasized tumors are considered malignant with certainty. Although some atypical histologic features (e.g. confluent necrosis, over 5 mitotic figures (MF)/ 10 high power field (HPF), capsular or vascular invasion), have been suggested as being associated with malignancy, histologic criteria for malignant PG are still elusive. Some recommend that a tumor with one or more of above atypical histologic features should be classified as borderline. To date, there are no molecular markers useful for distinguishing malignant from benign PGs. One candidate is PTEN, a tumor suppressor gene, for which we previously demonstrated high levels of PTEN expression in fetal and adult adrenal medulla and ganglion cells (AIMM 2002; 10:139-46). We now consider whether PTEN expression levels correlate with malignancy or borderline PGs.
Design: We retrospectively reviewed 39 PG cases from Montefiore Medical Center and immunostained all with a mouse monoclonal antibody against PTEN (Santa Cruz Biotech, 1:100 dilution). Case included one malignant (metastatic in bone), seven borderline and 31 benign PGs. PTEN immunostaining intensity (weak, moderate and strong, or 1,2,3+) and percentage of tumor cell staining were evaluated. Less than 5% of tumor cells with weak immunoreactivity were interpreted as negative.
Results: The malignant PG had no PTEN staining. Six of seven (86%) borderline PGs were also negative for PTEN and one had low PTEN staining (+, 10%). In contrast, 30 of 31 (97%) benign PGs showed moderate to strong PTEN immunostaining (2-3+, 10-90%) and only one showed weak staining (1+, <5%), which was interpreted as negative.
Conclusions: PTEN expression is present in benign PG, and mostly lost in borderline and malignant PGs, suggesting that it has a role in suppressing malignant behavior in this tumor. PTEN immunostaining is therefore potentially useful for identifying borderline and malignant PGs.

415 Cystic Metastases from Papillary Thyroid Carcinoma Have Frequent BRAF Mutations

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Background: Cystic neck masses can be the presenting symptom from an occult papillary thyroid carcinoma. Usually, the mass will be aspirated and the fluid sent for cytologic analysis. The diagnosis on cytology can be difficult, since epithelial tumor cells are scant and are present in a background of abundant cyst debris. Clinicians are alerted to the possibility of false negatives from the cytology and monitor these patients carefully. BRAF mutations have been recently described to be particularly prevalent in conventional papillary thyroid carcinomas and can be present in up to 60% in some series. We assessed cystic metastases for the present of BRAF mutations to determine whether this might be a useful marker in the workup of the cystic neck mass.
Design: Well-characterized cystic metastases from conventional papillary thyroid carcinoma were included in this study. The histologic slides were reviewed and the diagnosis was confirmed. Tissue was microdissected and DNA was extracted from these samples and PCR was performed for exon 15 of the BRAF gene. The PCR amplicon was then used for cycle sequencing using an automated sequencer and the BigDye Terminator kit (ABI, Foster City, CA). The sequence was assessed for the typical T1799A BRAF mutations.
Results: Ten cases of cystic metastases from conventional papillary carcinoma were included in this study. DNA extraction and PCR amplification was successful in all cases. The BRAF point mutation was identified in 4/10 (40%) of these cases.
Conclusions: The presence of BRAF mutations in cystic metastases from conventional papillary carcinoma suggests that this analysis could be useful in the workup of a cystic neck mass. Using the molecular test as a supplement to the conventional analysis, such as cytologic or histological sampling, may improve diagnostic sensitivity.

416 Differential Gene Expression Profiling in Differentiated Thyroid Neoplasms and Non-Neoplastic Thyroid Tissue

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Background: The pathologic diagnosis of thyroid tumors relies on a set of histomorphologic criteria that do not consistently correlate with clinical behavior especially with the follicular tumors. Attempts to identify biomarkers that may aid in the diagnosis and biologic stratification of these tumors have been unsuccessful to date. A broad investigation of the genome of these tumors may identify novel markers that can be used to predict biologic behavior. Microarray technology allows for screening of tumors for numerous genetic markers at one time. We performed gene expression analysis of thyroid tumors and non-tumorous thyroid tissue in an attempt to characterize the genetic events and correlate them with the known biologic behavior of the tumors.
Design: Fresh tissue from 26 thyroid neoplasms and 12 non-tumorous thyroid tissue samples from our institution were used. The tumors included 5 follicular adenomas (FA), 5 papillary thyroid carcinomas (PTC) and 11 follicular carcinomas (FC) and 5 clinically aggressive FC. Normal samples were obtained from 3 patients without thyroid tumors and 9 were from thyroids with co-existing tumors. RNA was extracted from these samples and hybridization with an oligonucleotide microarray was performed. The data was analyzed using bioinformatics software after normalization. The differentially expressed genes were correlated with the histologic diagnosis and clinical outcome using hierarchal cluster analysis.
Results: All non-neoplastic thyroid tissues clustered separately from the tumors and were distinguished by a set of 454 gene probes, the most significant of which are related to stromal proteins, collagen and various growth factors. One FA clustered with the FC and 2 non-aggressive FC clustered with the FA. Of the 5 aggressive FC , 4 clustered together while one clustered with the FA. Follicular variants of PTC clustered with the FA and FC while classic PTC clustered separately and were distinguished by a set of 66 genes.
Conclusions: A subset of genetic events involved in thyroid tumorigenesis appears to be separate and distinct from those in normal thyroid tissue. Suppression of genes related to stromal proteins and growth factors play an important role in thyroid tumorigenesis and distinguish tumor from non-tumorous thyroid tissue. Understanding the function of some differentially expressed genes may help stratify these tumors as they relate to clinical behavior.

417 Analysis of *BRAF* Mutation in Cutaneous Metastases from Thyroid Carcinomas

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Background: Cutaneous metastases from thyroid carcinoma are rare. Papillary thyroid carcinomas have been found to have *BRAF* mutation in nucleotide 1799. Papillary thyroid carcinomas associated with *BRAF* mutation are associated with more aggressive behavior. We analyzed a series of 15 patients with cutaneous metastases from thyroid carcinoma for *BRAF* mutation.
Design: Formalin-fixed paraffin embedded tissues from 15 patients with cutaneous metastases of thyroid carcinoma, including 10 cases of papillary thyroid carcinoma (PTC) and 5 cases of follicular thyroid carcinoma (FTC) were evaluated. Two PTC cell lines were used for controls, including NPA cells known with *BRAF* V600E gene mutation and PTC1 cells with known wild type *BRAF* genotype. All tumor samples and controls were analyzed for mutations at nucleotide 1799 in the *BRAF* gene exon 15. *BRAF* mutation analysis was performed by direct DNA sequencing of a PCR amplified 224 bp fragment of exon 15. *BRAF* mutation analysis was also performed with the Mutector colorimetric assay and by quantitative RT-PCR. Clinical and follow-up information was obtained by chart review.
Results: The histologic features in the cutaneous metastases were generally characteristic of the primary tumor, however 3 of the 10 papillary carcinoma metastases demonstrated cytoplasmic clearing not typical of classic papillary thyroid carcinoma. *BRAF* mutation (T1799A) was detected in 4 of 10 cases of PTC, but in none of the 5 FTC. Comparison of conventional DNA sequencing and colorimetric assay indicated 100% correlation between all three methods. The mutation and wild type *BRAF* genotypes were detected in NPA and PTC1 control cell lines, respectively. Thirteen of the 15 patients died of disease and 2 are alive with disease at last follow-up. Thirteen patients developed cutaneous metastases after being diagnosed with thyroid carcinoma. The time period from diagnosis of primary thyroid carcinoma to the cutaneous metastases ranged from 1 to 14 years with a mean of 6 years. Two patients with follicular thyroid carcinoma presented with cutaneous metastases.
Conclusions: These results indicate that *BRAF* mutations are frequently identified in cutaneous metastases of papillary thyroid carcinomas. The finding of *BRAF* mutations in these cutaneous metastases supports the more aggressive behavior associated with papillary carcinomas with this mutation.

418 Cytokeratin CK20 Is a Sensitive Marker for the Cytoskeletal Changes Associated with Hypercortisolism in the Pituitary Corticotroph Cell

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Background: Crooke's cells are nonneoplastic corticotroph cells which accumulate cytokeratin filaments in response to pathologic sources of hypercortisolism, as well as to exogenous steroid administration. Cytologically, the abnormal accumulation of cytokeratin filaments in the cytoplasm produces a characteristic hyaline appearance, often visible with routine hematoxylin and eosin stains. In two previous papers, we noted that Crooke's cells demonstrate CK20 immunopositivity, while normal pituitary gands are negative for CK20 expression. However, the presence of the classic Crooke's cell is not a sensitive marker for hypercortisolism. We evaluate the use of CK20 as a sensitive marker for the early and subtle cytoskeletal changes that occur within the corticotroph cell in response to hypercortisolism.
Design: We examined CK20 expression within the pituitaries from 15 hypercortisolic patients, including five corticotroph adenomas, one pituitary carcinoma, one ectopic corticotroph adenoma, one adrenal adenoma, one bilateral adrenal hyperplasia, and six documented cases of exogenous steroid administration. Five pituitaries from nonhypercortisolic patients served as controls. Each pituitary was formalin-fixed, paraffin-embedded, and cut at three-micron sections. H and E stains were performed as

well as immunohistochemical analysis. Immunohistochemistry was performed using a double-stain technique with both ACTH and CK20 (alkaline phosphatase red and iVIEW DAB).
Results: Intracytoplasmic CK20 immunopositivity was identified within corticotroph cells of 15/15 patients, although the number of CK20 positive corticotroph cells varied from case to case. Most notably, all six of the patients exposed to exogenous steroids lacked classic Crooke's cells on H and E stains, yet distinct CK20 immunopositivity was recognized within many of the corticotroph cells. None of the control pituitaries exhibited immunopositivity for CK20.
Conclusions: In response to both pathologic and exogenous sources of hypercortisolism, nonneoplastic corticotroph cells accumulate intracytoplasmic cytokeratin filaments, producing the distinctive hyaline change of Crooke's cells. However, classic Crooke's cells are often not identified with routine H and E stains within the pituitaries of these patients. Our study shows CK20 immunopositivity of corticotroph cells is a more sensitive and specific marker for the changes associated with hypercortisolism.

419 Narrow Repertoire of Gene Expression Including Aberrant Expression of MHC Type 2 Molecules Distinguishes FVPTC from Classic Morphology PTC and Benign Thyroid Tissue

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Background: The commonest variant of PTC is the so-called Follicular Variant (FVPTC). FVPTC is a particularly problematic lesion and can be challenging from a diagnostic viewpoint, even in resected lesions. In this study we have identified transcripts exclusively associated with the Follicular Variant using cDNA expression analysis and confirmed array findings using TaqMan RTPCR.
Design: Using a recently developed chemiluminescence based microarray platform we searched for potential biomarkers in a group of 25 thyroid samples comprising 11 benign lesions and conditions and 14 samples of PTC (6 classic morphology and 8 FVPTC). Statistical analysis included unsupervised clustering, ANOVA, and a false discovery rate analysis strategy.
Results: Unsupervised clustering segregated samples into benign and malignant groups with no tendency for the FVPTC group to cluster independently of classic morphology PTC, confirming the close relationship of these variants. Only 15 transcripts were significantly associated with FVPTC morphology. Remarkably these genes were associated with an extremely narrow repertoire of functions. Relatively increased expression of class 1 MHC genes (HLA-A) and aberrant expression of class 2 MHC genes, (HLA-DMA, HLADPB-1, HLA-DQB-1, HLA DRA) and associated genes (e.g. CD74 representing the invariant membrane bound moiety of class II HLA molecules and a regulator of the functions of MHC class II molecules and CD 14, a surface marker of monocytes/macrophages) were the most significant findings. Tumour infiltrating lymphocytes were not overrepresented in the follicular variant tumours compared to classic morphology PTC. Additionally relatively up regulated expression of members of the Cathepsin family was a feature of FVPTC.
Conclusions: This study confirms the close relationship between the 2 most common variants of PTC. Aberrant expression of MHC class II molecules in FVPTC is a novel finding and may represent a biomarker for this entity. Furthermore, as has been seen previously in ovarian serous carcinomas, expression of HLA DRA without co-ordinate expression of HLA DRB may represent a seemingly paradoxical mechanism for evasion of immune-surveillance.

420 SELDI-TOF Mass Spectrometry Analysis of Normal, Benign and Malignant Thyroid Tissue

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Background: In the field of endocrinology, thyroid cancer makes up more than 90% of all endocrine cancers and accounts for 63% of deaths from endocrine cancers, with an increasing incidence of the disease worldwide. Fine-needle aspiration cytology is the single most informative investigation tool. However, cytology often cannot accurately distinguish benign from malignant tumors and intraoperative consultation is also generally unhelpful to guide surgical management. Recently, the simultaneous measurement of a large number of expressed proteins has become an important screening tool for the discovery of new biomarkers that can diagnose early stage cancer. Proteomic analysis of cytological specimens obtained by thyroid fine-needle aspiration biopsy (FNAB) of individuals may potentially be developed as a screening test for thyroid cancer detection and to better determine the need for and extent of surgical therapy. We therefore tested the hypothesis that proteomic analysis would provide a fingerprint profile that would allow distinction between benign and malignant thyroid nodules.
Design: Frozen samples unequivocally classified as normal thyroid tissue (n=17), follicular adenomas (n=5) and papillary thyroid carcinoma (n=12) were analyzed by SELDI-TOF mass spectrometry. The tissues were ground in liquid nitrogen and lysed in RIPA buffer containing a protease inhibitor cocktail. Total cell lysates were loaded onto biochip surfaces and washed. The protein chips were then transferred into the TOF mass spectrometer (Ciphergen, Fremont, CA, USA) and spectra were generated. Analysis of the mass spectra was performed using the ProteinChip software (Ciphergen, Fremont, CA, USA).
Results: Each specimen analyzed by SELDI-TOF mass spectrometry provided a mass spectrum that was unique but the proteomic profiles of normal, benign and malignant lesions frequently overlapped and could not be separated.
Conclusions: These preliminary data suggest that the application of SELDI-TOF mass spectrometry may not provide informative diagnostic profiles that will allow accurate tumor classification for the common thyroid lesions. Further studies may provide leads for specific markers, and complementary technologies may be required to identify them.

421 An Essential Role for the Hematopoietic Transcription Factor Ikaros in Hypothalamic-Pituitary Mediated Somatic Growth

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Background: Ikaros transcription factors play critical functions in the control of lympho-hematopoiesis and immune regulation. Family members contain multiple zinc fingers that mediate DNA binding and homo- or hetero-oligomerization. Ikaros is abundantly expressed in pituitary mammosomatotrophs where it deacetylates histone 3 sites on the proximal growth hormone (GH) promoter to silence gene expression. Contrary to the prediction of enhanced growth, Ikaros-null mice display stunted growth with reduced circulating levels of the GH target factor insulin-like growth factor-I (IGF-I).
Design: The pituitaries of Ikaros-deficient mice were studied by morphology, immunohistochemistry and morphometry to determine somatotroph structure and function. The hypothalamus was also examined for GHRH development. GH administration was undertaken and response was evaluated by measuring IGF-1 levels and growth. The effects of Ikaros expression on GHRH were evaluated in hypothalamic N3 cells.
Results: Ikaros-deficient mice have small anterior pituitary glands with a reduced somatotroph population. Systemic administration of GH results in increased IGF-I levels and enhanced somatic growth. In contrast, reconstitution with wild-type lymphocytes is not sufficient to rescue the stunted growth phenotype of Ikaros-deficient mice. Ikaros was identified in mouse hypothalamic arcuate nuclei where it co-localized with GH-releasing hormone (GHRH). Over-expression of Ikaros enhanced GHRH promoter activity and induced endogenous GHRH gene expression.
Conclusions: These findings unmask a wider role for Ikaros in the neuroendocrine system, highlighting a critical contribution to the development of the hypothalamic-pituitary somatotrophic axis.

422 Delineation of Novel Diagnostics Markers of Papillary Thyroid Carcinoma by Molecular Profiling

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Background: The diagnosis of papillary thyroid carcinoma, particularly the follicular variant, is often problematic. Thus, new diagnostic tools that can be incorporated into both the cytologic and morphologic evaluation of thyroid nodules are needed. Gene expression profiling has become a powerful biomarker discovery tool, and could be successfully used to address the need to discover new thyroid diagnostic markers.
Design: Gene expression profiles of 95 benign and malignant thyroid tumors and 4 normal thyroid samples were generated by DNA microarray analysis using commercially available oligonucleotide arrays. Probe-sets corresponding to genes that were most preferentially expressed in the papillary thyroid carcinoma cohort compared to all the other thyroid tumor cohorts and normal thyroid were selected using simple statistics of t-tests and fold changes.
Results: Twenty-eight probe sets representing the most preferentially present transcripts in papillary carcinoma compared to the other thyroid tumors were identified. These probe sets correspond to a diverse group of 23 unique genes that represent potential diagnostic markers. The gene list includes some genes that have been previously reported to be upregulated in papillary carcinoma, such as MET and CITED1. One gene, CLDN1, was further evaluated and validated at the protein level by immunohistochemistry on thyroid tissue microarrays and whole sections of 240 consecutive thyroid nodules.
Conclusions: Molecular profiling in the form of gene expression profiling is a powerful biomarker discovery tool. Here, we used DNA microarray analysis of a large cohort of benign and malignant thyroid nodules to identify novel diagnostic markers of papillary carcinoma and validated one such marker. The availability of robust immunohistochemical markers of papillary carcinoma, including the follicular variant, will improve the overall diagnosis of thyroid nodules.

423 A Multi-Modality Approach to the Treatment of Primary Hyperparathyroidism

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Background: Parathyroidectomy is curative for a majority of symptomatic patients with primary hyperparathyroidism. Recent advances in pre-operative imaging and rapid intra-operative parathyroid hormone (RIOPTH) assays have cultivated a targeted minimally invasive (MI) surgical approach. Our institution has seen an almost 10 fold increase in the number of RIOPTH assay requests since its advent here in 2001, creating an ideal environment for the study of the utility of this assay in conjunction with traditional histopathological evaluation.
Design: Between September 2004 through July 2005, 150 consecutive cases of primary hyperparathyroidism were identified. Multiple factors were recorded such as age, gender, Tc^{99}-sestamibi and ultrasound results, gland location, gland weight, frozen section diagnosis, oil red O staining pattern, final diagnosis, baseline and RIOPTH values, and pre- and post-operative PTH, serum calcium, serum phosphate, and 24 hour urine calcium levels.
Results: Of the 150 cases, 86% (129/150) had a single adenoma, 7.3% (11/150) had a double adenoma, 4.7% (7/150) had multi-gland hyperplasia, and 1.3% (2/150) was diagnosed with an atypical adenoma. The mean age of the patients was 58.5 years with a female to male predominance of 3.4:1. The mean pre-operative and post-operative

RIOPTH levels were 25.0 pmol/L and 3.5 pmol/L, respectively. Of the 150 cases, 72.7% (109/150) had positive pre-operative imaging studies. In the cases of double adenomas with imaging studies identifying only one of two or neither aberrant gland (9/11 cases), the intra-operative PTH level remained elevated in 75% of cases following removal of the first hypercellular gland. However, in 37.5% of cases, the percent decrease in the RIOPTH level was greater than a widely accepted minimum threshold of 50%.
Conclusions: The success of MI parathyroidectomy is dependant on multiple factors including pre-operative imaging, surgical expertise, a reliable RIOPTH assay, and histological evaluation of the excised tissue by an experienced pathologist. Given the lack of standardized interpretation, it is our experience that normalization of RIOPTH values is the best indicator of curative surgical therapy. It is essential for the pathologist to maintain his role, not only to confirm the presence of parathyroid tissue, but to guide the appropriate use of this labor intensive assay, especially in the context of multi-gland disease.

424 The Conventional and Goblet Cell "Carcinoids" of the Appendix: A Proposal by Immunohistochemical Profiles

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Background: In our practice of surgical pathology, we occasionally encounter neuroendocrine tumors(NETs) in the appendix, most of which are composed of either conventional "carcinoids"(CCs) or goblet cell "carcinoids"(GCCs). It has been pointed out that GCCs tend to show more aggressive behavior. This study is aimed at to elucidate the nature of CCs and GCCs by immunohistochemical profiles.
Design: Two cases of CCs (less than 1 cm) and three cases of GCCs (with infiltration in the wall) were subjected to the immumnohistochemical (IHC) staining on the formalin fixed paraffin sections using Envision (DAKO Cytomation). The primary antibodies included NET markers (chromogranin A: CGA, synaptophysin: SNP and CD56: NCAM) and biomarkers (CEA, CK7, CK20, E-cadherin, MIB-1 and CDX-2).
Results: All cases of CCs and GCCs were diffusely positive for CDX-2 suggesting that both groups of "carcinoid" are derived from the intestinal-type cells, but GCCs with more colonic-committed cells by the facts of positive CEA (3 cases), CK20 and E-cadherin (2 cases) staining which was absent in CCs. All cases of CCs were positive for NET markers and hormones diffusely in the tumor cells. All cases of GGCs were positive for CGA in very limited tumor cells. One case of GCC was positive for CD56. MIB-1 was negative in CCs but it was detected in lower proportion (<5%) in GCCs. Positive CDX-2 is helpful in differentiating CCs from paragangliomas. Strong CEA staining in GCCs is helpful in detecting widely infiltrating tumor cells.
Conclusions: Our IHC study suggest as flollows. Both CCs and GCCs are intestinal-derived NETs. CCs are more neuroendocrine-differentiated tumors with indolent biologic activity fitting in the scope of the term well differentiated neuroendocrine tumor (WDNET) by WHO Tumor Classification (2004). In contrast, GCCs are more colonic-oriented tumors with focal neuroendocrine differentiation and more aggressive biological activity fitting in the well differentiated neurendocrine carcinoma (WDNEC).

IHC panels of carcinoid tumor of appendix

Case No.	1	2	4	5	6
Histological type	CC	CC	GCC	GCC	GCC
Serotonin	(+)	(+)	(-)	(-)	
Substance P	(+)	(+)	(-)	(-)	(-)
CGA	(+)	(+)	(+)	(+)	(+)
SNP	(+)	(+)	(+)	(-)	(-)
NSE	(+)	(+)	(-)	(-)	
CD56	(+)	(+)	(-)	(-)	(+)
CEA	(-)	(-)	(+)	(+)	(+)
CK7	(-)	(-)	(-)	(-)	
CK20	(-)	(-)	(+)	(+)	
E-cadherin	(-)	(-)	(+)	(+)	
MIB-1	0%	0%	5%	5%	
CDX-2	(+)	(+)	(+)	(+)	(+)

CC: conventional carcinoid, GCC: goblet cell carcinoid, FM: Fontana-Masson, CGA: Chromogranin A, SNP: synaptophysin, Blank indicates"not done"

425 Immunohistochemical Detection of Somatostatin Receptor(SSTR)2a in Duodenal and Pancreatic Gastrinomas

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Background: Gastrinomas are the tumors which occur in the duodenum and pancreas and show Zollinger-Ellson syndrome. The tumor cells show immunohistochemical localization of gastrin in the cytoplasm. These tumors, especially the ones in duodenum, show early metastasis even though the primary tumors are small. Recent molecular targeted therapy by somatostatin analogue(Octareotide) in the gastrinomas warrant the current investigation on SSTR2a in order to perform effective thepeutic results.
Design: The clinical features of three pancreatic gastrinomas and 5 duodenal gastrinomas were summarized in the table. Immunohistochemical detection was performed on formalin fixed paraffin sections using anti-gastrin antibody(DAKO Cytomation) and anti-SSTR2a antibody(Gramsch Lab).detected by Envision method and ABC method respectively.
Results: As it has been reported, the pancreatic gastrinomas are larger in size. Then duodenal gastrinomas are in smaller in size, sometimes multiple and showed early metastases. All pancreatic and duodenal cases showed the presence of gastrin in the cytoplasm of the tumor cells. All but one case showed the presence of SSTR2a on the cell membrane of the tumor cells. Positivity was graded as -:negative, +:-25%,+-++:25-50%,+++:50%-. In the SSTR2a-positive case, serum gastrin level was lowered by somatostain analoque administrtion. It is also emphasized that, in the cases of sporadic gastrin positivity, SSTR2a staining was diffuse on the cell membrane. Immunohistochemicdal detection of gastrin is important to predict the unfavorable biologic behavior of the pancreatic and duodenal neuroendocrine tumors. Positive membranous staining of SSTR2a, especially SSTR2a+++ suggests effective treatment by somatostatin analoque.
Conclusions: Gastrinomas are the group of neuroendocrine tumors with unfavorable biologic behavior. SSTR2a immunoreactivity is high and the cases with SSTR2a may be good candidates for SSTR2a therapy. Immunohistochemistry is essential not only in the diagnosis but in the molecular targeted therapy.

	Age / Sex	ZES	Primary site	Number	Size (mm)	Liver meta.	LN meta.	MEN	Result of IHC	
									Gastrin	SSTR2a
Case 1	77M	Yes	P	3	30.55	Yes	N/A	No	+++	+
Case 2	43M	Yes	P	1	30	No	No	No	+	-
Case 3	34M	Yes	P	1	15	Yes	1	No	+	+++
Case 4	53M	Yes	D	1	10	Yes	3	No	+	+++
Case 5	62F	Yes	D	1	2	No	No	No	+++	+++
Case 6	56F	Yes	D	4	1-3	No	No	Yes	+++	+
Case 7	45M	Yes	D	5	1-5	Yes	2	No	+++	+++
Case 8	41F	Yes	D	N/A	N/A	N/A	1	No	++	+++

All cases were immunohistochemically positive for gastrin
MIB-1 indices >10% in case 3 MIB-1 indices <1% in other cases
ZES: Zollinger-Ellson syndrome P: Pancreas, D: Duodenum, G: Gastrin

426 Expression of $BRAF^{WT}$ and $BRAF^{V600E}$: Its Significance in Tumorigesis of Thyroid Carcinomas

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Background: *BRAF* (7q24) encodes a serine/threonine protein kinase, and its expression level varies in different tissues. Gain of function *BRAF* mutations can induce constitutive activation of ERK1/2 signaling, and a high prevalence of BRAF genetic aberrations including $BRAF^{V600E}$, $BRAF^{K601E}$, $BRAF^{VK600\text{-}1E}$, and the AKAP9-BRAF rearrangement have been suggested to play crucial roles in thyroid carcinogenesis. However, little is known about the expression pattern of BRAF in various neoplastic and non-neoplastic thyroid lesions.
Design: In the present study, we focussed on the expression level of BRAF protein in various thyroid tumors and thyroid cancer cell lines and its relation to *BRAF* mutations. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded thyroid specimens using a polyclonal antibody for BRAF. Point mutations of *BRAF* in exon 15 were analyzed by PCR-direct sequencing (ABI3100) using DNA extracted from microdissected paraffin-embedded sections and thyroid carcinoma cell lines.
Results: Normal and hyperplastic thyroid lesions showed focal and faint immunoreactivity for BRAF, especially in cuboidal follicular cells of small follicles. In contrast, diffuse expression of BRAF was observed in neoplastic cells of follicular adenomas and well-differentiated carcinomas. Point mutation of *BRAF* ($BRAF^{V600E}$) was identified in 42% (13/31) of papillary carcinomas and 33% (5/15) of undifferentiated carcinomas, but not in normal (0/12), nodular hyperplasia (0/6), follicular adenomas (0/7) and follicular carcinomas (0/11). The immunohistochemical expression of BRAF did not correlate with BRAF mutation. Western blotting revealed BRAF protein expression in all thyroid carcinoma cell lines irrespective of *BRAF* mutational status.
Conclusions: Heterogeneous focal expression of wild-type BRAF ($BRAF^{WT}$) in non-neoplastic lesions may play a role in the growth or functional activity of follicular cells. In contrast, diffuse expression of $BRAF^{WT}$ and/or $BRAF^{V600E}$ may be involved in the tumorigenic process through constitutive activation of the ERK signaling pathway in cooperation with other genetic abnormalities.

427 Divergent Expression and Action of Fibroblast Growth Factor Receptors (FGFRs) 1 and 2 in Thyroid Cancer

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Background: The fibroblast growth factor (FGF) family of ligands plays fundamental roles in development and the tumorigenetic process. The cellular effects of FGFs are mediated by FGF receptors (FGFRs 1-4) in mammals. The prototypic FGFR consists of three extracellular immunoglobulin (Ig)-like domains, a single transmembrane domain, and an intracellular tyrosine kinase domain. A major alternative splicing occurs within the first Ig-like domain of FGFRs 1-3, resulting in "b" and "c" receptor isoforms that exhibit distinct ligand binding specificities. The individual and combined functions of different members of the FGFR family in thyroid cancer progression remain unknown.
Design: In this study we focussed on the expression of the two principal members of the FGFR family (1 and 2) that transduce most FGF signals in human thyroid tissue and tumor cell lines. We used siRNA-mediated downregulation and CMV promoter-mediated over-expression of FGFRs to determine the individual or combined impact of FGFR1 & 2 on cancer cell proliferation and growth in xenografted SCID mice.
Results: In a tissue array of primary human thyroid samples, FGFR1 was variably expressed in a subset of papillary carcinomas that displayed lymph node metastasis and in poorly-differentiated and anaplastic carcinomas. In contrast, expression of FGFR2 was not identified in neoplastic thyroid specimens. Instead, FGFR2 was selectively expressed in unaffected normal thyroid tissue. Similarly, FGFR1 was noted in the thyroid carcinoma cell lines TPC-1 (papillary), NPA (papillary), and WRO (follicular) but not in the anaplastic carcinoma cell lines (ARO and DRO) as determined by multiple techniques. FGFR2 was not detected in any of the cell lines tested. Re-expression of the

FGFR2b isoform interrupted BRAF phosphorylation and consequently ERK1/2 in WRO cells. Moreover, siRNA-mediated FGFR1 knockdown in WRO cells resulted in decelerated S-phase entry and tumor suppression in xenografted SCID mice.

Conclusions: These findings suggest a unique FGF receptor isoform profile in thyroid cancer with a predominantly anti-tumor differentiating effect assigned to the FGFR2b isoform. In contrast, FGFR1 appears to mediate a proliferative signaling cascade. These findings underscore the complex network of this family of tyrosine kinases in modulating cancer cell growth and predict the need for highly selective inhibitors in the control of disease progression.

428 Correlation between Clinico-Pathologic Features of Papillary Thyroid Microcarcinomas and Tumor Behavior

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Background: Papillary microcarcinomas are tumors measuring 1cm or less in size. They frequently represent an incidental finding and are common in human populations. The clinical management of those tumors is controversial, particularly with respect to the completion of thyroidectomy and post-surgical radioiodine treatment. Although most of these tumors are indolent, some give metastases or recur, and therefore need to be treated more aggressively. No clinical or pathologic features associated with more aggressive tumor behavior have been reported.

Design: We studied a series of 65 papillary microcarcinomas. The follow-up ranged from 1 month to 15 years, with average follow-up of 4.3 years. At the time of surgery or during the follow-up, 11 patients had lymph node metastasis, one of whom had also local tumor recurrence, and one patient developed lung metastasis. These 12 tumors were placed in the aggressive group. The other 53 cases were placed in the non-aggressive group. Histologic slides were reviewed to classify tumors and examine specific pathologic features. Statistical analysis was performed using the two-tailed Fisher exact test or Student's t test for continuous data.

Results: The results of the analysis are summarized in Table1.

Conclusions: In summary, papillary microcarcinomas with more aggressive behavior had significantly more common tumor fibrosis. They also had a tendency for association with male gender, larger tumor size, tumor multicentricity, infiltrative border, and subcapsular location, although the differences for those features did not reach statistical significance, probably because of the limited number of cases in the aggressive group. No correlation was observed with patients age, histologic variant of the tumor, and extrathyroidal extension.

Table 1. Correlations between the aggressiveness and clinico-pathologic features of papillary microcarcinomas

	Aggressive n=12	Non-aggressive n=53	P value
Age, mean+/-SD (years)	48 +/- 17	47 +/- 14	1.00
Female :male ratio	7 : 5	42 : 11	0.15
Tumor size, mean+/-SD (mm)	6.0 +/- 2.8	4.7 +/- 3.3	0.21
Classic papillary variant	67%	50%	0.35
Follicular variant	33%	51%	0.35
Multicentricity	58%	36%	0.20
Infiltrative border	100%	75%	0.10
Tumor fibrosis	83%	47%	0.03
Extrathyroidal extension	17%	11%	0.63
Subcapsular location	42%	25%	0.29

429 Cytokeratin 19 Is Not a Powerful Prognostic Marker in Well Differentiated Pancreatic Endocrine Tumors

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Background: Well differentiated pancreatic endocrine tumors (WD-PETs) are a heterogeneous group of neoplams including benign, malignant, and borderline subtypes and their prognosis is difficult to establish on a pure morphological background. In recent years, several attempts have been made to identify prognostic markers that could be of help in predicting patients' outcome. Among them, the expression of cytokeratin 19 (CK19) has been recently proposed as an indicator of unfavorable outcome. However, this finding remains to be verified and to be compared with more used prognosticators such as Ki67 and mitotic index, vascular and/or perineural invasion. The aim of our study was to evaluate the expression and the role of CK19 as prognostic marker in a large series of WD-PETs.

Design: 140 well characterized WD-PETs have been immunohistochemically studied using two different anti-CK19 monoclonal antibodies (clones BA17 and RCK108 by Dako). Results were statistically compared with clinicopathological features, Ki67 index, mitotic count and vascular and perineural invasion.

Results: Using both clones we observed an increase of CK19 expression from benign to malignant WD-PETs. By univariate analysis, only the CK19-immunoreactivity obtained with the RCK108 antibody correlated with prognosis. In particular, RCK108 staining correlated with prognosis when the whole group of WD-PETs was considered and in the group of insulinomas. On the contrary, RCK108 staining did not show a prognostic meaning for nonfunctioning neoplasms. Ki67 index, mitotic count, vascular and perineural invasion were all statistically correlated with prognosis at the univariate analysis. At the multivariate Cox test analysis, only the Ki67 index (>2%) proved to be an independent prognostic marker.

Conclusions: CK19 expression, when detected using the RCK108 antibody, correlates with outcome of patients with WD-PETs, and especially for those harboring insulinomas . However, Ki67 index appears to be a more significant prognostic predictor. Although the expression of CK19 by WD-PETs opens new speculative ideas about the tumorigenesis of such tumors, it doesn't seem to be a reliable prognostic marker to use in the diagnostic practice

430 Mutational Spectrum of Paragangliomas Obtained from Archival Paraffin Sections

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Background: Paragangliomas are vascularized tumors arising from neural crest-derived cells which contribute to the autonomic nervous system. Mutations in genes that contribute polypeptide subunits of the succinate-ubiquinone oxidoreductase (SDH) complex, particularly the *SDHD* and *SDHB* genes, have been associated with hereditary susceptibility to develop paragangliomas. Most paragangliomas have been felt to arise 'sporadically,' although good measures of the relative distribution of 'sporadic' and hereditary paraganglioma are not available.

Design: To probe this distribution, we sought series of consecutive unselected paragangliomas from surgical pathology files of several institutions for DNA sequencing of the *SDHB*, *SDHD* and *SDHC* genes from archival paraffin sections. Ninety-nine cases (82 head/neck and 17 others) were examined.

Results: Full sequence information for the *SDHD* and *SDHB* genes was obtained from 55 samples, and partial sequence, ranging from few to many exons, from the remaining 44 tumors. *SDHC* was sequenced in a subset of 14 samples. Variants judged to be mutations of *SDHB* or *SDHD* were found in 14 cases; 6 in the *SDHD* gene and 8 in the *SDHB* gene. Eight (3 *SDHD*, 5 *SDHB*) mutations have not been described previously to our knowledge. No mutations were observed in *SDHC*. Previously described and novel polymorphisms were identified in all 3 genes. Some investigators have hypothesized that *SDHD* mutations are more prevalent in head/neck paragangliomas and *SDHB* mutations in abdominal tumors. In this study 7 of 13 head/neck tumors had *SDHB* mutations. The role of SDH gene mutations in sporadic paragangliomas is not well understood. Five patients whose tumors showed SDH gene mutations had normal tissue available for analysis. For each patient the mutation in the tumor was also present in normal tissue, indicating hereditary origin of the paraganglioma.

Conclusions: This fact, along with an overall mutation incidence of at least 14%, is consistent with the idea that new sequence variants in SDH genes are commonly associated with hereditary paragangliomas but less frequently or uncommonly with sporadic paragangliomas. Accurate assessment of the incidence of SDH gene mutations in hereditary versus sporadic paraganglioma will require longitudinal studies with careful attention to family history, clinical followup as well as molecular analysis of tumor and normal tissue.

431 Reevaluation of Immunohistochemical Markers in Diagnosing Papillary Carcinoma (PC) of the Thyroid: Evidence for Utility of HBME1 Combined with CK19 Immunostaining

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Background: The follicular variant of PC can be difficult to distinguish from cellular adenomatous nodules. Prior studies have advocated various antibodies to diagnose PC but there is little agreement on their utility. We undertook this study to reevaluate immunohistochemical markers in the diagnosis of PC.

Design: To identify potentially useful markers, formalin-fixed, paraffin-embedded tissue from 10 cases of PC was initially stained with 10 antibodies, including HBME1, CK19, fibronectin, Ki-67, Calretinin, p16, SFTPB, CITED and antibodies to two novel molecules that have been found to be upregulated in PC (CST6 and EPS8). Of these, only HBME1, CK19 and fibronectin showed diagnostic utility. These 3 markers were then further evaluated in 51 PC and 57 benign thyroids. PC cases included 20 classic PC, 10 follicular variants and 21 papillary microcarcinomas. Benign thyroids comprised 10 normal thyroids, 10 cellular adenomatous nodules, 8 Graves' disease, 4 papillary hyperplastic nodules, 6 follicular adenomas and 19 Hashimoto's thyroiditis. For all antibodies, more than 10% staining was considered positive, and for HBME, only basolateral membrane staining was accepted.

Results: CK19 positivity was seen in all 51 PC but also in most (39/57) benign thyroids (10/10 normals, 5/10 adenomatous nodules, 2/8 Graves', 1/4 papillary hyperplastic nodules, 5/6 follicular adenomas and 16/19 Hashimoto's). Membrane positivity for HBME1 was found in 49/51 (96%) PC and in 4/57 (7%) benign thyroids (0/10 normals, 0/10 adenomatous nodules, 0/8 Graves', 0/4 papillary hyperplastic nodules, 0/6 follicular adenomas, 4/19 Hashimoto's). Fibronectin was positive in 35/51 PC (69%) and 4/57 (7%) benign thyroids. Cytoplasmic Ki-67 positivity was present in 18/26 PC and 3/16 benign thyroids. Calretinin stained only 3/10 PCs. SFTPB showed weak and focal staining in 5/26 PC and 1/16 benign thyroids. CITED was positive in 26/26 PC and 15/16 benign thyroids. p16, EPS8 and CST6 stained the majority of both PC and benign thyroids evaluated.

Conclusions: Basolateral membrane staining for HBME1 is highly sensitive and specific for PC. CK19 is very sensitive but nonspecific, therefore negative staining for CK19 is reliable evidence against PC. The most useful combination for the diagnosis of PC appears to be HBME1 and CK19. Fibronectin is specific but less sensitive than HBME1 and its utility is hampered by high background staining. CITED, SFTPB, CST6 and EPS8 do not appear to be useful.

432 Analysis of Differential $BRAF^{V600E}$ Mutational Status in Multifocal Papillary Thyroid Carcinoma: Evidence of Independent Clonal Origin in Distinct Tumor Foci

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Background: Papillary thyroid carcinomas (PTCs) often present as multifocal tumors, and the presence of multifocal tumor foci has been viewed as a poor prognostic factor, because multifocal tumors are believed to result from intraglandular metastasis within a single tumor. However, recent studies indicate that a significant portion of multifocal PTCs arise independently from unrelated clones.

Design: To investigate whether multifocal tumors arise from independent clones or from intraglandular metastasis within a single tumor, we examined $BRAF^{V600E}$ mutation statuses in individual tumor foci of 61 patients with multifocal PTC using polymerase chain reaction/restriction enzyme polymorphism analysis and direct sequencing.
Results: Multifocal PTCs were characterized by an older age at presentation, a higher frequency of lymph node metastasis, and a background of lymphocytic or Hashimoto's thyroiditis. The $BRAF^{V600E}$ mutation was found in 67.9% of the tumor foci of 140 papillary tumor foci in 61 patinets with multifocal PTC. Among the 61 patients, 29 were $BRAF^{V600E}$ positive at all foci (47.6%), eight (13.1%) were all-negative, and 24 (39.3%) were mixed (i.e. $BRAF^{V600E}$–positive and $BRAF^{V600E}$-negative tumors coexisted in the same patient). Patients with $BRAF^{V600E}$-all-negative multifocal PTCs were younger than those with $BRAF^{V600E}$-all-positive or -mixed multifocal PTCs. And the histology of individual tumors was more different in $BRAF^{V600E}$-mixed cases.
Conclusions: In conclusion, our results show that the status of the $BRAF^{V600E}$ mutation was heterogeneous in 39.3% of multifocal PTCs, which suggests that individual tumors arise independently in a significant subset of multifocal PTCs.

433 Immunohistochemical Analysis of Hurthle Cell Neoplasms of the Thyroid: A Hierarchical Clustering Approach

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Background: Morphologic similarities between Hurthle cell adenoma (HCA) and carcinoma (HCC) of thyroid make their distinction very difficult. To date, the search for definitive biomarker/s largely remains inconclusive. Given the large number of genetic and chromosomal alterations in these neoplasms, a combined approach using multiple diagnostic criteria may be necessary. We adopted a hierarchical clustering approach combining morphologic and immunohistochemical data to distinguish HCA from HCC.
Design: Paraffin-embedded thyroid tissue from eighteen HCC and nine HCA cases was analyzed for six potential biomarkers using immunohistochemical staining: N-cadherin, c-met and insulin-like growth factor binding protein 3 (IGFBP3), showed differential expression between HCA and HCC on gene array (Finley et al, 2004). Matrix metalloproteinases –1 (MMP-1) and –2 (MMP-2) have a proven role in tumor metastasis. Ki-67, a marker for proliferation, has consistently shown higher expression in HCC. The staining results were interpreted by three independent observers based on the percent staining (N-cadherin, c-met, IGFBP3), number of positive cells per high power field (HPF) (Ki-67), and staining pattern (MMP-1, MMP-2). The staining was also graded as –2, 1 and 2 for software analysis. Tumor size, an important indicator of clinical behavior, was combined with the markers in the clustering analysis. A software assisted (GeneCluster by Eisen M, Stanford U, CA) hierarchical cluster analysis was performed using biomarkers and tumor size.
Results: MMP-2, c-met and Ki-67 showed differencial expression between HCA and HCC. c-met was detected in 12/18 HCC and 4/9 HCA cases. MMP-2 showed increased juxta-capsular staining in 5/18 HCC and 0/9 HCA cases. Ki-67 was detected at higher levels in HCC (median: 118/10 HPF) compared to HCA (median: 28/10 HPF). The hierarchical cluster analysis using ki-67, c-met, MMP-2 and tumor size successfully separated the cases into two distinct groups. Group 1 (n=10) contained 8/9 HCA and 2/18 HCC cases, whereas group 2 (n=17) contained 16/18 HCC and 1/9 HCA cases. The sensitivity of the clustering approach was 89%, whereas the specificity was 80% for HCA and 94% for HCC.
Conclusions: The successful separation of HCAs and HCCs shows that the hierarchical clustering approach can be very useful in tumors with incompletely understood biology, especially when used in combination with morphologic criteria. Further studies are being performed to validate the statistical strength of this analysis model.

434 Clinical Significance of Tall Cell Morphology in Papillary Thyroid Carcinoma: Clinicopathologic Study of 92 Cases

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Background: Papillary thyroid carcinoma (PTC) with tall cell features has been regarded as a histologic subtype associated with a worse prognosis than conventional PTC.
Design: 92 cases of PTC displaying tall cell features were retried from the surgical pathology files at the Ohio State University. Clinical and demographic features, as well as size of the tumor, percentage of the lesion with tall cell features, extrathyroidal extension, evidence of lymph node or distant metastases, recurrences and survival were analyzed. For comparison, a control group of 109 cases of PTC without tall cell features matched for sex, age and clinical stage was also analyzed. Significant associations were identified using Chi-square and Fisher's exact test. Multivariate analysis was determined by Cox's proportional hazard model. Actuarial survival was calculated by the Kaplan-Meier method. The statistical significance of survival was determined by the log rank test. A p-value of less than 0.05 was considered significant.
Results: There were 70 women and 22 men, aged 18-94 years (mean= 49.1). Twelve cases showed <29% tall cell component; 8 had 30-49% tall cell component; 29 had 50-84%, and 43 had >85% tall cell component. No statistically significant association could be found between presence of tall cell component and pathologic stage, lymph node metastases, local recurrence and survival (p>0.5). A significant association was observed for tall cell component with extrathyroidal extension (p= 0.009). In both the tall cell group and the control conventional PTC group extrathyroidal extension showed a strong association with clinical stage (p= 0.0001). Recurrences were more frequent in men than in women in both groups. Multivariate analysis showed that presence of lymph node metastases and clinical staging were important independent variables that predicted recurrence (p=0.001 and p=0.038, respectively), but tall cell component was not statistically significant (p>0.5). Survival at 200 months was 80% for both groups, without there being any significant differences.
Conclusions: The most important prognostic parameter in both groups was pathologic stage. The results of our study appears to indicate that prognosis in PTC with tall features is related to extrathyroidal extension and that the presence of tall cell features in the absence of extrathyroidal extension may not influence prognosis.

435 Role of NFκB and HIF-1α in Follicular Thyroid Neoplasm Progression

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Background: Several gene abnormalities have been described in follicular thyroid carcinomas (FTC), but no study evaluates topographically vascular (hypoxia inducible factor-1α, HIF-1α) and inflammatory signaling pathways (nuclear factor κB, NFκB) by tumor phenotype.
Design: We selected 9 hyperplastic nodules, 22 adenomas, 14 minimally-invasive FTC, 24 widely-invasive FTC, and 13 anaplastic carcinomas (WHO criteria). Total RNA was extracted from normal and neoplastic tissues by hot acidic phenol, DNase I-treated, phenol extracted and cleaned (RNeasy columns). T7-(dT24) oligomer was used for priming the first-strand cDNA synthesis and the resultant cDNA was phenol/chloroform extracted, and used as template for cRNA synthesis (T7 MegaScript In Vitro Transcription Kit). The cRNA was fragmented, Cy3- and Cy5-labeled, and hybridized to the human GeneChip X3P Array noncompetitively. Cross-validated gene expression analyses (CGEA) were performed (expression factor>2, significance<0.01), and variables studied regarding the histological diagnosis. Significant variables were evaluated immunohistochemically using standard protocols for the pathway last effector.
Results: Tumor infiltrating lymphoxytes were absent in 68/73 (93%) neoplasms, regardless of histologic subtype. CGEA showed down-regulation of ubiquitin C and sequestosome 1 in FTC, which directly correlated with CDC34, SKP1/2 (HIF-1α degradation pathway), and inversely with IκBα (inhibition of NFκB pathway). Across all neoplasms the positive cell percentage was higher in the internal compartments. NFκB-p50 and p65 (p<001) as well as HIF-1α (p=0.049) immunoexpressions increase with increasing neoplastic grade accordingly, in particular in internal compartments. The expression of NFκB was low across all the lesions, the greatest degree of immunoexpression seen in internal compartments of anaplastic carcinomas (4%). The hyperplastic nodules showed an exceptional level of expression of HIF-1α in the internal compartment (over 16%, ~25x the highest percentage observed in any other lesion)
Conclusions: Ubiquitylation down-regulation of follicular tumor cells results in peripheral down-regulated NFκB pathways (p65 and p50) and HIF-1α, but increase expression in internal compartments with more aggressive tumor behavior suggesting an involvement in follicular thyroid tumor progression.

436 Parasitic Nodules of the Thyroid, a Metastasis Simulator: A Study of 76 Cases

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Background: Parasitic nodules are thyroid tissue separated from the gland, considered as an expression of nodular hyperplasia. They can be easily confused with metastatic carcinoma in a lymph node. The aim of this study is to evaluate the clinical and morphologic features of this entity, with emphasis in the distinction from metastasis. To the best of our knowledge, this is the larger series of this entity.
Design: We reviewed the slides of 76 cases retrieved from the consultation files of 1 of the authors. The cases were evaluated for clinical parameters and submitting diagnoses. Follow-up were retrieved contacting the sender pathologist.
Results: ***Clinical features:*** patient's age ranged from 15 to 83 year-old (median: 51.06). There was predominance for females (3.68 : 1). Most of cases showed a single nodule (82.9%). The distribution was from the submandibular to the mediastinal area, including the retroclavicular area, the sternocleidomastoid and sternohyoid muscles. Contributor's diagnoses: The main differential diagnoses were metastatic thyroid carcinoma (40.7%), parasitic nodule (21.0%), and hyperplasia (5.32%). ***Morphologic features***: The size of the nodules ranged from 0.5 to 6.5 cm. Microscopically they showed normal tissue (90.81%), Hashimoto's thyroiditis (32.9%) and nodular hyperplasia (31.57%). Malignancy was found in 9.19% of the cases; the diagnoses were: papillary and follicular carcinoma, and well-differentiated tumor of uncertain malignant potential (UMP). These cases showed no clinical or histological evidence of tumor in the main gland. Histological study of the main gland was available in 52 cases; the main diagnoses were nodular hyperplasia (61.53%) and Hashimoto's thyroiditis (34.61%). Malignancy was found in 26.9% of the cases, the diagnoses were: papillary, follicular and medullary carcinomas and oncocytic tumor UMP.
Conclusions: Parasitic nodules were more frequent in middle aged women, as a single nodule. Association with nodular hyperplasia and Hashimoto's thyroiditis is common. The 9.19% of the cases showed malignancy without evidence of tumor in the main gland. This suggests that parasitic nodule can originate a primary tumor. The main differential diagnosis was metastatic carcinoma.Useful features for a correct interpretation are the presence of nodular hyperplasia and the absence of cytological features of papillary carcinoma and lymph node remnants, as sinuses. The latter is an important clue for cases with Hashimoto's thyroiditis. The knowledge of this entity is important for avoid a misdiagnosis of metastatic thyroid carcinoma.

437 Hypoxia Inducible Factor in Pheochromocytomas and Paragangliomas

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Background: Hypoxia inducible factor 2 alpha (HIF2a) is a part of transcription complex which is regulated by hypoxia. Hypoxia has a significant role in tumor angiogenesis and the role of HIF2a in the development of pheochromocytoma and paraganglioma is unknown.
Design: Tissue microarrays from 70 patients with benign (n=45) or malignant paragangliomas (n=25) and 83 patients with benign (n=54) or malignant pheochromocytomas (n=29) were analyzed immunohistochemically with antibodies to HIF2a, vascular endothelial growth factor (VEGF), cyclooxygenase 2 (COX2), S100

protein and Ki-67 to study the role of angiogenesis and proliferation in the development of pheochromocytomas and to distinguish benign from malignant tumors. All cases of malignant tumors had proven metastatic disease.
Results: Malignant paragangliomas expressed less HIF2a than benign paragangliomas ($p<0.05$). Differences in the level of HIF2a expression were not identified in benign and malignant pheochromocytomas. Benign pheochromocytomas expressed more VEGF than the malignant tumors ($p<0.05$). COX2 expression was similar in both benign and malignant paragangliomas and pheochromocytomas. Ki-67 proliferative index was higher in both malignant paragangliomas and pheochromocytomas compared to their benign counterparts. S100 protein expression in sustentacular cells was not significantly different between benign and malignant tumors.
Conclusions: These results show that malignant paragangliomas are associated with down regulation of HIF2a expression which suggests a role for HIF2a in tumor progression of malignant paragangliomas. HIF2a, VEGF and Ki-67 may be useful in helping to separate benign from malignant pheochromocytomas and paragangliomas.

438 Differential Gene Expression in Hereditary Pheochromocytomas/Paragangliomas

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Background: Hereditary pheochromocytomas/paragangliomas are tumors of the autonomic nervous system arising from neuroectodermal cells and occur due to mutations in a number of unrelated genes including *NF1*, *RET*, *VHL*, and the succinate dehydrogenase subunits B, C and D that encode for components of the mitochondrial complex II. Microarray analysis has permitted the segregation of these hereditary tumors into two clusters using an oxidoreductase signature. One cluster consists of *VHL*, *SDHB* and *D* mutant tumors that demonstrate decreased levels of oxidoreductase function compared to *RET* and *NF1* mutants and non-syndromic pheochromocytomas.
Design: For correlation between this genetic signature and detectable levels of oxidoreductase components from hereditary pheochromocytomas, we performed immunohistochemical analysis for the SDHA and SDHB subunits of mitochondrial complex II on six non-syndromic, five *RET*, two *NF1*, four *SDHB*, three *SDHD*, and seven *VHL* mutant pheochromocytomas from the archives of Brigham and Women's Hospital and consultation material. Tissue was processed according to a standard protocol using primary antibodies (Molecular Probes, Eugene, OR) for either SDHA (1:8,000 dilution) or SDHB (1:1000 dilution) and the Envision Plus Detection System (Dako, Carpinteria, CA) for antigen-antibody detection. Immunoreactivity was graded semiquantitatively as 0, no staining; 1+, <5% of tumor cells reactive; 2+, 5 to 25% of tumor cells reactive; 3+, 25 to 50% of tumor cells reactive; 4+, >50% of tumor cells reactive-weak intensity; 5+, >50% of tumor cells reactive-moderate intensity; 6+, >50% of tumor cells reactive-strong intensity.
Results: We demonstrated that expression of SDHB protein is markedly reduced in all tumors with SDHB and SDHD mutations suggesting that low SDHB protein expression can serve as a molecular surrogate for demonstrating disruption of complex II. Interestingly, decreased SDHB levels were also noted in a subset of tumors with VHL mutations (3 of 7), suggesting that *VHL* and *SDH* mutants may share similar mechanisms of tumorigenesis.
Conclusions: There is a significant correlation between the immunohistochemical and transcriptional profiling data, suggesting that staining for SDHB in tissue sections in routine pathology practice may be diagnostically useful for the identification of a subclass of hereditary pheochromocytomas as well as for those with underlying deficiencies in oxidoreductase function.

439 BRAF Mutations Are Uncommon in Thyroids with Chronic Lymphocytic Thyroiditis

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Background: Chronic lymphocytic thyroiditis (CLT) or Hashimoto's thyroiditis has an epidemiological relationship to papillary thyroid carcinoma (PTC). The follicular epithelium in CLT can be markedly atypical, with cytologic changes ranging from oncocytic morphology to clearing and overlapping. At the molecular level, the association between CLT and PTC is more controversial. Some studies indicate that RET/PTC translocations can be detected in the atypical follicular epithelial cells, while other studies indicate that this mutation is absent. BRAF, a mutation that is very common in PTC, has not been examined in the atypical epithelium of CLT.
Design: Well-sampled thyroids that showed evidence of CLT, some of which also had PTC, were studied. Microdissection targets were selected to include normal tissue, atypical follicular epithelium of CLT, and when present, PTC. Histologic slides were reviewed and diagnoses confirmed. DNA was extracted from the microdissected tissue fragments and PCR was performed for exon 15 of the BRAF gene. The PCR amplicons were then subjected to cycle sequencing using an automated sequencer and the BigDye Terminator kit (ABI, Foster City, CA). The sequence was assessed for the typical T1799A BRAF mutations.
Results: Twenty-seven patient samples without carcinoma were included, from which 47 different targets of atypical nuclear features in CLT were microdissected. An additional 28 patient samples with carcinoma and CLT were studied (12 conventional papillary carcinomas, 13 follicular variants, and 3 tall cell variants). Only four of the conventional PTCs (2 microscopic and 2 clinically sized) had BRAF mutations. The overall BRAF mutation rate in PTCs with CLT was 4/28 (14%). No BRAF mutations were identified in any of the targets from the atypical follicular epithelium in CLT (0/47).
Conclusions: BRAF mutation was identified in a low percentage (14%) of PTCs that arose in a background of CLT. There was no evidence of BRAF mutation in any of the areas with atypical follicular epithelium in CLT. These data suggest that BRAF is a less frequent mechanism of tumorigenesis in a background of CLT. Furthermore, these data demonstrate that BRAF mutation is not present in the atypical follicular epithelium of CLT.

440 Prognostic Immunohistochemical Markers in Pancreatic Endocrine Tumors Assessed by Tissue Micro Array Analysis

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Background: The only definite criterion for malignancy at the time of diagnosis in pancreatic endocrine tumors (PET) is the presence of metastases. Yet it is well known that PET may also metastasize ten to twenty years after resection. Many studies have reported prognostic significance of different immunohistochemical markers, but only few of them have been verified by analysis of independent patient collectives. Our aim was to examine the prognostic significance of the markers MIB1, CK19, CD99, COX2 and p27 in PET.
Design: A tissue micro array (TMA) comprising 185 PET from archival tissues from the files of the Departments of Pathology Zurich and Basel (1974 – 2004, median follow-up 72 months, mean follow-up 88 months) was constructed. Clinical follow-up data of 90 patients could be obtained by contacting the responsible general practitioners. All histological slides were reviewed and classified according to the most recent WHO classification into well differentiated PET, well differentiated PET of uncertain behavior, well differentiated pancreatic endocrine carcinomas and poorly differentiated pancreatic endocrine carcinomas. Sections of the TMA were stained with antibodies against MIB1, CK19, COX2, p27 and CD99. The stainings were scored quantitatively for MIB1 and semiquantitatively for CK19, COX2, p27 and CD99 according to the proposals in the respective publications.
Results: Time to tumor relapse as well as tumor-specific survival were significantly shorter in patients with tumors exhibiting either an elevated MIB1 index or CK19 positivity. The proposed prognostic significances of COX2, p27 and CD99 could not be confirmed. The WHO classification allowed stratification of patients into four different risk groups regarding time to relapse as well as tumor-specific death.
Conclusions: We correlated the clinical follow-up and survival data of 90 PET-patients with clinical, morphological and immunohistochemical findings. A prognostic significance could be confirmed for the immunohistochemical markers MIB1 and CK19, while the significance of COX2, p27 and CD99 could not be reproduced. Overall, for clinical practice the most significant system predicting tumor-free and tumor-specific survival proved to be the WHO classification allowing a stratification of PET-patients into four risk groups.

441 Encapsulated Follicular Lesions with Partial Features of Papillary Thyroid Carcinoma: The Diagnostic Utility of HBME1, Keratin 19, and Galectin-3

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Background: Using tissue array panels consisting of 133 unequivocal cases of follicular adenoma (FA) and papillary thyroid carcinoma (PTC), both classic type (PTCC) and follicular variant (PTCFV), we established previously that simultaneous 2-3+ immunohistochemical staining (IHC) of keratin 19 (CK19) and galectin 3 (GAL3) was seen in >90% of PTCC, 70% of PTCFV, and <10% of FA. To further increase the diagnostic value of IHC, HBME1 staining was evaluated in this tissue array. The utility of these 3 antibodies was then tested in 11 cases of encapsulated follicular lesions with partial features of PTC (FL/QPTC).
Design: Tissue arrays were constructed, consisting of 49 PTCC, 30 PTCFV, 54 FA, and 11 FL/QPTC. IHC was performed using monoclonal antibodies against CK19, GAL3, and HBME1. Positive staining was defined as 2-3+ staining intensity in >10% of cells.
Results: Positive HBME1 staining was seen in 43/49 of PTCC, 22/30 of PTCFV, and 3/54 of FA. In comparison, CK19 was positive in 49/49 PTCC, 26/30 PTCFV, and 11/54 FA. GAL3 was positive in 47/49 PTCC, 23/30 PTCFV, and 11/54 FA. HBME1 staining is thus less sensitive but more specific. Moreover, HBME1 showed cleaner background, with negative staining of adjacent normal thyroid tissue in all cases. Combining HBME1, CK19, and GAL3, 2-3+ staining of all 3 antibodies was seen in 42/49 PTCC, 20/30 PTCFV, and none of FA. Positive staining with 2 of the 3 antibodies was seen in 6/49 of PTCC, 3/30 of PTCFV and 6/54 FA. However, single antibody staining, particularly if seen focally, is less specific, with 1/49 of PTCC, 5/30 of PTCFV, and 13/54 of FA showing 2-3+ staining with only 1 of the 3 antibodies. With the exception of 2 PTCFV, negative staining (0-1+) of all 3 antibodies was only seen in FA (35/54). Based on these findings, 11 cases of FL/QPTC were evaluated. Four cases were positive for all 3 antibodies, thus favoring the diagnosis of PTCFV. Of the remaining 7 cases, 2 were positive for 2 antibodies, and 5 were positive for 1 antibody only. Although a definitive diagnosis is arguable in these cases, the combined histological and IHC findings favor the diagnosis of FA in 6 cases and PTCFV in 1 case.
Conclusions: HBME1 is more specific than CK19 and GAL3 in the diagnosis of PTC and shows less background. For the evaluation of FL/QPTC, the panel of HBME1, CK19, and GAL3 is superior to any single antibody, as most PTC are positive for at least two antibodies, whereas FA only occasionally shows diffuse positivity for more than a single antibody.

442 *BRAF* Mutation Frequency in Papillary Thyroid Carcinoma with Mixed Papillary-Follicular Patterns

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Background: Despite their similarity in clinical manifestation and prognosis, conventional papillary thyroid carcinoma (PTCC) and its follicular variant (PTCFV) are different in their molecular pathogenesis. BRAF mutation, described in up to 69% of PTCC, occurs very infrequently in PTCFV (0-20%), and BRAF mutation-positive and mutation-negative tumors were found to have distinctive gene expression profiles, implying possible differences in biological behavior. In this regard, it is of interest to investigate whether PTC with mixed papillary and follicular morphology (PTCM) is pathogenetically closer to PTCC or PTCFV.

Design: Ten cases each of PTCC, PTCFV, and PTCM were analyzed. PTCM was defined as cases in which papillary and follicular architecture each comprised at least one third of the lesion. Tumor cells were isolated by laser microdissection from paraffin sections and DNA was prepared and analyzed for BRAF mutation in exon 15. In PTCM cases, the follicular and papillary areas were microdissected and analyzed separately. Each sample was evaluated by nested PCR followed by bidirectional DNA sequencing, and mutated sequences were detected using the Mutation Surveyor™ software. In addition, BRAF mutations at V599E were also detected by using a primer-extension based mutation detection kit (Mutector™, TrimGen).

Results: BRAF V599E mutations were found in 10 of 10 cases of PTCC, 1 of 10 cases of PTCFV, and 4 of 10 cases of PTCM. The papillary and follicular areas in PTCM showed identical mutated or wild-type sequences in all 10 cases, confirming its clonal origin. All mutations were of V599E and were confirmed by Mutector™. No additional mutations were revealed by DNA sequencing.

Conclusions: Our findings confirmed that BRAF mutation is frequent in PTCC and rare in PTCFV. PTCM, often clustered with PTCC group in previous BRAF studies, had a BRAF mutation frequency closer to that of PTCFV. The mutation frequency observed in this small series of pure PTCC (100%) is higher than previously reported. This may be attributed to microdissection and increased sensitivity in mutation detection, or it might suggest that the previously reported frequencies were lower as the result of inclusion of PTCM cases. Our findings thus indicate that PTCM might be closer to PTCFV than to PTCC in its molecular pathogenesis. It would be worthy to extend this comparison to a larger series, to evaluate PTCM cases for RET-PTC translocation frequency, and to compare the gene expression profiles of PTCM to PTCC and PTCFV by DNA microarray analysis.

443 cDNA Microarray Analysis of Thyroid Carcinoma Cell Lines Harboring ret/PTC Oncogenes and BRAF V600E Mutations

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Background: The use of cDNA microarrays is a powerful method for the quantitative analysis of disease-specific gene expression that can detect altered gene expression associated with the pathology or the altered biology of a disease entity. Although several other studies have examined genes that may be involved in the pathogenesis of thyroid disease, the complete repertoire of genes, signalling pathways and other basic mechanisms remain poorly defined. Another shortcoming remains the inadequacy of diagnostic and prognostic biomarkers of disease sub-categorisation and progression. This study was undertaken as a step toward identifying previously uncharacterised molecular genetic mechanisms in thyroid carcinoma and to further understand the roles played by ret/PTC oncogenes and BRAF V600E mutation in said disease.

Design: Gene expression profiles for a panel of thyroid cell lines were compared using a whole genome microarray system from Applied Biosystems. The panel comprised cell lines characteristic of various papillary and anaplastic thyroid carcinomas harbouring genetic abnormalities consistent with those found *in vivo* such as ret/PTC and BRAF mutations, in addition to an SV40 transformed normal thyroid cell line.

Results: Genes involved in cell structure and motility and the extracellular matrix were consistently found to be differentially expressed between compared groups. This, coupled with the discovery of over-expressed histones in the V600E mutated groups, led to speculation that these genes may be responsible for the structural peculiarities of certain types of PTC. Escape of apoptosis was also a consistent theme with its inducers and inhibitors frequently found to be down- and up-regulated respectively. Individual genes from inter-group comparisons were also evaluated.

Conclusions: Target genes identified have the power to stratify normal from malignant thyrocytes and further to stratify between ret/PTC and BRAF aberrations. Validation using larger independent panels including clinical samples, is ongoing. The identified gene targets, together with known tumour-specific chromosomal translocations, could prove to be a powerful molecular adjunct to thyroid tumour pathology for the purpose of general diagnosis and/or differentiating histologic subtypes of thyroid carcinoma.

444 BRAF Mutations and RET/PTC Rearrangements in Dominant Nodules of Hashimoto's Thyroiditis

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Background: The histopathology of Hashimoto's thyroiditis (HT) is variable and includes distinct subtypes: HT with classic features of chronic autoimmune thyroiditis; HT associated with hyperplastic/adenomatous lesions; and HT associated with unequivocal thyroid carcinomas. Nodules within HT, defined as dominant nodules, can present with unique morphological findings of carcinoma, including features suggestive of papillary thyroid carcinoma. Activating point mutations in *BRAF* and activating *RET* rearrangements are mutually exclusive events in the oncogenesis of papillary thyroid carcinoma. *RET* rearrangements have been previously described in dominant nodules of HT. We hypothesize that *BRAF* mutations and *RET* rearrangements may distinguish between an abnormal histologic appearance seen in HT dominant nodules and true papillary thyroid carcinoma.

Design: Twenty-eight cases diagnosed as Hashimoto's thyroiditis with a dominant nodule, from 345 consecutive HT thyroidectomies were identified from BWH archives. Seventeen cases were microdissected. Genomic DNA analysis for *BRAF* mutation from paraffin embedded cases of HT with a dominant nodule was performed using PCR, denaturing high performance liquid chromatography, and automated sequencing. Screening for *RET* mutation was performed by FISH, with multicolor probes to detect RET_PTC rearrangements.

Results: Of twenty-eight cases examined, patients ranged in age from 9 to 76, with 1 male and 16 females. Nodule size ranged from 1.5 to 6.2 cm. Ten of these cases contained separate, coincidental papillary carcinomas and 1 had a follicular carcinoma. No *BRAF* mutation was present in a dominant nodule. Of the ten cases with incidental papillary carcinoma, three had a V600E point mutation in *BRAF*, and one case had an exon 15 deletion (600-604E). All the cases tested for RET-PTC rearrangement were negative.

Conclusions: Dominant nodules in HT with histopathological features of carcinoma are not associated *BRAF* mutation or RET-PTC rearrangement within the dominant nodule. *BRAF* mutation was present in 4 of 10 separate, coincidental papillary carcinomas in a setting of HT, supporting the belief that dominant nodules are not malignant and that strict histologic, clinical and molecular criteria must be met for the diagnosis of papillary thyroid carcinoma.

445 Immunohistochemical Detection of Somatostatin Receptor (SSTR) 2A in Clinically "Non-Functioning" Pituitary Adenomas

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Background: SSTR2 and 5 have been detected in GH producing and TSH producing pituitary adenomas as target molecules for somatostain analogue (Octreotide) therapy. Our immunohistochemical study is aimed at to elucidate the expression of SSTR2A, the most significant target molecule for therapy, in the clinically "non-functioning" pituitary adenomas in order to explore the possibility of the targeted therapy. Expression of SSTR in non-functioning adenomas was already reported in previous many reports. This study focused on membanous localization of SSTR 2A expression in addition to its frequency.

Design: Total 43 cases of the clinically non-functioning adenomas (18 cases of gonadotropin:Gn positive adenomas, 11 cases of silent somatotroph cell adenomas and 14 cases of null cell-immunonegative adenomas) were subjected to immunohistochemcial study for SSTR2A on the 4% paraformaldehyde fixed paraffin sections. ABC method was used by using polyclonal primary anti-SSTR2A antibody(Gramsch Laboratories, Schwabhausen, Germany) and Vectastain ABC systems (Vector Laboratories, Burlingame, CA) after antigen retrieval by autoclaving for 10minutes in citrate buffer at pH6.0.

Results: The staining was interpreted as positive for SSTR2A when more than 5% of tumor cell were immunostained on the cell membrane. 9 of 18 Gn producing adenomas, 8 of 11 silent somatotroph cell adenomas and 3 of 14 null cell adenomas were immunopositive on their cell membranes for SSTR2A. Especially in silent somatotroph cell adenomas, their most cells were strongly immunostained on their cell membranes.

Conclusions: Our study suggests that clinically "non-functioning"pituitary adenomas, especially silent somatotroph cell adenomas, could be the candidates for effective somatostatin analogue therapy when SSTR2A is immunohistochemically deteceted on the cell membrane.

446 ACTH and aSU Positive Human Pituitary Adenomas-Morphology, Transcriptional Profiles and Histogenesis of the Rare Tumors with Unique Functional Differentiation

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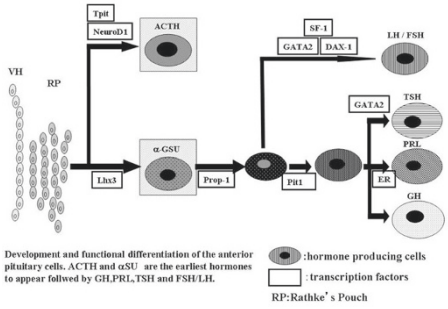
Background: It has been well known that ACTH producing adenomas are mono-hormonal and produce only ACTH and POMC-derived peptides. Very rarely ACTHomas are positive for GH or aSU in addition to ACTH. This study is aimed at to elucidated the nature of ACTH-aSU positive adenomas, and their morphology, transcriptional profiles and histogenetic process are discussed.

Design: Five cases (3 cases of Cushing's pituitary adenomas and 2 cases of silent corticotroph adenomas out of total cases of 46 ACTH producing(including 13 cases of silent corticotroph adenomas) showed the immunopositivity for aSU in addition to ACTH. These cases were extracted from our series of over 1000 cases of human pituitary adenomas obtained by trans-sphenoidal surgery. Characteristics of these ACTH-aSU positive cases were elucidated. Immunohistochemical staining for various pituitary transcription factors were detected by ABC method on paraffin sections after antigen retrievals by autoclaving. Hormones were detected by indirect immunoperoxidase method. Immunohistochemical double staining was also performed.

Results: These cases show immunopositivity for only ACTH and aSU but not other anterior pituitary hormones. By doulble immunohistochemical staining, ACTH and aSU were present in the different tumor cells, but in all cases, some tumor cells contained both ACTH and aSU. Morphological characteristics included large and pleomorphic cytology of the tumor cells with occasional rosette formation (in one case). Nuclei were mildly atypical, Crooke's hyaline was absent. Immunohistochemically, the tumor cells showed the following transcription factors; SF-1 and GATA2 which are related to LH/FSH cell lineage in addition to NeuroD1 and T-pit which related to ACTH cell lineage.

Conclusions: ACTH-aSU positive pituitary adenomas are rare tumors and exhibit unique morphology and transcriptional profiles. As ACTH and aSU are the earliest hormones to appear during development, it is speculated that these ACTH-aSU positive adenomas are derived from the early pituitary-committed progenitor cells.



447 Loss of Heterozygosity of DNA Repair Gene, hOGG1, in Papillary Thyroid Carcinoma and Hashimoto Thyroiditis

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Background: Chronic inflammatory conditions have long been postulated to play significant roles in the development of malignancy. Hashimoto Thyroiditis (HT) is an autoimmune-mediated chronic inflammatory condition in the thyroid gland and has been seen frequently to co-exist with papillary thyroid carcinoma (PTC). The activation of ret/PTC-1, frequently seen in PTC has been detected in HT, raising the possibility that long-standing HT may predispose thyroid epithelia to malignant transformation. To further explore the relationship between HT and PTC, we perform loss of heterozygosity (LOH) analysis of hOGG1, a major repair gene for oxidative DNA damages, in PTC and HT.

Design: Ten cases of PTC with co-existing HT, 14 cases of pure HT (without co-existing PTC) and 8 normal thyroid tissues were included in this study. DNA samples from PTC, HT and normal thyroid tissues were obtained from tissue sections collected using laser-capture microdissection (LCM). All DNA samples were subjected to PCR amplification with 5 fluorescent-labeled microsatellite makers adjacent to the hOGG1 locus (3S1297, 3S1289, 3S1300, 3S1261, 3S1274), followed by fragment analysis using ABI PRISM 3100 Genetic Analyzer.

Results: Ten of 10 (100%) PTC are informative for at least 1 of the 5 markers used and all 10 (100%) PTC showed evidence of hOGG1 LOH. In HT collected in the same gland that contains PTC, 8 of 10 cases (80%) showed evidence of hOGG1 LOH. In these 8 HT cases, the LOH patterns are identical to those seen in the corresponding PTC from the same gland. In the remaining 2 cases, PTC showed hOGG1 LOH at one of the 5 markers whereas the corresponding HT from the same gland showed no evidence of LOH. Eleven of 14 cases of pure HT (78.5%) are informative for at least 1 of the 5 markers used and among these 11 cases, 8 (72.7%) showed evidence of hOGG1 LOH. In 8 normal thyroid tissues studied, none (0%) showed evidence of hOGG1 LOH.

Conclusions: We have shown the high prevalence of hOGG1 LOH in pure HT and identical patterns of hOGG1 LOH observed between PTC and adjacent HT. While the direct cause-effect relationship between HT and PTC remains to be proven, our data indicate strongly that thyroid follicular epithelia can accumulate aberrant genetic changes in long-standing HT, and under some circumstances, these genetically altered epithelia may even progress to PTC.

448 Overall Molecular Classification of Thyroid Tumors as Defined by DNA Microarray Analysis

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Background: Gene expression profiling has become a valuable tool for the molecular classification of human cancer. The classification of thyroid carcinoma is occasionally problematic and subject to inter- and intra-observer variability. To better refine the classification of thyroid tumors, we applied gene expression profiling to a large cohort of benign and malignant thyroid tumors that represent the common types, as well as normal thyroid.

Design: Gene expression profiles of 95 benign and malignant thyroid tumors and 4 normal thyroid samples were generated by DNA microarray analysis using commercially available oligonucleotide arrays. Statistical analysis of the gene expression profiles was performed using principal components analysis.

Results: Principal components analysis of all samples recapitulated the overall classification of thyroid tumors. Undifferentiated carcinomas and medullary carcinomas were most distinctive. Papillary carcinomas formed a distinctive group with significant intra-group variation with several identifiable subgroups. Oncocytic tumors formed another distinct subgroup, with some separation between oncocytic adenomas and carcinomas. The follicular adenomas, follicular carcinomas and normal thyroid were most similar, with some distinction of the follicular carcinomas with the PAX8-PPARG translocation.

Conclusions: These results demonstrate that gene expression profiling can recapitulate the overall classification of thyroid tumors. The most difficult distinctions were between benign and malignant follicular and oncocytic tumors, a result that mimics the difficulties observed in routine morphologic assessment. This approach should improve the classification of thyroid tumors, as well as instill it with a strong foundation in biology.

449 Immunohistochemical Detection of XIAP in Thyroid Lesions and Disorders

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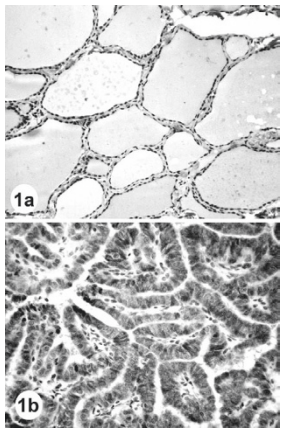
Background: The X-linked inhibitor of apoptosis (XIAP) is the most potent member of the family of IAPs, a group of structurally related caspase inhibitors. XIAP expression correlates with clinical aggressiveness of certain tumors, including renal carcinomas and hematopoietic malignancies. We examined the expression of XIAP in neoplastic and other disorders of the thyroid.

Design: 5 μm sections from routinely processed thyroid specimens with diagnoses of papillary (n=32), follicular (n=7, 2 with oncocytic features), medullary (n=4), and anaplastic (n=3) carcinoma, follicular adenoma (n=11, 3 with oncocytic phenotype), Hashimoto's thyroiditis (n=16), and adenomatous goiter (n=12), were subjected to citrate-based antigen retrieval, followed by incubation with monoclonal anti-XIAP antibody (BD Biosciences) and Envision Plus reagents (Dako). Granular cytoplasmic staining was considered positive and was evaluated for extent and intensity of staining (1+, 2+, and 3+).

Results: XIAP was not detected in normal thyroid tissue (fig 1a) but was detected in 27/32 papillary thyroid carcinomas (fig 1b) (including 1 fine needle aspiration cell block). The staining ranged from 1-3+ in intensity and extent. In contrast, follicular adenomas, follicular, medullary, and anaplastic carcinomas, Hashimoto's thyroididis, and adenomatous goiters were all non-staining with the exception of scattered oncocytic foci in 2/16 cases of Hashimoto's thyroiditis and 3/12 cases of adenomatous goiters.

Conclusions: XIAP was selectively detected in papillary thyroid carcinoma. These findings suggest possible diagnostic utility, for example in interpreting thyroid fine needle aspirations, as well as offering clues to the tumor biology of papillary thyroid carcinoma compared to other thyroid neoplasms.



450 *BRAF* Mutation Analysis in Microscopic Papillary Thyroid Carcinomas

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Background: Constitutive activation of the RAF/MEK/MAP kinase pathway by the T1799A point mutation in exon 15 of the *BRAF* gene is the most common genetic event in the pathogenesis of papillary thyroid carcinoma (PTC). Some studies now suggest, however, that there is a different prevalence based on variant histologies that can be seen in PTC. The contribution to these differences from the size of the tumor (i.e., < 1cm vs. > 1 cm) is also not fully understood.

Design: 83 cases of PTC were included in the study, including 27 conventional (CPTC), 38 follicular variants (FV), and 18 tall cell variants (TCV). Tumors were classified as microscopic (<1 cm) or clinically sized (>1 cm). DNA was extracted from microdissected tumor fragments from paraffin-embedded tissue and exon 15 of the *BRAF* gene was amplified by PCR. Automated cycle sequencing was performed and results were analyzed for the presence of the characteristic point mutation.

Results: Overall, 52% of the CPTCs, 53% of the FVs, and 39% of the TCVs that were analyzed were microscopic tumors. Of the CPTCs, 50% of the microscopic tumors and 23% of the clinically sized tumors had the *BRAF* mutation. Of the FVs, 5% of the microscopic and 6% of the clinically sized tumors had the mutation. Of the TCVs, 57% of the microscopic tumors and 55% of the clinically sized tumors harbored the mutation.

Conclusions: These data show that mutation rates for *BRAF* are reasonably well conserved by histologic subtype in microscopic and clinically sized tumors, with the exception of the difference seen in conventional PTC. These data also demonstrate that the *BRAF* mutation rate is very low in FVs, both in microscopic and clinically sized tumors and support prior evidence that the *BRAF* mutation is common in TCV, regardless of the tumor size.

451 Molecular Alterations in the Lymph Node Metastases of Papillary Thyroid Carcinoma

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Background: The molecular events participating in papillary thyroid carcinogenesis include point mutations in the BRAF and RAS genes and RET/PTC rearrangements. Approximately 40% of thyroid papillary carcinomas harbor BRAF, ~20% RET/PTC, and ~15% RAS mutations. The prevalence of these molecular alterations in lymph node metastasis of papillary thyroid carcinoma is unknown.

Design: We studied a series of 60 lymph node metastases of papillary carcinoma for BRAF, RAS and RET/PTC mutations. In 47 cases frozen tissue and in 13 cases paraffin-embedded tissue from the lymph nodes involved with metastatic carcinoma were available for the study. DNA and RNA were extracted and the PCR and RT-PCR assays were used to detect the mutations.
Results: Among 47 metastatic papillary carcinomas where frozen tissue was available, 21 (45%) were found to have BRAF point mutations, 12 (26%) had RET/PTC-1 or 3 rearrangement, and only 2 (4%) had RAS point mutations. Another 12 lymph node metastasis had no detectable genetic alterations. Among 13 cases where paraffin tissue was available, 8 (62%) revealed BRAF mutations and 3 (23%) revealed RAS mutations, while no RET/PTC rearrangement was detected. By combining the two groups, 48% of papillary carcinomas metastatic to lymph nodes revealed BRAF mutation, 20% RET/PTC rearrangements and 8% RAS mutations, whereas 24% of lymph nodes demonstrated none of these genetic alterations. All 5 metastatic tumors harboring RAS mutations were classified as the follicular variant of papillary carcinoma.
Conclusions: Our data demonstrates that the majority of lymph node metastases from papillary thyroid carcinoma contain either BRAF point mutations or RET/PTC rearrangements. A higher prevalence of BRAF mutations in metastases than in primary tumor nodules supports the previously suggested association between BRAF and more aggressive behavior of papillary carcinomas. A lower frequency of RAS mutations in lymph node metastases correlates with the recent findings demonstrating the association between RAS and the follicular variant of papillary carcinoma, which has an overall lower prevalence of lymph node metastasis.

Gastrointestinal

452 "Seedling" Mesenchymal Tumors (Gastrointestinal Stromal Tumors and Leiomyomas) Are Very Common in the Esophagogastric Region

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Background: Gastrointestinal stromal tumors (GIST) are the most common non-epithelial neoplasm of the gastrointestinal tract and show a predilection for the stomach and esophagogastric junction (EGJ). Most are detected because of symptoms, but some are incidental findings at autopsy or surgery for other reasons. Incidental GIST tend to be smaller at diagnosis, but even small (<1 cm) GIST have been shown to harbor activating *KIT* mutations at rates similar to advanced GIST. However, the prevalence and characteristics of small GIST in surgical resections of the EGJ remains unclear.
Design: We studied 150 esophagogastric resections for esophageal or EGJ carcinomas (100 with pre-operative chemoradiation and 50 untreated cases) that had been extensively embedded for histologic examination (mean 30 sections/case). Number, size, morphology, and location of all GIST and leiomyomas were recorded. All potential GIST were evaluated with CD117 (c-kit) and CD34 immunohistochemistry, and a subset (6) leiomyomas with smooth muscle actin.
Results: 19 incidental GIST were found in 15 (10%) patients; 4 patients harbored 2 separate lesions. Prevalence of GIST was identical in treated (10/100) and untreated (5/50) cases. All (100%) showed diffuse positivity for both CD117 and CD34 and all were of spindle cell morphology. Lesions ranged from 0.2-3.0 mm in size (mean 1.3 mm). Eight (42%) were present in the outer muscularis propria, 8 (42%) in inner muscularis, and 3 (16%) between the muscle layers. Lesions clustered near the EGJ, with 9 (47%) on the gastric side, 9 (47%) on the esophageal side, and 1 (5%) undetermined due to overlying ulceration. Leiomyomas were even more common than GIST, occuring in 47% of patients (44% of treated and 52% of untreated, p= 0.39), with a mean of 3 leiomyomas/patient (range 1-13) and mean size of 1.7 mm (range 0.2-12 mm). Unlike colorectal leiomyomas, most (91%) EGJ leiomyomas were located in the inner muscularis propria and only rarely (1%) in muscularis mucosa.
Conclusions: Both GIST and leiomyomas are common incidental "seedling" lesions of the EGJ, found in 10% and 47% of patients, when carefully evaluated. The universal positivity for c-kit in small incidental GIST suggests that additional genetic or epigenetic alerations are needed for neoplastic progression. Further, the large difference in prevalence between incidental and clinically significant GIST suggests that most do not progress.

453 Duplication of the Muscularis Mucosae and Adenocarcinoma Staging in Barrett's Esophagus

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Background: Depth of invasion is one of the most important prognostic indicators in esophageal adenocarcinoma. Unlike other regions of the gastrointestinal tract, the esophagus in Barrett's metaplasia frequently develops a duplicated muscularis mucosae (MM). The effect of this MM duplication on appropriate staging of superficially invasive adenocarcinoma, however, is unclear.
Design: The study was carried out in two parts. First, 50 resections for high grade dysplasia or T1 adenocarcinoma in Barrett's esophagus (BE) were evaluated for the presence and histologic characteristics of MM duplication, including 1) % of BE segment involved by MM duplication, 2) origin of the duplicated muscle layer, and 3) appearance of the tissue between duplicated MM. Next, we studied 30 resections for BE that had superficial invasion confined to regions of MM duplication. These cases were classified as to 1) depth of invasion (inner MM, space between duplicated MM, or outer MM), 2) angiolymphatic invasion, and 3) rate of lymph node metastasis. For comparison, we used recently published data (*Am J Surg Pathol* 2005;29:1079-85) for cases of lamina propria invasion and superficial submucosal invasion, respectively.
Results: 46 of 50 (92%) BE resections showed MM duplication, involving 5% to >90% of the Barrett's segment. In 5 (10%) cases MM was focally triplicated. The outer MM was in continuity to a single muscle layer beneath squamous epithelium, suggesting that outer MM represents the "original" muscle layer. The space between duplicated MM predominantly consisted of loose fibrovascular tissue similar to submucosa; in 15 (30%) cases, there were also areas of fibrosis or thin muscle strands joining the 2 MM layers. Of 30 adenocarcinomas invading duplicated MM, 10 (33%) invaded only inner MM, 12 (40%) invaded between the MM layers, and 8 (27%) into outer MM. Angiolymphatic invasion was seen in 5 (17%) cases, and nodal metastases in 3 (10%, 1 case each of inner MM, between MM, and outer MM invasion).
Conclusions: MM duplication is a characteristic finding in BE but can pose difficulty in proper staging of superficial adenocarcinoma. The rates of angiolymphatic invasion (17%) and nodal metastases (10%) in cases with invasion into duplicated MM are higher than published rates for lamina propria invasion (each 0%) but similar to those for superficial submucosal invasion (25% and 8%, respectively), suggesting that these tumors can behave aggressively despite their technically "intramucosal" location.

454 True Smooth Muscle Neoplasms of the Stomach: A Clinicopathologic Study

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Background: Gastric leiomyomas and leiomyosarcomas are distinct from the more common c-kit positive gastrointestinal stromal tumors (GIST). Due to their rarity, a comprehensive review has not been undertaken to evaluate their incidence, histopathologic features and clinical behavior. The objective of this study was to analyze true gastric smooth muscle neoplasms, define their clinicopathologic features and criteria for malignancy.
Design: All gastric spindle cell tumors diagnosed at two institutions between 1980-2005 were evaluated. Immunohistochemical stains including c-kit, SMA, desmin, S-100, CD34, caldesmon and vimentin were performed. Tumors that were c-kit negative, >2 cm in size and expressed at least one smooth muscle differentiation marker were included in the study. Mitotic rate, atypia, coagulative necrosis and presence of mucosal ulceration were evaluated and follow-up was obtained. Sequencing of KIT exons 9, 11, 13, and 17 and PDGFRA exons 12 and 18 was performed in selected cases.
Results: Of 1881 tumors evaluated, 16 met the inclusion criteria. The mean tumor size was 5.4 cm (2-12 cm) and the mean age was 53 years (5-88 yrs.). Using gastric GIST criteria, 5 tumors were classified as benign, 7 as having uncertain malignant potential and 4 as malignant. The appearance of the benign lesions was homogeneous and resembled smooth muscle neoplasms at other sites. None showed more than 1 mitosis per 50 HPF. The malignant tumors showed significant heterogeneity, pleomorphism, an increased mitotic rate (12-22/50 HPF) and coagulative necrosis. 3/4 malignant tumors showed adjacent organ invasion, and one had metastasized to the liver at diagnosis. KIT and PDGFRA analysis revealed no mutations in 7 cases. Follow-up was available in all but one case (mean 67 months, range 2-207). One tumor recurred widely throughout the peritoneum during the follow-up. None of the patients died of their disease.
Conclusions: Less than 1% of all gastric spindle cell neoplasms are true smooth muscle tumors. Despite a large mean tumor size (5.4 cm), the majority of tumors behaved in a benign fashion. The only reliable criteria for malignancy was the presence of an increased mitotic rate and adjacent organ invasion. These results suggest that the criteria for malignancy in gastric smooth muscle tumors is likely to be different from those in c-kit positive GIST's. Evaluation of additional cases is needed to confirm this observation.

455 Expression of Annexin A1 in Esophageal and Esophagogastric Junction Adenocarcinomas: Association with Poor Outcome

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Background: The prognosis of patients with esophageal and esophagogastric junction (EGJ) adenocarcinoma is poor even after a curative surgical resection. Annexin A1 (ANXA1) is a calcium binding protein involved in arachidonic acid metabolism and EGFR tyrosine kinase pathway. ANXA1 has been implicated in early esophageal carcinogenesis, particularly in squamous cell carcinoma. However, the role of ANXA1 in esophageal adenocarcinoma and its clinical significance is not clear.
Design: Expression of ANXA1 was assessed by immunohistochemistry and quantitated by percentage of tumor cells with positive staining in tissue microarrays constructed from triplicates of 1 mm tumor tissue cores of 104 (11 stage I, 24 stage II, 53 stage III, and 16 stage IV) surgically resected esophageal and EGJ adenocarcinomas. ANXA1 expression was defined by ≥25% of the tumor cells with positive staining, and was correlated with clinicopathologic features, recurrence-free survival and overall survival.
Results: ANXA1 has site-specific expression in the esophagus and stomach, being expressed in normal squamous epithelium but not in normal columnar esophageal glands or gastric mucosa. ANXA1 expression was detected in 39% (41/104) of adenocarcinomas and was associated with higher T stage (46%, 36/78 in T3 tumors vs 19%, 5/26 in T1/T2 tumors, p=0.03) and with the presence of distant metastasis (63%, 10/16 with distant metastasis vs 35%, 31/88 without metastasis, p=0.04). There was no association between ANXA1 expression and tumor differentiation (p=0.65), or presence of lymph node metastasis (33/76 in N1 vs 8/28 in N0, p=0.17). ANXA1 expression correlated with decreased recurrence-free (p=0.003) and overall survivals (p=0.003) in univariate analysis. In multivariate analysis ANXA1 expression (p=0.008 and p=0.02) and N stage (p=0.001 and p<0.001) were prognostic factors for recurrence-free and overall survivals independent of T stage.
Conclusions: ANXA1 is expressed in a subset of esophageal and EGJ adenocarcinomas. ANAX1 expression is associated with higher T stage and distant metastasis, and is an independent prognostic factor for patient survival.