Results: In 2008, the resident team undertook the problems of less than optimal surgical pathology turn-around time (TAT) and resident dissatisfaction with surgical pathology teaching. Root cause analysis showed a lack of standardization in service schedules and practices in all units (e.g., gross room, histology, transcription). The residents redesigned the service in a step-wise fashion by altering service schedules and duties of faculty, support staff, technicians, and trainees. All changes occurred through group-oriented sessions attended by all personnel. TAT was tracked prior to and during the implementation process. The team first created a service in which more pathologists signed out cases followed by a service in which fewer pathologists signed out cases. Prior to implementation, the percentage of cases signed out in 2 and 3 days was 61% and 77%, respectively. In the initial implementation, when more pathologists were added to the service, the percentage of cases signed out in 2 and 3 days was 80% and 90%, respectively. In the second phase, with a reduction in the number of pathologists, the percentage of cases signed out in 2 and 3 days remained unchanged. All residents showed high levels of satisfaction with the redesigned services.
Conclusions: Our residency quality improvement educational program was effective in improving quality and in leading residents in transformational change processes. As senior residents are involved in almost all front-line activites and are familiar with the system, their education and process involvement is critical in improving healthcare quality.

510 Diagnostic and Educational Support to Pathologists in the Developing World through Telepathology

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Background: The practice of pathology in the developing world presents challenges in terms of limited resources, shortage of trained personnel and lack of continuing education programs. Telepathology holds great promise as a way to offer diagnostic support, 2nd opinions and ongoing training. We report our experience with a recently established static telepathology program between 2 hospitals in the United States and Tanzania, a country of 38 million people served by only 15 pathologists without access to immunohistochemistry (IHC) or molecular diagnostic testing.
Design: An Olympus BH-2 6-headed microscope and SPOT Insight digital camera were donated to an 80-bed Tanzanian multispecialty hospital with an average specimen volume of 800 cytology & surgical pathology cases/year. 2 local pathologists were given on-site training on image acquisition. Images were uploaded to the iPath open source telepathology server. A US pathologist reviewed images in consultation with subspecialist colleagues, in order to provide both diagnostic and educational support to submitting pathologists as the program's main objective.
Results: During the initial 5 months of the program, 15 cases were submitted for 2nd opinion consultation in subspeciality areas of cytology (4), GI (4), breast (2), head & neck (2), hematology (2) and soft tissue (1). Static images enabled a complete or partial diagnosis in 12 cases (80%). 4 entirely diagnostic cases included a soft palatal pleomorphic adenoma, a breast giant hamartoma, a rectal tonsil and a rectal cavernous hemangioma. 4 partially diagnostic cases were favored to be malignant and 4 were favored to be benign/reactive. Among the 8 partially diagnostic and 3 non-diagnostic cases, factors precluding a definitive diagnosis included absence of confirmatory IHC/flow cytometry (1 soft tissue, 2 hematology cases), air-drying (4 cytology cases), tangential tissue sectioning (1 head & neck case) and non-technical issues (3 cases). Responses posted to completely and partially diagnostic cases included a diagnosis, discussion of the differential diagnosis and additional information (e.g. gross findings, diagnostic pitfalls and/or publications about the entity).
Conclusions: Telepathology is well-suited to provide both diagnostic support and continuing education to pathologists in the developing world. Factors precluding a definitive diagnosis are mainly technical and can be overcome by additional training or building local capacity for basic ancillary testing.

Endocrine

511 Geographical Mapping of a Multifocal Thyroid Tumour Using Genetic Alteration Analysis & MiRNA Profiling

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Background: Papillary thyroid carcinoma (PTC) frequently presents as multiple tumour-foci within a single thyroid gland or pluriform, with synchronous tumours comprising different histological variants, raising questions regarding its clonality. Among genetic aberrations described in PTC, BRAF V600E mutation and ret/PTC activation occur most commonly. Several studies have investigated the genetic alteration status of multifocal thyroid tumours, with discordant results. To expand on the question of clonality the objective of this study was to examine disparate geographical and morphological areas from a single PTC for the presence of ret/PTC or BRAF mutations and correlate it with miRNA expression profiles.
Design: A multicentric PTC containing classic PTC, insular and anaplastic foci, & tumour cells adjacent to vascular invasion and lymphocytic infiltrate was examined for the presence of ret/PTC & BRAF mutations. Geographical data was correlated with expression profiles of 330 miRNAs. Hierarchical clustering analysis of the profiles, miRNA gene target prediction, & immunohistochemistry were also performed.
Results: Each morphological area proved negative for ret/PTC 1 rearrangement. Two distinct foci with classic morphology harboured the BRAF mutation. All other regions, including the insular and anaplastic were negative for the mutation. MiRNA profiles were found to distinguish tumours containing the BRAF mutation from the other tumour types, & differentiate between insular and anaplastic tumours. Profiles also included miRNAs previously discovered in this cancer, and miRNAs linked to various processes involved in tumour growth and proliferation.
Conclusions: The initial genetic alteration analysis indicated that pluriform PTC did not necessarily evolve from classic PTC progenitor foci. MiRNA profile analysis, however provided an interesting interpretation to the answer of the clonality question. The hierarchical clustering analysis indicated that the multiple foci may not have arisen due to the clonal metastasis of tumour cells or of independent mutational events, but perhaps both phenomena can occur simultaneously within the one tumour to enhance cancer progression. Putative gene targets were also obtained for the differentially regulated miRNAs raising the question as to the utility of these RNAs as either biomarkers or biological mediators.

512 Pancreatic Specific Transcription Factors and CK19 in Pancreatic Endocrine Tumors

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Background: The role of transcription factors (TFs) **Isl-1, Pax6, Nkx2.2, Nkx6.1, MafB and Pdx-1** in pancreas development was recently described. They regulate commitment to individual cell lineages and maintain terminally differentiated phenotype. Their disruption results in impaired development of pancreatic endocrine structures. Few data are available about their expression in pancreatic endocrine tumors (PETs). PETs are classified in WHO categories as tumors with benign behaviour (WDET-B), uncertain behavior (WDET-U), well differentiated carcinomas (WDEC) and poorly differentiated carcinomas (PDEC). No absolute histopathological criteria are available to predict clinical course. Therefore, identification of immunohistochemical markers that could predict the biological behavior would be extremely helpful in surgical management and adjuvant therapy of PET. The expression of the intermediate filament cytokeratin 19 (CK19) has been recently proposed as predictor of survival in PETs.
Design: To evaluate in a large series of PETs the expression of TFs (Isl-1, Pax6, Nkx2.2, Nkx6.1, MafB, Pdx-1) and CK19 and analize their correlation with WHO categories. TFs expression was immunohistochemically evaluated in a series of 131 PET (48 WDET-B, 32 WDET-U, 41 WDEC and 10 PDEC); TF score was defined as follows: Low (LS), 0-3 TFs immunopositive per case; High (HS), 4-6 TFs immunopositive per case. The same series was evaluated for CK19 expression and its correlation with WHO categories and TFs expression investigated.
Results: HS for TFs (4 or more) was observed in 90% of WDET-B, in 69% of WDET-U, and in 49% of WDEC; all PDEC showed LS ($p<0.05$). Nkx6.1 was the most frequently TF associated with WDET-B (79%) in contrast to WDET-U with 50% of positive cases. Low reactivity was observed for Nkx6.1 in WDEC (20%) and PDEC (10%) ($p<0.05$). CK19 expression was higher in WDEC (83%) than in WDET-B and WDET-U tumors (56%) ($p<0.05$).
Conclusions: Endocrine specific TFs are coexpressed in most WDET-B and WDET-U; on the contrary, a progressive loss of expression is detected in WDEC and PDEC: the TFs biological role in pancreatic endocrine specification is maintained in PET and run in parallel with WHO PET categories. Among these TFs, Nkx6.1 is the most sensitive marker of tumor differentiation, with very limited reactivity in WDEC and PDEC. These changes in TFs expression in PET are inversely correlated with CK 19 expression. The combined analysis of TFs and CK19 could offer a better prognostic index in the clinico-pathologic evaluation of PET.

513 Gene Expression Profiles in Archival Thyroid Carcinoma Using Pre-amplification RT-PCR, Immunohistochemistry and MicroRNA Expression Analysis

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Background: Thyroid cancer is the most frequently occurring endocrine malignancy, with world wide thyroid cancer incidence rates increasing year after year. Furthermore thyroid nodules are a common occurrence and distinguishing benign specimens from malignant can be problematic. The development of a robust molecular expression signature encompassing transcriptomic, protein and microRNA expression analysis would greatly aid in the diagnosis of thyroid malignancy.
Design: Two hundred and five formalin fixed paraffin embedded (FFPE) thyroid samples were laser capture microdissected and a novel pre-amplification technique was used to facilitate gene expression analysis using TaqMan® Q-RT-PCR of a panel of 5 targets including MMP11, BAX, APOE, TOP2A and LYN plus endogenous control. A tissue microarray (TMA) was constructed using the same two hundred and five samples and immunohistochemical analysis was carried out for MMP11, BAX, APOE, TOP2A and LYN. Expression of microRNA let-7a was analysed in one hundred and four FFPE thyroid tissue samples using a novel *in situ* (ISH) hybridisation technique for miRNA expression in archival samples.
Results: All five targets were found to significantly ($p < 0.05$) discriminate a range of benign from malignant thyroid disease states at mRNA and protein expression levels. Four out of the five targets were also shown to significantly differentiate, at the gene and protein level, corresponding groups of matched samples. Of these four targets, MMP11 and APOE, which were over-expressed in malignant tissues, were found to be targets of let-7a using miRNA target prediction databases. Expression of let-7a was significantly ($p < 0.05$) down-regulated in all malignant and neoplastic groups, and subsequently was also found capable of discriminating benign from malignant thyroid disease.
Conclusions: This robust panel of molecular markers could prove extremely useful in assisting the classification and diagnosis of thyroid malignancy.

514 Significance of Ki-67 Proliferative Index in Midgut Carcinoid Tumors

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Background: Gastrointestinal carcinoid tumors may show variable clinical behavior despite similar morphology. A high Ki-67 proliferative index has been shown to be associated with metastatic disease and poor survival. A Ki-67 index of 2% is one of the proposed cutoffs for grading carcinoid tumors. Since the clinical behavior is site-specific and most of the metastatic midgut carcinoid tumors have a low proliferative index, the aim of this study was to determine the clinical significance of Ki-67 index in these tumors.

Design: Ki-67 immunohistochemical stain was performed on both primary and metastatic midgut carcinoid tumors from 60 patients. The slides were "eyeballed" and the area of interest ("hot spot" in variably stained cases) was circled. Ki-67 index was determined by computer assisted image analysis using Ariol system. The results were correlated with presence or absence of nodal and/or distant metastases, and disease progression defined by increasing size, appearance of new lesions, or worsening symptoms. Spearman rank order correlation and Wilcoxon scores were used for statistical analysis.

Results: There were 32 females and 28 males ranging in age from 29-95 years (mean: 53 years). Fifty patients had metastatic disease, of which 48 had nodal metastasis and 23 had distant metastasis. The mean and median Ki-67 indices were 5.26 ($\pm$6.71) and 2.21 for patients with any metastatic disease, and were 1.68 ($\pm$1.17) and 1.59 for those without (p=0.044). Similarly, there was a significant difference in the mean and median Ki-67 values for patients with and without distant metastases (7.45$\pm$7.77 and 5.0 *versus* 2.93$\pm$4.44 and 2.0; p=0.003). Nine patients developed progressive disease. All of these 9 patients had distant metastases. Among patients with distant metastases (n=23), the median time to progression for patients with Ki-67 index of >10% was 1.9 years versus 12.5 years for patients with Ki-67 of <10% (p=0.0004).

Conclusions: Ki-67 proliferative index in midgut carcinoid tumors has significant correlation with metastatic disease (local or distant). Patients with distant metastases have a significantly higher Ki-67 index than patients without. Patients with distant metastases and a Ki-67 index of >10% are highly likely to undergo clinical progression.

515 Interobserver Variability in Assessing Ki-67 Proliferative Index in Gastrointestinal Well-Differentiated Neuroendocrine Neoplasms

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Background: Gastrointestinal well-differentiated neuroendocrine neoplasms (carcinoid tumors) with similar morphologic features may show variable biological behavior, which may be predicted by Ki-67 proliferative index (>1% Ki-67 associated with worse clinical outcome). Many practicing pathologists just "eyeball" immunostaining for reporting Ki-67 index, which may not be accurate or reproducible, particularly in tumors with a low proliferative index. Thus, using computer-assisted image analysis to assess Ki-67 index may be more desirable. This study examined interobserver variability in "eyeballing" Ki-67 proliferative index in carcinoid tumors and compared this method with computer-assisted image analysis.

Design: Sixty-eight Ki-67 immunostained slides from midgut carcinoid tumors were circulated among 5 pathologists for "eyeballing". In cases where Ki-67 labeled cells were heterogeneously distributed, "hot spots" were circled to ensure that the same areas were evaluated. Ki-67 index was categorized as <1%, 1-2%, and numerical value for higher indices. Kappa statistics were calculated using 1% and 2% cutoffs. Computer-assisted image analysis was performed using Ariol system.

Results: With a cut off of 2%, an agreement was achieved by all 5 pathologists in 46 cases (68%) with a k-value of 0.66 (95% confidence interval: 0.59-0.74); indicating a substantial degree of agreement by "eyeballing". With a cut off of 1%, the agreement was reduced to 35 cases (52%) and the k-value was in the moderate range (0.52). When individual pathologist performance was compared with computer-assisted image analysis, k-value was much lower (0.39 if the cut off was 2% and 0.36 if 1%). Computer-assisted image analysis generated a higher Ki-67 index in 21% of the cases comparing with individual pathologists, due to counting Ki-67 labeled lymphoid cells within the tumors.

Conclusions: There is a moderate to substantial agreement among pathologists in "eyeballing" Ki-67 index in carcinoid tumors, when the cut off for Ki-67 was kept as 1% or 2%. There is a stronger agreement among pathologists than between pathologists and computer. "Eyeballing" is thus a simple and relatively reliable method for assessing Ki-67 proliferative index in most carcinoid tumors. However, in borderline cases, computer-assisted image analysis may be helpful for more accurate counting if the areas examined contain no or only rare lymphoid cells.

516 Stem Cell Features Segregate to the Peripheral Compartment of High-Grade Follicular Thyroid Carcinomas

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Background: Previous studies have looked at the expression of some stem cell markers in the thyroid, but no topographical analysis is available. We aim to assess topographically stem cell markers in differentiated follicular cell thyroid proliferative lesions by gene expression and immunohistochemistry.

Design: We selected 15 hyperplastic nodules, 22 adenomas, 14 minimally-invasive carcinomas, 24 widely-invasive carcinomas, 15 papillary carcinomas and 13 anaplastic carcinomas (WHO criteria). Total RNA was extracted from normal and neoplastic tissues (peripheral and internal compartments) 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 U133A Array noncompetitively. Cross-validated gene expression analyses were performed (expression factor≥2, significance≤0.01), and variables studied regarding the histological diagnosis and stem cell markers. Telomerase immunostaining and FISH-PNA of telomere were also analyzed. Significant variables were evaluated immunohistochemically.

Results: Statistically significant topographic differences were observed for CD44 for PTC and FTA, being significantly higher for widely invasive FTC in both peripheral and internal compartments. CD44 expression correlated positively with IL18 receptor accessory protein, PAX8, MAPK10, AKT3, and ELK3, and negatively with MCM4, Histone deacetylase 2, Transcription factor 3, Chromodomain helicase DNA binding protein. Using these variables, 96.6% of follicular neoplasms and 54% PTC were correctly classified. Telomerase was significantly higher in internal compartments (p<0.001) and in malignant lesions (p<0.001), correlating with PNA-FISH detectable telomeres in internal compartments. PNA-FISH detectable telomere in more than 20% of peripheral tumor cells was observed in high-grade lesions (widely-invasive and anaplastic carcinomas).

Conclusions: Stem cell markers showed higher expression in peripheral compartments of follicular cell thyroid carcinomas, controlling the MAPK-ELK and WNT pathways (MAPK10, TCF3) and cell replication (MCM4, HDAC2, helicase). Maintained stem cell features in peripheral follicular cells would contribute to higher metastatic potential.

517 DNA Copy Number Aberrations in Thyroid Tumors Determined Using SNP Arrays

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Background: Changes in DNA copy number, such as deletions and amplifications, are common in cancer and frequently play an important role in tumor origination and progression. Single-nucleotide polymorphism (SNP) microarrays (SNP arrays), particularly those of high-density, allow effective detection of copy number changes in the entire genome. DNA copy number aberrations have not been systematically studied in thyroid cancer using high-density SNP arrays.

Design: We used Affymetrix Genome-Wide Human SNP Array 6.0 that contains 1.8 million genetic markers for the detection of copy number variation in 12 thyroid papillary carcinomas (PC) (including 6 classic papillary (PC,CL) and 6 follicular variants (PC,FV)), 6 follicular carcinomas (FC), and 5 follicular adenomas (FA). In addition, 9 matching normal thyroid tissues were used. For all samples, DNA was isolated from snap frozen tissue. Copy number estimates were calculated using Partek Genomics Suite (Partek, St. Louis, MO) with the averaged 9 normal samples as a baseline. All tumor samples were genotyped for *BRAF* mutation.

Results: On principle component analysis, FAs formed a separate cluster from malignant tumors, whereas PC,CL, PC,FV, and FC showed no particular grouping. Further analysis of PC revealed 16 regions of deletion and 24 regions of amplification frequently present in these tumors. Among those, deletions on 1p13.3 were found in 50% of PC,CL and 50% PC,FV, whereas all other deletions were more common in PC,FV than in PC,CL, including loci on 3p26.3, 3p26.1, 13q21 and 13q31 (all found in 67% of PC,FV vs 17% PC,CL), and locus on 3p24.1 – (83% vs 0). Multiple deletions were found in one PC,CL tumor that carried BRAF mutation. Most common loci of amplifications were on chromosomes 7q11, 10q21, and 19p13. Multiple amplification events were found in 33% of PC,CL (both *BRAF* positive tumors) and 83% of PC,FV.

Conclusions: Our results show that in thyroid tumors, DNA copy number aberrations, including both deletions and amplifications, are more common in follicular variant papillary carcinomas than in classic papillary carcinomas. The most dramatic differences between the two tumor subtypes were observed in deletion on 3p24.1 and in amplification of 7q11.23 and 10q21.3. Among classic papillary carcinomas, multiple DNA copy number aberrations were more common in tumors with *BRAF* mutation, consistent with the known correlation between this mutation and more aggressive tumor behavior.

518 Overexpression of p21 Is a Marker of Tumor Metastasis in Primary Pancreatic Neuroendcrine Neoplasms

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Background: p21 is an inhibitor of cyclin-dependent kinases and is down-regulated by p53 to inactivate cell proliferation. Some studies have failed to demonstrate a correlation between p21 expression and the status of p53 gene in cancer suggesting that p21 might also be regulated by p53-independent pathways. Using an Affymetrix platform, we identified p21 as one of the leading metastasis-associated genes in primary pancreatic endocrine carcinomas (PECAs) that had already metastasized to the liver. Expression of p21 has not been studied in metastatic PECAs and non-metastatic primary pancreatic endocrine tumors (NMP-PETs).

Design: To elucidate the expression of p21 gene product in MP-PECAs, NMP-PETs, and normal pancreatic islets, we constructed Tissue Microarrays (TMAs) from archival tumor blocks. In order to study the phenotypic/genotypic heterogeneity of the tumors and their relation to normal islets, 5 tumor cores and up to 5 adjacent pancreatic tissue cores with islets (as tissue controls) were selected for each case. The 65 cases in the TMAs included 22 MP-PECAs, 35 NMP-PETs and 8 cases of poorly differentiated neuroendocrine carcinoma (PD-NECAs) of the lung and other sites. Standard Avidin-Biotin IHC techniques were used to stain TMAs for p21 $^{(WAF1, CIP)}$ antibody (Calbiochem-Oncogen). Based on evaluation of all cores in the TMA, a p21 index was calculated as percentageage (%) of the stained cells.

Results: Only nuclear staining was considered positive result. Percentages and ranges of positively stained nuclei (PN) were calculated for all 4 types of specimens as shown in Table 1.

Table 1: Results of nuclear staining

	Pancreatic Islet	NMP-PETs	MP-PECAs	PD-NECAs
Number of cases	20 (8 M/12 NM)	18	19	8
Range of PN	6-21	1-25	3-63	2-80
Average of PN	11	9	21	34

The mean p21 expression index (%) for MP-PECAs was 21.0 vs. 8.72 for NMP-PETs (p=0.01). The mean p21 expression index (%) for all (both metastatic and non-metastatic) neoplasms vs. matched control islets was 14.8 vs. 7.9 (p=0.04).

Conclusions: We have validated p21 protein expression as a marker of metastatic phenotype in pancreatic endocrine tumors. High nuclear expression of p21 appears to have a potential clinical utility as a marker of tumor progression.

519 Palladin Is a Novel Marker of Metastasis in Primary Pancreatic Endocrine Neoplasms

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Background: Based on gene expression profiling we identified a number of novel metastasis-associated genes in primary PECAs (MP-PECAs) with liver metastases as compared to clinically localized primary PETs (CLP-PETs). One of the top genes, palladin, is intimately involved in cell structure and motility. The purpose of this analysis was to validate the expression of palladin in pancreatic endocrine neoplasms at protein level and to determine its association with the presence of liver metastasis.

Design: A pancreatic endocrine tumor tissue microarray (TMA) was constructed using well-differentiated pancreatic endocrine tumors/carcinomas (PETs/PECAs) and non-neoplastic pancreatic tissue/islets. The TMA was immunostained with rabbit anti-human Palladin polyclonal antibody (dilution 1:50) (ProteinTech Inc. Chicago IL). The staining results were expressed as low or high expression based on the intensity and distribution of staining and was correlated with presence or absence of liver metastases.

Results: Our study included 24 males and 32 females, age range: 17 to 79 (mean age 54), with tumor size ranging from 0.6 cm to 11.5 cm (mean 3.0 cm). All tumors were well-differentiated PETs/PECAs (WHO 2004). Palladin staining was cytoplasmic. High palladin expression was found in 73% of PECAs but in only 21% of PETs. Conversely, low palladin expression was seen in 11% of PETs but in only 3% of PECAs. Histologically normal pancreatic islets were negative or showed weak palladin expression [Table1]. High expression of palladin was associated with liver metastasis (p = 0.017).

Expression of Palladin in PECAs/PETs

H-score	Intensity of Palladin Staining	Primary PETs with no liver metastases (CLP-PETs) no. cases (%)	Primary PECAs with liver metastases (MP-PECAs) no. cases (%)
Low expression	≤1	11 (79)	3 (27)
High expression	>1	3 (21)	8 (73)

Conclusions: Palladin appears to be a promising marker of metastatic potential in primary pancreatic endocrine neoplasms. The over-expression of this protein in primary endocrine tumors suggests that cytoskeletal abnormalities may contribute to the invasiveness and metastatic phenotype in these neoplasms.

520 Expression of E-Cadherin and β-Catenin in Gastro-Intestinal Neuroendocrine Tumours

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Background: β-catenin is a multifunctional molecule that acts as transcription factor resulting in cell proliferation and differentiation in the Wnt pathway. It interacts with E-cadherin, a transmembrane adhesion molecule.

Design: To examine the expression of β-catenin and E-cadherin in gastrointestinal neuroendocrine tumours (GI NETs). Nineteen cases were retrieved from the archives of the department of Pathology, UHN and stained with a β-catenin and two antibodies (Abs) (against extracellular (ECD) and cytoplasmic domains(CD)) to E-cadherin. Parameters examined were localization (membrane vs cytoplasm); intensity, 0 (negative), 1+ (weak), 2+ (moderate), 3+ (strong); percentage positive cells, <5% (0), 5-30% (1+), >30-70% (2+), > 70% (3+). An index of staining was calculated (intensity by % positive cells).

Results: Age range: 36-93 years, 14 were males. E-cadherin immunoreactivity with the Ab against the CD was cytoplasmic in all cases, being weak to moderate in more than 80% of the cases. Nuclear positivity was seen in 3/19 cases, while membranous immunoreactivity was seen in 10. E-cadherin immunoreactivity with Ab against the ECD was cytoplasmic in 18/19 cases and also membranous in 12, without any nuclear staining. One case was negative. Membranous staining was seen in more than 50% cells in 9/10 cases with CD Ab and 9/12 cases with ECD one. There was not significant difference in intensity of immunoreactivity between the two Abs. There was a progressive loss of membranous immunoreactivity from T1 to T4 tumors, while cytoplasmic staining was seen in all the cases irrespective of the stage. No correlation was seen between nuclear staining and stage. TNM staging was not possible in two cases. Dot like E-cadherin cytoplasmic positivity was seen in more advanced tumors (T2-T4 and N1), with both the Abs. β-catenin immunoreactivity was cytoplasmic in 5/19 cases, cytoplasmic and nuclear in 5, cytoplasmic and membranous in 1, nuclear and membranous in 1, and 7/19 cases showed only membrane immunoreactivity. 10/11 tumors showed cytoplasmic or nuclear positivity in more than 30% cells, whereas membrane positivity was from weak to moderate. There was a progressive loss of membranous staining and increase of nuclear/cytoplasmic staining from T1 to T4 tumors.

Conclusions: GI NETs show dysregulation in Wnt pathway with aberration in E-cadherin and β-catenin: progressive loss of membranous immunoreactivity with all the three Abs with increasing stage of the tumors. The two Abs against E-cadherin show a similar pattern of staining.

521 Analysis of microRNA let-7a and mir-140 Expression Levels Using Laser Capture Microdissected (LCM) Samples of Thyroid Formalin Fixed Paraffin Embedded (FFPE) Tissues

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Background: Archival formalin-fixed paraffin-embedded (FFPE) tissues represent an abundant source of clinical specimens. Our previous study demonstrated that miRNAs can be detected in FFPE samples and its expression level is reliable in FFPE compared with snap frozen paired samples. Thyroid cancer incidence has increased significantly during the past decades and has become one of the leading cancer types in females. This study is aimed to examine microRNA expression across a spectrum of thyroid diseases including benign, malignant, inflammatory, and hyperplastic.

Design: 182 samples of thyroid FFPE tissues, representing different diseases, were laser captured microdissected and then were undergone RNA extractions using Ambion RecoverAll Total Nucleic Acid Isolation Kit according to manufacturer's protocol. Applied Biosystems TaqMan® microRNA (miRNA) assays and protocol were utilised in this study, including two human targets, let-7a and mir-140, and one endogenous control, RNU6B. Nonparametric Mann–Whitney statistical analysis was performed on the relative quantification (RQ) values using Analyse-it® statistical software for Microsoft® Excel®.

Results: We found Let-7a has a higher expression level in PTC versus Hashmoto's thyroiditis or thyroiditis and mir-140 has a higher expression level in neoplasm and malignancy compared with non-neoplasm, with P value < 0.05.

Conclusions: microRNAs were constantly detected in FFPE tissue samples, and even in the limited available materials, for example in LCM. The different expression levels of let-7a and mir-140 in thyroid disease settings demonstrated these microRNAs could be the potential biomarkers functioning in the thyroid signalling pathways.

522 MicroRNA Analysis in Common Thyroid Neoplasms as an Aid for Diagnostic Evaluation

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Background: Although diagnosis of the majority of thyroid neoplasms is straightforward by histological evaluation, the differential diagnosis of a number of lesions such as follicular variant of papillary thyroid carcinoma (FVPTC), follicular carcinoma (FC) and follicular adenoma (FA), remain difficult due to morphologic similarity and lack of diagnostic ancillary studies. Studies found a group of small RNA molecules (~22 nucleotides long), namely microRNA (miR) derived from non-coding sequence of RNA, bind to specific mRNA targets to modulate translation or RNA degradation process, and important to tumor growth. It has been also reported recently that overexpression of miR-221, -222, and -181b were detected in thyroid papillary carcinomas (PTC) using real-time PCR (RT-PCR) technique and freshly frozen surgical specimens.

Design: Based on previous studies of miR extraction from paraffin-embedded samples, we quantified miR-221 and -222 by RT-PCR (Taqman assay) on miR isolated from paraffin-embedded thyroid specimens, and extended the analysis to a broader category of thyroid neoplasms including PTC, FVPTC, FC and FA, as well as normal thyroid tissue. miR-RNU is used as an internal control for calculation of Ct (cycle threshold) values. The expression of miR-221 and -222 in normal and several thyroid neoplasms are illustrated in the following table.

	Normal	PTC	FVPTC	FC
n	9	10	18	10
ΔCt (miR-221)	-0.18 ± 0.83	-3.96 ± 1.15***	-2.05 ± 2.5*	-2.20 ± 2.24*
ΔCt (miR-222)	-1.11 ± 0.71	-4.81 ± 1.18***	-2.85 ± 2.70	-2.34 ± 2.05

t-test was used for statistical analysis. *** p<0.0005, * p<0.05

Results: Our results showed miR-221 is significantly overexpressed in PTC compared to normal thyroid, and slightly overexpressed in FVPTC and FC. miR-222 expression level is also highly up-regulated in PTC, but did not show a statistical significance in FVPTC or FC.

Conclusions: In conclusion, miR-221 and -222 are highly sensitive diagnostic markers of PTC, but are less sensitive to FVPTC and FC, and may be helpful in understanding the biology as well as aid in evaluation of thyroid neoplasms.

523 Cribriform-Morular Variant of Thyroid Carcinoma: Distinct Immunohistochemical Profile from Other Papillary Thyroid Carcinoma Variants

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Background: Papillary thyroid carcinoma (PTC), the most common thyroid tumor, is typically well-differentiated and expresses HBME-1 and galectin-3; in contrast, poorly differentiated and anaplastic thyroid tumors are characterized by expression of p53 and Bcl-2 and high MIB-1 proliferative index. The cribriform-morular variant of thyroid carcinoma (CMv-TC) typically occurs as an extraintestinal manifestation of familial adenomatous polyposis (FAP), although rare sporadic cases have been reported. It occurs almost exclusively in young females, is well-differentiated, often multifocal, is characterized by cribriform, solid, and morular areas lacking typical nuclear features of papillary thyroid carcinoma, and is associated with germline and somatic mutations in the APC or beta catenin genes. In contrast to conventional PTC, CMv-TC rarely metastasizes and carries a benign prognosis.

Design: We reviewed nine cases of CMv-TC from our two institutions, and performed immunohistochemical analysis on a total of fourteen lesions from six cases. Clinical history and genetic test results were obtained from the medical record.

Results: All patients with CMv-TC were female and ranged in age from 18 to 51. All CMv-TC tumors showed strong nuclear and cytoplasmic accumulation of beta-catenin, expressed CK19, p53 and Bcl-2, and showed weak or absent expression of HBME-1 and galectin-3 (Table 1). MIB-1 index, and expression of S100 and 34BE12, were variable and did not distinguish CMv-TC from conventional PTC.

Table 1. APC Mutation and Immunohistochemical Profile of CMv-TC

Age/Sex	APC mutation	CK19	HBME-1	Galectin-3	β-catenin (nuclear)	p53	Bcl-2
24/F	NK	++	+/-	+	+++	++	+++
24/F	+PTT seg. 2	++	-	+/-	+++	++	+++
18/F	R302X	++	-	-	+++	++	+++
51/F	R564X	++	-	-	+++	++	+++
49/F	+PTT seg. 2	++	-	+	+++	++	+++
18/F	D1948X	++	+/-	+	+++	++	++

NK, not known; PTT, protein truncation test.

Conclusions: CMv-TC is characterized by nuclear and cytoplasmic accumulation of beta catenin, immunoreactivity for CK19, p53 and Bcl-2, and lack of immunoreactivity for HBME-1 and galectin-3; these features are distinct from PTC, including classical, tall cell, and diffuse sclerosing variants. Expression of Bcl-2 and p53 in a well-differentiated tumor is unexpected, as these markers indicate dedifferentiation in PTC. Our findings suggest that CMv-TC should be classified as a distinct category of thyroid carcinoma arising in a familial setting, rather than as a variant of PTC.

524 Does p53 and MIB-1 Immunostaining Help in the Diagnosis of Poorly Differentiated Thyroid Carcinoma Using the Turin Criteria?

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Background: The recent Turin proposal (2007) to diagnose poorly differentiated thyroid carcinoma (PDTC) used the following criteria : presence of a solid/trabecular/insular growth pattern, absence of nuclear features of papillary thyroid carcinoma, and the presence of at least one of the following features: convoluted nuclei, mitotic activity ≥ 3 per 10 HPF, and tumor necrosis. Using these criteria, along with MIB-1 and p53 immunostaining, we attempted to validate a series of poorly differentiated thyroid tumors. Our goal is to determine if these markers can assist in further classifying these tumors, especially in cases where the proposed criteria were not met.

Design: Forty one cases of PDTC were selected and retrieved from the files at BWH over a two year period (2005-7). The cases were reviewed and the above criteria were applied. The following characteristics were analyzed: classification of the main tumor, percentage of the poorly differentiated component, mitotic rate (per 10 HPF), and the presence or absence of convoluted nuclei and necrosis. In addition, MIB-1 and p53 stains were evaluated.

Results: The average age of patients with PDTC was 56 years (range 33-83). Male and female patients were roughly present in equal proportions (20 M, 21 F). A total of 14 tumors were associated with papillary thyroid carcinoma of diverse variants, while 14 were designated pure. Six oncocytic neoplasms were present, along with one tumor arising from a follicular carcinoma. The average percentage of the poorly differentiated component was 65% (range 5-100%). Mitotic rates ranged from 0-15/10 HPF (average 2.7). Necrosis was present in 27 of 41 cases. p53 was over-expressed in 27 of 28 cases. Convoluted nuclei were seen in 27 of 41 cases. Of the remaining 14 cases without convoluted nuclei, three contained areas of necrosis, 4 contained ≥ 3 mitoses/10 HPFs, and three contained both increased mitoses and necrosis. A total of four cases lacked convoluted nuclei, increased mitoses, and necrosis, but were classified as PDTC based on increased MIB-1 staining (≥15%) and p53 overexpression.

Conclusions: The recently proposed Turin criteria can reliably diagnose PDTC, and should be incorporated in routine practice. Furthermore, the addition of p53 and MIB-1 stains can assist in cases where the diagnosis of PDTC is suspected based on growth pattern, but without of convoluted nuclei, increased mitoses, or necrosis. We conclude that p53 and MIB-1 stains complement and support the Turin criteria for the diagnosis of PDTC.

525 Immunohistochemical and Molecular Characteristics of Follicular Patterned Thyroid Nodules with Incomplete Papillary Thyroid Carcinoma-Like Nuclei

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Background: Follicular patterned thyroid nodules with incomplete papillary thyroid carcinoma (PTC)-like nuclei (FTN-IPTCNs) are difficult to diagnose, and are often designated well-differentiated tumors of uncertain malignant potential in encapsulated follicular lesions. They are characterized by well-formed follicles with atypical nuclei mimicking those of PTC, but the extent of atypia is incomplete for definite diagnosis of PTC. Their biological behavior and association with follicular variants of PTC (FVPTC) have not yet been established.

Design: We investigated immunohistochemical features (galectin-3, HBME-1, CK19, fibronectin-1, CITED1), *BRAF* V600E mutation and *RASSF1A* promoter methylation status in 30 FTN-IPTCN cases, along with 26 FVPTCs, 21 follicular adenomas (FAs) and 14 nodular hyperplasias (NHs).

Results: Expression of galectin-3, HBME-1, CK19 and CITED1 was significantly higher in FTN-IPTCNs than in FAs or NHs, but expression of galectin-3, CK19 and fibronectin-1 was lower in FTN-IPTCNs than in FVPTCs. The *BRAF* V600E mutation was not detected in the benign nodules or FTN-IPTCNs, whereas 57% of FVPTCs had the mutation. *RASSF1A* promoter methylation was higher in FTN-IPTCNs than in benign nodules but there was no difference between FTN-IPTCNs and FVPTCs.

Conclusions: We conclude that FTN-IPTCN is an intermediate lesion between a benign nodule and an FVPTC immunohistochemically, and that its immunohistochemical features and high rate of *RASSF1A* promoter methylation as that in FVPTCs indicate that it is pathogenetically related to FVPTC.

526 miRNA Markers of Aggressive Behavior of Papillary Thyroid Carcinoma

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Background: Papillary thyroid carcinoma (PTC) is a relatively indolent tumor, although in 10-15% of cases it can develop recurrence or distant metastases and may cause tumor-related death. The aggressive biological behavior can not always be predicted based on histopathologic features. microRNAs (miRNAs) are dysregulated in various tumors types, and some of them serve as markers of tumor progression. In this study, we searched for miRNA markers of more aggressive behavior of PTCs.

Design: First, six PTCs with either local tumor recurrence or distant metastases (aggressive group) were matched by sex, age, and mutational status (4 *BRAF* positive, 1 *RET/PTC* positive and 1 with no mutations) with 6 PTCs with no complications on similar length follow-up (non-aggressive group) and studied for expression of 328 human mature miRNAs with Flexmir MicroRNA Human Panel (Exiqon) on Luminex 200. Next, 12 additional PTCs (4 aggressive and 8 non-aggressive) were analyzed for expression of specific miRNAs using real-time PCR on ABI 7500.

Results: Overall, the tumors in aggressive PTC group and non-aggressive PTC group revealed relatively similar miRNA expression profiles, with most highly overexpressed miRNAs being miR-146b, miR-221, and miR-222 and most downregulated miRNAs being miR-153, miR-448, miR-325, miR-382 and miR-495. However, the levels of miR-221 and miR-222 expression were 2.3 and 2.5 fold higher in aggressive group as compared to non-aggressive group. This correlates with the recent finding of miR-221 and miR-222 association with progression and metastatic behavior in melanoma. In addition, miR-155, miR-205, miR-337 were significantly more overexpressed in aggressive PTCs (2-4 fold difference) and miR-376a, miR-376b, miR-422a, and miR-130b were downregulated only in aggressive PTCs. Several miRNAs targeting the *MET* gene, including miR-34b, miR-206, miR-1 and miR-198 were expressed at significantly lower levels in aggressive tumors, which may provide a mechanism for *MET* overexpression found in PTCs.

Conclusions: We identified several miRNAs that were differentially expressed in aggressive PTCs as compared to non-aggressive tumors. Among those were miR-221 and miR-222, recently implicated in aggressive behavior of other tumor types, and several miRNAs targeted the *MET* gene. These data suggest that a panel of miRNAs may be used to predict a more aggressive behavior of PTCs.

527 Spectrum and Prevalence of *BRAF* Mutations in Thyroid Tumors

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Background: Papillary carcinoma (PC) is the most common type of thyroid malignancy. *BRAF* mutations are found in 40-45% of these tumors and serve as an unfavorable prognostic marker. In addition to the most common point mutation, *BRAF* V600E, several other mutations in this gene have been reported. In this study, we investigate the spectrum and prevalence of *BRAF* mutations detected in a large series of thyroid tumor samples.

Design: We analyzed 324 thyroid tumors, including 242 papillary carcinomas (PC), 39 follicular carcinomas (FC), and 43 follicular adenomas with atypical nuclear features present focally and not sufficient for the diagnosis of PC (FAA). DNA was isolated from fleshly frozen tissue (32 samples) or paraffin-embedded tissue (292 samples). Mutations in codon 600 and adjacent codons of the *BRAF* gene were detected by real-time PCR and post-PCR melting curve analysis on LightCycler (Roche). Direct nucleotide sequencing on ABI 3130 (Applied Biosystems) was used to confirm the mutation type.

Results: *BRAF* mutations were identified in 97 (40%) of PC and in none of FC and FAA. Of 97 mutations, 95 (98%) were the most common *BRAF* V600E mutation, one was a *BRAF* A1801G (K601E) point mutation and one was a novel complex mutation *BRAF* T599I, I599_V600insL. Among tumors with *BRAF* V600E mutation, 74% were classic PC, 15% were tall cell variant of PC, and 7% were follicular variant of PC. The tumor with *BRAF* K601E mutation was a follicular variant of PC and the tumor with *BRAF* T599I, I599_V600insL mutation was a solid variant of PC.

Conclusions: Although V600E accounts for the vast majority of all *BRAF* mutation in thyroid tumors, about 2% of unusual *BRAF* mutations can be found, including a novel *BRAF* T599I, I599_V600insL mutation identified in the solid variant of PC. In this series, all types of *BRAF* mutation were specific for PC and were not found in nodules with slightly atypical nuclear features.

528 Thrombospondin-1 Is Modulated by the B-Raf^{V600E} Pathway in Papillary Thyroid Cancer

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Background: B-Raf^{V600E} is the most common genetic alteration in papillary thyroid cancer (PTC), especially in aggressive subtypes such as tall cell PTC, and in those with extra-thyroidal extension. However, mechanisms by which B-Raf^{V600E} induce tumor aggressiveness are still not fully understood. Thrombospondin (TSP-1) is a multifunctional extracellular protein that has been positively associated with tumor cell migration. We chose to look at whether B-Raf^{V600E} modulates TSP-1 in PTC, both *in vitro* and *in vivo*.

Design: We applied Gene Set Enrichment Analysis (GSEA) to gene microarray data from 26 PTCs-Raf^{V600E}, 14 PTCs-wild type (wt) Braf, and 10 normal thyroid (NT) tissue samples to look at significantly altered genes (leading edge). Short hairpin RNAs were used to knock-down (KD) wtBraf and Braf^{V600E} mRNA.

Results: The leading edge of gene sets significantly associated to B-Raf^{V600E} PTCs included several adhesion molecules, such as TSP-1, which is a member of the *Breast Cancer Signaling* gene set (BCS). Validation for TSP-1 was performed by immunostaining in tissue microarrays (TMAs) of a cohort of thyroid tissue samples including PTC-Raf^{V600E}, PTC wtBraf and benign/NT samples. PTC-Raf^{V600E} showed higher tumoral TSP-1 expression levels, whereas benign/NT samples showed consistently lower levels. KD of B-Raf^{V600E} in an aggressive human PTC cell line 8505 (homozygous for B-Raf^{V600E}) resulted in TSP-1 mRNA and protein down-regulation, as well as in a reduction of cell migration/invasion. KD of TSP-1 in 8505 cells also resulted in a similar reduction in cell migration/invasion. Both B-Raf^{V600E} and TSP-1 KD vs control reduced cell proliferation. Conversely, wt Braf KD in the human PTC cell line TPC-1 (RET/PTC1 and wtBraf) did not affect TSP-1 mRNA and protein levels, and neither cellular proliferation nor migration/invasion.
Conclusions: B-Raf^{V600E} 's role in the aggressiveness of thyroid cancer may be linked to adhesion molecules in the BCS gene set, in particular TSP-1. Further studies with TSP-1 and other BCS genes should be performed to identify new therapeutic targets.

529 A Paucity of Colonic Endocrine Cells Characterizes a Subset of Patients with Malabsorption and/or Diarrhea (M/D)

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Background: The gut is reputed to be the largest endocrine organ in the body. A generalized absence of enteroendocrine (EE) cells characterizes two malabsorptive diseases, namely enteroendocrine cell dysgenesis (ECD) and autoimmune polyglandular syndrome 1 (APS1). In both diseases, the enterocolonic mucosa demonstrates a paucity of endocrine cells. Because it is far from routine practice to examine small bowel and colonic mucosa for EE cells, the diagnosis of either entity is rarely made by pathologists.
Design: We prospectively examined the colonic mucosa for EE cells by chromogranin A (CGA) immunohistochemistry (IHC) in patients with unexplained chronic malabsorption and nearly normal mucosa. We hypothesized that loss of endocrine cell subsets such as enterochromaffin (EC) cells might be associated with other M/D conditions. Serotonin (5HT) IHC was employed to examine for the subset of EE cells that are EC cells. We additionally hypothesized that a paucity of EE and/or EC cells might be found in mild colonic GVHD. Two methods of enumerating endocrine cells were employed. One was a manual count of endocrine cells and the other was a quantification by image analysis.
Results: There were 7 patients with paucity of endocrine cells recovered over a 9 month period. Three patients were found to have nearly absent EE cells; they were later characterized as having ECD (n=1) and APS1 (n=2) based in large part on the pathological findings. Four patients with reduced EC cells but normal EE cell numbers were also identified. These 4 suffered from diverse conditions such as congenital diarrhea, mild GVHD, toddler diarrhea, and diarrhea post lung transplant. Six cases were suitable for image analysis. The image analysis and manual count methods agreed in 4 of the 6 cases, but low signal intensity hampered image analysis in our hands. The patient with GVHD was found to have markedly reduced EC cells by both techniques. Archival cases of mild colonic GVHD showed no aberration of EC cells, but did show a statistically significant increase in EE cells.
Conclusions: The pathologist can be of great assistance in the diagnosis of APS1 and ECD by examining colonic mucosa for EE cells in patients with unexplained M/D. Loss of EC cells characterizes a subset of acquired M/D conditions. A paucity of EC cells is not seen in most cases of GVHD.

530 Heat Shock Protein (HSP) Expression in Malignant Thyroid Tumors: A Potential Therapeutic Target?

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Background: HSPs are molecular chaperones which facilitate tumor growth and resistance to chemotherapy and radiation. HSP27, 70 and 90 have been studied in numerous cancers but rarely in thyroid cancers (TCs). There are isolated reports of an association between HSP90 and RET/PTC-related tyrosine kinase in papillary thyroid carcinoma (PC). HSP90 antagonists have also been recently used in the therapy of various cancers, with promising results. We evaluated the expression of HSP27, 70 and 90 in follicular carcinoma (FC) and PC with lymph node metastasis (PTCLN) and without nodal metastasis (PTC), to assess their potential as future targets of therapeutic HSP antagonists.
Design: Paraffin blocks from 15 FCs, 28 PCs (14 PTCLN,14 PTC) were stained with HSP27, 70 and 90 antibodies. Staining was graded: negative (-), weak (1+), moderate (2+) or strong (3+) and distribution was (-), focal (<10%), patchy (10-50%) or diffuse (>50%). Ten follicular adenomas (FAs) were used as controls.
Results: HSP27 was (+) in 63% of TCs. HSP70 was diffusely and strongly (+) in 100% of TCs. HSP90 was (-) in the majority (65%) of TCs. HSP90 showed (+) staining in 16% of FCs, 5% of PTCs and 14% of PTCLN. FAs were (+) for HSP27 (70%), HSP70 (100%) and HSP90 (30%). Using Fisher's exact test, we found no statistically significant difference in HSP27 (p=0.854), 70 (p=1.0) and 90 (p=0.281) stain distribution and intensity in the TCs and FAs tested

HSP Stain Distribution by Tumor Type

	HSP27		HSP70		HSP90	
Tumor Type	Negative	Positive	Negative	Positive	Negative	Positive
FC	6 (14%)	9 (21%)	0	15 (100%)	8 (19%)	7 (16%)
PTC	6 (14%)	8 (19%)	0	14 (100%)	12 (28%)	2 (5%)
PTCLN	4 (9%)	10 (23%)	0	14 (100%)	8 (19%)	6 (14%)
Total	16 (37%)	27 (63%)	0	43 (100%)	28 (65%)	15 (35%)

HSP Staining in PTC with and without Nodal Metastasis

	HSP27(+)	HSP70(+)	HSP90(+)
PTC	19%	100%	5%
PTCLN	23%	100%	14%

HSP90 showed a trend towards greater (+) staining in PTCLN compared to PTC but was not statistically significant (p=0.209).
Conclusions: HSP27, 70 and 90 showed no significant difference in staining in TC subtypes tested, or FAs. HSP90 was not highly expressed in TCs and therefore, unlike other cancers its therapeutic antagonists may not be helpful in TC. HSP70 was strongly expressed by all FCs, PTCs and PTCLNs tested, and should therefore be investigated as a potential therapeutic target.

531 Molecular Characterization of Radioactive Iodine Refractory (RAIR) FDG-PET Positive Thyroid Carcinoma (TC)

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Background: RAIR FDG-PET positive TC represent the major cause of deaths from TC and are therefore the main focus of novel target therapies. Aim: To perform a comprehensive screen for known TC-associated oncogenic mutations in RAIR tumors and correlate genotype with histotype.
Design: Sixty samples of locally recurrent/metastatic (n=56) and primary tissue (n=4) from 43 RAIR PET positive patients were selected for histologic and molecular analysis. At least one biopsied metastatic site corresponded to a FDG-PET positive lesion sampled within 2 years of the PET scan. Poorly differentiated thyroid carcinomas (PDTC) were defined on the basis of high mitotic activity (≥ 5 mitosis/10 high power fields) and/or tumor necrosis. Paraffin tissue samples were genotyped using a highly multiplexed Sequenom mass spectrometry-based mutation assay that screened for 111 known mutations in 16 different genes: *BRAF*, *RET*, *NRAS*, *HRAS*, *KRAS*, *PIK3CA*, *MAP2K1*, *AKT1*, *MET, IKBKB, PIK3R5, PRKCZ, RHEB, RPS6AK3, RPS6KB1, FRAP1*.
Results: The mutation frequency in primary and locally recurrent/metastatic samples was as follows: *BRAF* (n=39; 65%), *RAS* (n=7; 12%), *AKT1* (n=3; 5%), *PIK3CA* (n=2; 3%), *MET* (n=1; 2%), *RET* (n=2; 3%). Overall 34/43 (79%) patients had at least one mutation. In 13 patients with multiple tumor specimens, their mutational status was conserved in 7 (54%), and *BRAF*^{T1799A} status was homogeneous within patients in 11 individuals (85%). In 34 patients whose FDG-PET positive recurrence/metastases was genotyped, 23 (68%) were positive for any mutations, of which *BRAF* was the most common (20/34, 59%). The histotype of the 34 FDG-PET positive recurrence / metastases was as follows: PDTC: n=20; 59%-Papillary thyroid carcinoma (PTC) tall cell variant (TCV): n=6;17%- Well differentiated PTC (WDPTC): n=4;12%; Hurthle cell carcinoma (HCC):n=3;9%- Anaplastic carcinoma:n=1;3%. Of the 20 PDTC, 9 (45%) had *BRAF*^{T1799A}, 2 (10%) *RAS*, 1 *MET* (5%) and 8 (40%) were wild type. All 6 TCV and 3 WDPTC had *BRAF*^{T1799A} while 1 WDPTC had a *BRAF*$^{V600/K601del}$. All 3 HCC were wild type and the anaplastic tumor contained *BRAF*^{T1799A}. There was a very strong correlation between *BRAF* mutation and the presence of tall cells in the recurrence/metastatic site (P=0.0003).
Conclusions: 1) *BRAF*^{T1799A} is frequently seen in RAIR thyroid carcinomas 2) Distinct RAIR thyroid carcinoma sites are genetically homogeneous for *BRAF*^{T1799A} within a patient. 3) The above findings make *BRAF* an attractive target for therapy in incurable RAIR thyroid carcinomas.

532 Encapsulated Thyroid Tumors of Follicular Cell Origin with High Grade Features (EFHG): A Clinico-Pathologic and Molecular Study of 25 Cases

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Background: EFHG are unusual neoplasms. Non-invasive EFHG create a major therapeutic and diagnostic dilemma stemming from lack of studies with long term follow up (FU) and controversy regarding their true nature.
Design: EFHG were defined as encapsulated tumor of follicular cell origin with >=5 mitosis/10 high power fields and/or tumor necrosis. Tumors with extra-thyroid extension were excluded. Available paraffin tissues from these cases were subjected to a thyroid cancer-specific platform for mass spectrometry high-throughput genotyping. The latter consisted of 111 known mutations in 16 different genes: *BRAF*, *RET*, *NRAS*, *HRAS*, *KRAS*, *PIK3CA*, *MAP2K1*, *AKT1*, *MET, IKBKB, PIK3R5, PRKCZ, RHEB, RPS6AK3, RPS6KB1, FRAP1*.
Results: Necrosis was present in 56.0% (n=14) and extensive in 32.0% (n=8) of the 25 cases found over a 23 year period. Eight (32%) of 25 tumors were completely non-invasive. Eighty eight percent (n=22) of the patients were free of disease with a median FU of 8.5 years. All 8 non-invasive tumors did not recur despite the presence of focal/extensive necrosis in 3 cases (37.5%) and a median FU of 11.9 years. EFHG with no vascular invasion (VI) did not recur or metastasize while 3 (21%) of 14 patients with VI had distant metastases (DM). In the subgroup without DM at presentation (n=24), 2 (33%) of 6 patients with extensive VI relapsed while none of the 18 patients with absent or even focal VI recurred (p=0.054). Overall mutations were found in 9 (41%) of 22 cases tested. There was a significantly higher frequency of N-RAS codon 61 mutation (8 of 22, 36%) than B-RAF V600E mutations (1 of 22, 4.5%) (p=0.02). Three (37.5%) of 8 non-invasive tumors harbored mutations. The N-RAS positivity rate in our non-invasive cases (25%) is similar to the N-RAS codon 61 mutations frequency reported in atypical adenoma and minimally invasive follicular carcinoma (23.3 % and 22.2% respectively) but different from the one published for typical adenomas (0%). (Vasko et al. *JCEM* 88:2745, 2003)
Conclusions: 1) Non-invasive EFHG have an indolent behavior even in the presence of extensive tumor necrosis and the molecular data suggest they have started their malignant progression 2) In cases without DM at presentation, EFHG with absent or focal VI have an excellent prognosis 3) Mutations of *NRAS* are the most frequent oncogenic event in EFHG, which as opposed to BRAF mutations in non-encapsulated high grade tumors, are associated with a favorable prognosis.

533 VHL Inactivation Is an Important Pathway for the Development of Sporadic Pancreatic Endocrine Tumors

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Background: A small subset of familial pancreatic endocrine tumors (PET) arises in patients with von-Hippel-Lindau syndrome. A loss of function of the *VHL* gene can lead directly to an accumulation of the Hypoxia-Inducible Factor alpha (HIF-1α), resulting in the transcription of a series of hypoxia inducible genes such as CA-9 and GLUT-1. Sporadic PET very rarely harbour somatic *VHL* mutations, but the chromosomal location of the *VHL* gene is frequently deleted in sporadic PET and a subset of sporadic PET shows active hypoxia signals on mRNA and protein level. The aim of the present study was to correlate epigenetic and genetic VHL alterations to hypoxia signalling in order to identify the frequency of functional *VHL* inactivation in sporadic PET.

Design: A total of 152 paraffin-embedded sporadic PET were included in the study and used for immunohistochemistry and FISH analysis. Fresh frozen tissue of 37 of these tumors was available for RNA and DNA analyses. *VHL* gene mutation, *VHL* deletion as well as promoter methylation analyses were performed. Quantitative RNA expression levels of VHL and CA-9 were studied. The HIF target proteins CA-9 and GLUT-1 were investigated by semiquantitative immunohistochemistry.

Results: *VHL* mutations were absent in all 37 PET examined. In 14 of 79 cases (18%) *VHL* deletion by FISH and in 2 of 35 cases (6%) methylation of the *VHL* promoter region was detected. CA-9 and GLUT-1 protein was expressed in 27% and 30% of the tumors, respectively. Protein expression of the HIF-1α downstream target CA-9 correlated significantly with the expression of CA-9 mRNA ($p < 0.05$) and *VHL* loss ($p < 0.001$). Moreover, CA-9 immunohistochemistry correlated significantly with GLUT-1 immunohistochemistry ($p < 0.001$).

Conclusions: We conclude that *VHL* gene inactivation by promoter methylation and *VHL* hemizygous deletion in nearly 25% of PET leads to an activation of the HIF-pathway. Our data suggest that *VHL* inactivation is an important mechanism for the development of a subset of sporadic PET.

534 The Role FGFR4 in Pancreatic Endocrine Tumors

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Background: Fibroblast Growth Factor Receptors (FGFRs) are 4 transmembrane kinases. FGFR4, which is the subject of a germline SNP (G388R), interacts with N-Cadherin and NCAM, adhesion molecules implicated in tumor invasion and metastasis. We examined the expression of FGFR4, N-cadherin and NCAM in pancreatic endocrine tumors (PETs) and their relationship to the FGFR4 G388R SNP, clinical and pathological parameters.

Design: Paraffin blocks of 83 PETs classified by WHO criteria were retrieved from Pathology files of University Health Network with ethics consent. FGFR4 SNP was determined by polymerase chain reaction-restriction fragment length polymorphism of somatic DNA. A tissue microarray composed of cores of tumors, corresponding normal pancreas and lymph node metastases was stained for FGFR4, N-Cadherin and NCAM, and evaluated for localization (membrane/cytoplasm), intensity (0-3), and percent positive cells.

Results: Tumors from 83 patients (36 males, 47 females, ages 23-80 mean 52.7 years) ranged from 0.8 cm to 11 cm (mean 3.4 cm). Insulin was localized in 32, glucagon in 14, vasoactive intestinal peptide (VIP) in 8, and 26 tumors were plurihormonal. Local invasion was seen in 22, 36 had lymphovascular invasion, 18 had lymph node spread and 13 had liver metastasis. FGFR4 immunoreactivity was cytoplasmic only and higher in PETs with lymph node spread ($p=0.003$), liver metastasis ($p=0.08$), and mitotic count >2/10HPF ($p=0.03$). It was lowest in insulinomas ($p=0.045$). FGFR4 expression increased from tumors of benign behavior (TBB) to low-grade carcinoma (LGC) ($p=0.005$); lower immunoreactivity was found in TBB compared to tumors of uncertain behavior (TUB) and LGC ($p=0.022$). FGFR4 was overexpressed in LGC compared to TBB and TUB ($p=0.002$). N-Cadherin immunoreactivity was exclusively cytoplasmic and stronger in PETs with liver metastases ($p=0.01$) and with mitotic count >2/10HPF ($p=0.003$). Membranous NCAM (MB-NCAM) was higher in insulinomas ($p=0.002$) and decreased in glucagonomas ($p=0.002$). It was also progressively reduced from TBB to TUB to LGC ($p=0.007$). It was higher in TBB compared to TUB and LGC ($p=0.003$); it was reduced in LGC compared to TBB and TUB ($p=0.025$). MB NCAM was overexpressed in patients with homozygous Arg388 compared to homozygous Gly388 ($p=0.039$). Cytoplasmic NCAM was overexpressed in PETs with liver metastasis ($p=0.06$).

Conclusions: FGFR4 is implicated in PET progression. Aggressive tumor behavior is associated with FGFR4, N-cadherin and NCAM cytoplasmic overexpression and with loss of MB NCAM, validating the RIP-TAG/NCAM(-/-) mouse model of tumor progression.

535 Evaluating the Specificity of CD56 as a Neuroendocrine Immunohistochemical Marker

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Background: CD56, an immunohistochemical (IHC) marker that belongs to the family of neural cell adhesion molecules, is widely used in surgical pathology. It is known that CD56 positively stains natural killer cells, activated T-cells, neuroendocrine (NE) tumors, and tissues of the central nervous system. There are multiple NE IHC markers such as NSE, synaptophysin, chromogranin and MAP-2 that are frequently used by pathologists, however the use of CD56 is increasing. In our experience, CD56 appears to be a sensitive NE marker. In this study, we aim to evaluate multiple tumors of various origins in order to determine the specificity of CD56 as a NE IHC marker.

Design: Paraffin embedded surgical specimens of 15 tumor types were retrieved from our files, including 5 cases each of infiltrating ductal breast carcinoma, colonic adenocarcinoma, pancreatic ductal adenocarcinoma (PDA), hepatocellular carcinoma (HCC), transitional cell carcinoma, leiomyosarcoma (LMS), inflammatory pseudotumor, squamous cell carcinoma, Merkel cell carcinoma (MCC), melanoma (MM), paraganglioma (PG), carcinoid tumors of the lung and gastrointestinal tract (CTLG), 6 small cell carcinomas (SCC), and 3 basaloid squamous cell carcinomas. All cases were stained for CD56, synaptophysin, and MAP-2. The slides were reviewed, and staining recorded as negative or strongly, moderately, or weakly positive.

Results: Strong and diffusely positive staining for CD56, synaptophysin, and MAP-2 was seen in 100% of cases of SCC, MCC, PG, and CTLG. MM showed weak to strongly positive staining for all three markers. Weakly positive CD56 staining was noted in a small number of LMS (2/5) and PDA (3/5) cases. CD56 had 100% sensitivity and 78% specificity for NE neoplasms. CD 56 was negative in the other non-NE neoplasms. Synaptophysin and MAP-2 stained weakly to moderately positive in 33% and 44% of non-NE tumors studied, with 67% and 56% specificity, respectively.

Conclusions: CD56, synaptophysin, and MAP-2 all proved to be highly sensitive NE markers. Our results show that CD56 is a highly specific marker with 78% specificity for NE tumors, and is more specific than synaptophysin or MAP-2. The pattern of staining in NE neoplasms appears more intense and diffuse, but the interpretation of CD56 should be approached with caution when evaluating MM, PDA, and LMS. We believe that CD56 is a sensitive and specific IHC marker that should be readily utilized when differentiating NE tumors from other neoplasms.

536 Detection of Somatostatin Receptors, SSTR2A and SSTR5, in TSH Producing Pituitary Adenomas: Patho-Clinical Correlation

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Background: Somatostatin analogue (octreotide) targets SSTR subtypes 2 and 5. In order to predict the response of the SSTR targeted therapy by somatostatin analogue (octreotide) in the pituitary adenomas, immunohistochemical detection of SSTRs is essential for appropriate treatment. Even though TSHomas are rare, they usually are associated with Graves' disease and macroadenomas which sometimes require octreotide for the residual tumors. Therefore, in particular, this study is aimed at to elucidate the expression of SSTRs in TSHomas with the special reference to the pre-operative response to octreotide.

Design: The pituitary adenomas from total 14 cases of TSHomas (female:12 cases,male:2 cases) were subjected to the following study. Nine cases were macroadenomas and five cases were microadenomas. Preoperative serum TSH levels were 0.464-9.802μU/ml (average 6.28). The formalin-fixed paraffin embedded (FFPE) sections were immunohistochemically stained for SSTR2A and SSTR5 (the antibodies supplied from Gramsch Laboratories, Germany) by ABC method after appropriate antigen retrieval with autoclave. The staining results were classified into 1+ (<25%), 2+ (<75%), 3+ (>75%) by the ratio of positive cells. Response of serum TSH by preoperative somatostatin analogue suppression test was also measured. In three cases, real time RT-PCR was performed for SSTR2 and SSTR5.

Results: Immunohistochemically, all cases were positive for SSTR2A. The staining was interpreted as "positive" if it is localized on the cell membrane. Incidence of the positive staining for SSTR2A was as follows;1+ (14.2%), 2+ (35.7%), 3+ (50.0%). SSTR5 was positive in eight cases (57.1%). Incidence of the positive staining for SSTR5 was as follows; cell membrane (35.7%), cytoplasm (21.4%). In real time RT-PCR analysis, SSTR2mRNA was overexpressed in 2 cases. When these staining were compared with pre-operative suppression test, the cases with low serum TSH suppression were correlated with weak (1+) immunohistochemical staining for SSTR2A. Good response to preoperative octreotide was well correlated with 3+ immunohistochemical staining for SSTR2A.

Conclusions: Our studies showed that TSHomas expressed SSTR2A and SSTR5 by immunohistochemistry which predict the response to the somatostatin analogue (octreotide). It is suggested that the cases with high (3+) SSTR2A, being as a better target molecule, expect better response to the octreotide.

537 MLH1 Down-Regulation during the Neoplastic Progression of Follicular Cell Thyroid Neoplasms

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Background: The contributions of mismatch repair proteins (MLH1 and MSH2) to topographic tumor heterogeneity and progression remain unknown in differentiated follicular cell thyroid neoplasms.

Design: We selected 15 hyperplastic nodules, 22 adenomas, 14 minimally-invasive carcinomas, 24 widely-invasive carcinomas, 15 papillary carcinomas and 13 anaplastic carcinomas (WHO criteria). Total RNA was extracted from normal and neoplastic tissues (peripheral and internal compartments) 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 U133A Array noncompetitively. Cross-validated gene expression analyses were performed (expression factor≥2, significance≤0.01), and variables studied regarding the histological diagnosis and MLH1/MSH2 expression.

Results: MLH1 and MSH2 expression was homogeneous in peripheral and internal compartments, with a significant inter-lesional variability for MLH1 in peripheral and internal compartments across the spectrum of follicular thyroid lesions. Additionally, expression of MLH1 in internal compartments is greater than the periphery. MLH1 was greatest between FTHN and FTA, declining with neoplastic progression. MSH2 shows no statistically significant topographical heterogeneity within follicular thyroid

lesions and there is also no variance across the spectrum. MLH1/MSH2 expression was positively correlated with SWI/SNF related, matrix associated, actin dependent regulator of chromatin member 1, Nuclear fragile X mental retardation protein, Cell division cycle 2-like 5, RecQ protein-like (DNA helicase), and protein expressed in non-metastatic cells 4, and negative correlated with Heterogeneous nuclear ribonucleoproteins L, D-like and R, Excision repair cross-complementing rodent repair deficiency, BCL2-associated transcription factor 1, Forkhead box O1, Interleukin 1 receptor, type II, and Laminin, alpha 3.

Conclusions: MLH1 expression is homogeneously down-regulated during the neoplastic progression of follicular cell thyroid neoplasms. MLH1 down-regulation contributes to impaired genetic repair (mismatch and excision repair), abnormal chromatin (heterogeneous nuclear ribonucleoproteins, SWI/SNF, DNA helicase) and stromal interaction (laminin).

538 Pathological Characterization of TSH Producing Adenomas and So-Called Silent Thyrotroph Adenomas

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Background: Slient pituitary adenomas are a subtype of adenomas characterized by positive immunoreactivity for one or more hormones classically secreted by normal pituitary cells but without clinical expression. Thyrotropin (TSH) producing adenomas (TSHomas) are a rare cause of hyperthyroidism. So-called silent thyrotroph adenomas (STA) have recently been reported but more rarely. The pathological characterization of STA has never been fully described.

Design: 10 patients with so-called STA and other 20 TSHomas followed at the two centers among 1200 patients with pituitary adenomas between 1990 and 2008 have been selected. We reviewed clinical data, histopathological pattern, ultrastructural features, and anterior pituitary hormones, transcripation factors, somatostatine receptors using immunohistochemistry or RT-PCR to character these so-called STA cases.

Results: Ten so-called STAs were from 7 females, and 3 males. Clinical symptoms were regularly headache, visual defected, vertigo and nausea. Acromegaly was noted in one young adult male and amenorrhea was noted in one premenopausal women. All of ten so-called STAs were macroadenomas and cavernous sinus invasion and sphenoidal sinus invasion were noted in 2 and 1 tumors respectively. SITSH has never been detected in all ten cases. However, slightly or moderate elevated serum levels of PRL have been detected in 5 cases. Notable, in four patients, postoperative serum TSH, fT3 and fT4 were significantly reduced once then 2 of them recover to normal range. Generally they resemble common morphological features, immunohistochemical profiles and ultrastructural features of TSHoma. Ten so-called STAs mainly and strongly showed TSH immunostaining. Both Pit-1 and GATA2 expressed in all 10 so-called STAs identified their thyrotroph specific as in 20 TSHoma. SSTR2A and SSTR5 also were detected in both so-called STAs and TSHoma.

Conclusions: Summary, so-called STA could be noted by clinical characters, morphological features and could be identified by immunohistochemical staining of anterior pituitary hormones. Transcription factors, Pit-1 and GATA2 may be useful marker to confirm so-called STA when cases showed demonstrated immunoreactivities for multiple pituitary hormones.

539 Platelet Derived Growth Factor Receptor Beta (PDGFRβ) Is Widely Expressed in Thyroid Neoplasms of Both C-Cell and Follicular Origin

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Background: Platelet derived growth factor receptor beta (PDGFRβ) is targeted by multiple tyrosine kinase inhibitors (such as sorafenib and sunitinib) currently in clinical trials for thyroid cancer and other tumors. However, the frequency and pattern of PDGFRβ expression in tumors of follicular or c-cell origin are not well characterized. We sought to examine the incidence of PDGFRβ in thyroid tumor cells by immunohistochemical analysis to determine which subsets of thyroid neoplasms may benefit from these targeted agents.

Design: Fifty-seven thyroid tumors including 14 medullary carcinomas, 4 anaplastic carcinomas, 14 papillary carcinomas, 21 follicular carcinomas, and 4 adenomas comprised two tissue microarrays. Expression of PDGFRβ was evaluated on 4 micron thick paraffin sections by immunohistochemical evaluation (sc-339, Santa Cruz Biotechnology, Inc., 1:50 dilution) performed on the Bond Automated Immunohistochemistry System. Intensity of tumor staining was graded from absent (0) to weak (1+), moderate (2+), or intense (3+). Distribution of expression within the tumor cells and within the tumor was documented.

Results: All 57 tumors expressed at least weak (1+) PDGFRβ expression in primarily a diffuse pattern (>80% tumor staining) in a cytoplasmic distribution with nuclear staining also noted. Pure membranous staining was not identified. Expression patterns observed were: medullary thyroid carcinomas 10 (71%) with high expression (2-3+) and 4 with low expression (1+); anaplastic carcinomas 3 (75%) with high expression and 1 with low expression; papillary carcinomas 9 (64%) with high expression and 5 with low expression; follicular carcinomas 16 (76%) with high expression and 5 with low expression; and adenomas 1 (25%) with high expression and 3 with low expression. Overall high expression (2-3+ staining) of PDGFRβ was noted in 39 (68%) of the 57 tumors.

Conclusions: 1) PDGFRβ is widely expressed in tumors of follicular or c-cell origin. 2) The receptor expression pattern is predominantly diffuse cytoplasmic with high expression (2 to 3+) within tumor cells. 3) The presence of PDGFRβ suggests a possible target for tyrosine kinase inhibitors for the spectrum of thyroid neoplasms.

540 Stem Cell Marker Kit Is an Independent Prognostic Marker for Pancreatic Endocrine Tumors – A Novel Finding Derived from Analysis of Islet Cell Differentiation Markers

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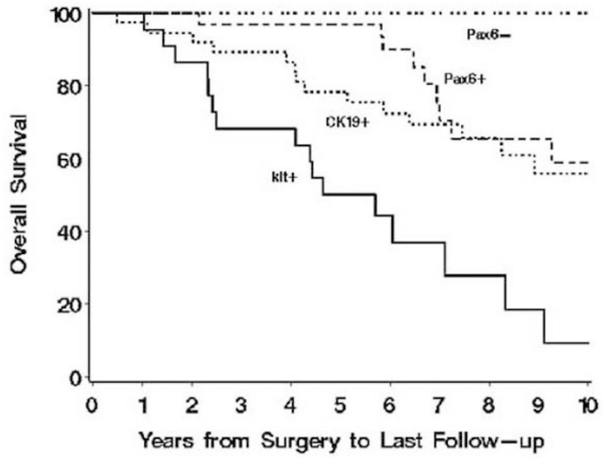
Background: The biologic behavior of pancreatic endocrine tumors (PETs) is difficult to predict based only on histologic features. CK19 has been shown to be a reliable prognostic marker for PETs. The underlying mechanism explaining why CK19 positive PETs have worse prognosis is unknown. Recently, the differentiation of islet cells from stem cells has been studied extensively, and a few proteins have been shown to play key roles or to serve as markers in different stages of differentiation. Kit is a stem cell marker in a variety organs including pancreas. CK19 is the first cytokeratin expressed in differentiating cells. Pax6 is usually expressed in more mature cells. The aim of study was to stage tumors along the line of islet cell differentiation and to correlate with biological behavior of PET.

Design: One hundred and two patients surgically treated for PETs between 1987 and 2002 were identified. Immunostains were performed using following antibodies: Kit, CK19, and Pax6. Associations of immunohistochemical features with survival were evaluated using Cox proportional hazards regression models.

Results: Forty-three patients died of disease at a mean of 5.4 years. Overall survival rates at 5 and 10 years were 79.6% and 50.6%, respectively. The molecular markers Kit, CK19, and Pax6 were combined to test their ability to predict death as follows: Kit + tumors (n=22); Kit-/CK19+ tumors (n=38); Kit-/CK19-/Pax6+ tumors (n=35); and Kit-/CK19-/Pax6- tumors (n=6). The overall survival differed significantly among all four groups (p<0.001; see Figure).



Conclusions: We found that molecular markers can be used to determine the degree of PET differentiation. The stem cell marker Kit positive PETs have the most aggressive behavior because tumor cells are at a relatively primitive stage. Ck19 positive tumors with some degree of differentiation have somewhat better prognosis followed by Pax6 positive tumors with the best prognosis. By understanding the molecular pathway of islet cell differentiation, a new and strong predictive marker Kit was identified. A similar strategy may be applicable to other tumor types in order to define new prognostic markers.

541 RAP1GAP Is a Novel Marker of Malignancy for Thyroid Tumors

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Background: RAP1 GTPase activating protein, also known as RAP1GAP, functions by switching off RAP1, the RAS-like protein that has been implicated in cell adhesion and migration. Recent findings suggest that RAP1GAP is frequently inactivated in several tumor types and may function as a tumor suppressor. The aim of this study was to investigate the alterations of the RAP1GAP gene and its expression in thyroid tumors and its potential value as a diagnostic marker for thyroid cancer.

Design: We studied 197 thyroid nodular lesions, including 40 hyperplastic nodules (HNs), 48 follicular adenomas (FAs), 28 follicular carcinomas (FCs), 78 papillary carcinomas (PCs), and 3 anaplastic carcinomas (ACs). Loss of heterozygosity (LOH) in the *RAP1GAP* region was studied using three microsatellite loci located on 1p36.1-p35 within or close to the location of the *RAP1GAP* gene. *RAP1GAP* mRNA expression levels were detected by qRT-PCR and protein levels by western blot. Immunohistochemistry was performed using RAP1GAP antibody (Santa Cruz, dilution 1:100).

Results: LOH at the *RAP1GAP* region was found in 21% of FCs, 40% of PCs, and 67% of ACs. *RAP1GAP* mRNA levels were significantly decreased in malignant thyroid tumors, down to 0.45 from the expression level in normal thyroid cells in FCs, 0.25 in PCs, and 0.04 in ACs. The presence of LOH correlated with the decreased expression levels of mRNA (p=0.039). Western blot analysis confirmed the decrease or complete loss of RAP1GAP protein in 3 PCs as compared to adjacent normal thyroid tissue. Using immunohistochemistry, significant decrease or loss of RAP1GAP expression was observed in 0/28 HNs, 2/32 (6%) FAs, 6/16 (38%) FCs and 21/29 (72%) of PCs. Among PCs, the decreased or loss of RAP1GAP immunostaining was observed in 20/21 (95%) of invasive PCs but only in 1/9 (11%) encapsulated PCs. Among FCs, the decrease or loss of immunostaining was found in 5/5 (100%) widely invasive FCs and 1/11 (9%) minimally invasive FCs.

Conclusions: *RAP1GAP* is likely to serve as a tumor suppressor gene which is frequently affected by LOH and lose expression in a significant proportion of thyroid cancers, especially in those with invasive growth. The loss of the RAP1GAP protein can be detected by immunohistochemistry and may serve as a diagnostic marker of malignancy in thyroid nodules.

Gastrointestinal

542 Quantitative Analysis of Intramucosal Mast Cells in Inflammatory Bowel Disease (IBD) and Symptomatic Non-IBD Patients with Histologically Uninflamed Colon Biopsies

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Background: Increased intestinal mast cell count, as recently proposed by >20 mast cells per high power field (HPF), has been reported in patients with irritable bowel syndrome (IBS). Mast cell activation has also been implicated in IBD but whether the number of intramucosal mast cells is quantitatively increased remains controversial. We aimed to study the density of mast cells in histologically unremarkable or uninflamed colon biopsies obtained from IBD and non-IBD patients to look for any particular disease group(s) that might show an increased mast cell count.

Design: Random colon mucosal biopsies (n=118) showing no histopathologic abnormality (including quiescent IBD) were immunostained with CD117 (c-kit) and mast cell tryptase (MCT). Intact mast cells with an identifiable nucleus were quantified in three 400X fields in areas with the highest density for each stain. The mean values of each case were analyzed according to patient groups based on clinical history and endoscopic findings.

Results: Both CD117 and MCT stains were equally sensitive in detecting intramucosal mast cells with similar mean counts (P=0.864), and thus only CD117 results were subjected to subsequent analyses. The overall mast cell counts in all biopsies ranged from 2-31 per HPF (mean: 16.9±6.2; median: 17). No significant difference was demonstrated between patients with IBD (mean=15.6± 5.6; n= 47) vs non-IBD (mean=17.8±6.4; n=71; P=0.060), Crohn (mean=14.1±6.0; n=21) vs ulcerative colitis (mean=17.0±5.1; n=25; P=0.084), non-IBD with diarrhea (mean=18.3±6.1; n=33) vs without diarrhea (mean=17.4±6.8; n=38; P=0.550), non-IBD with abdominal pain (mean=17.1±6.0; n=19) vs without abdominal pain (mean=18.1±6.6; n=52; P=0.567). Thirty-six biopsies (30.5%) showed >20 mast cells/HPF, which appeared to be equally distributed among various patient groups (23.4% in IBD, 36.4% in non-IBD with diarrhea, and 34.2% in non-IBD without diarrhea; $P \geq 0.221$).

Conclusions: There is no significant difference in the number of intramucosal mast cells in patients with or without IBD, diarrhea, or other digestive symptoms. Increased mast cell counts were observed in various patient groups with uninflamed colon biopsies, suggesting a nonspecific finding that may not be reliable in segregating patients with IBS.

543 Minichromosome Maintenance Protein 7 and SMAD 4 Expressions in Squamous Cell Carcinoma of the Esophagus

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Background: Minichromosome maintenance protein-7 (MCM–7) plays a critical regulator of DNA replication as a component of the DNA replication licensing complex. Recently it has been shown that MCM 7 protein could be correlated with the clinicopathological profiles of some human tumors. In the present study, we evaluated the expression of MCM7 in squamous cell carcinoma of esophagus to clarify the pathobiological significance including prognostic relevance, correlation with clinicopathological characteristics and with that of one of tumor suppressor gene, SMAD4.

Design: We examined the immunohistochemical expression of MCM7 and SMAD4 in 67 surgically resected esophageal specimens, which consisted of 16 early cancer and 47 late-stage(II-IV) cases. Twenty-seven cases(40.3%) showed lymph node metastasis. The overall 5-year survival rate was 56.7%. Distinct nuclear staining of MCM7 and cytoplasmic staining of SMAD4 was considered as positive. The percentage of tumour cells positive for MCM7 was classified into four groups: 1 (< 5-25%), 2 (25–50%), 3 (51–75%) and 4 (> 75%). For statistical analysis, those with negative and positive cases were compared first. The labeling indices of each four group were also compared.

Results: The positivity of MCM7 was 79.1% and significantly correlated with the T status (P=0.008), N status(P=0.032), UICC stage (P=0.03), survival period(p=0.036) and survival(P=0.047). The positivity of SMAD4 was 26.9%, and was not significantly associated with clinicopathological characteristics. Kaplan-Meier survival curves showed that the patients with positive- MCM2 had a poorer prognosis ($P < 0.05$), however those with positive-SMAD4 had no prognostic significance.

Conclusions: These results indicate the expression of MCM7 may be a useful poor prognostic marker in squamous cell carcinoma of esophagus. However, the clinical implication of SMAD 4 expression could not be demonstrated here.

544 Colorectal Lymph Node Examination: How Extensive Should It Be and Why Is "12" the Magic Number? A VAMC Experience

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Background: Twelve lymph nodes (LN) is a current benchmark in LN retrieval in colorectal resections according to the National Quality Forum. Data shows that more extensive lymphadenectomy improves overall survival (OS). These are multi institutional studies which have many variables such as length of colonic fat resected by many surgeons, and gross examination by many path labs. By using one surgeon's case log and one pathology group, we have the opportunity to reduce these variables. Our aim is to determine the impact of extensive LN search on OS, and the impact of 12 LN as a QA standard.

Design: We reviewed 157 patients from one surgeon's case log between 1994-2007. Within the 12 years, one surgeon applied strict guidelines to remove approximately the same distribution of pericolonic fat and the lab followed a 2 step approach to LN dissection. Step 1 is careful search for grossly identifiable LN, and step 2 is overnight immersion in Carnoy's solution to find smaller LN. We used Statview, Kaplan Meier Analysis and SAS Institute software to analyze the following variables: 1) The total number of LN identified and its impact on AJCC stage and OS 2) The OS and its association with finding of >12 LN 3) The impact of number of positive LN on OS.

Results: Of 157 cases, the mean number of identified LN for all resections was 14.75 1) The total number of LN identified did not correlate with AJCC stage (p=0.42) Mean number of LN per stage: I=13.4 (N=41); II=16.3 (N=56); III=14.3 (N=38); IV 14.2 (N=22). Regardless of stage, total number of LN identified was not statistically significant in predicting OS (p=0.24) 2) Regardless of tumor stage, no statistically significant impact on OS was found if <12 or >12 total LN were identified (p=0.06) 3) Regardless of tumor stage, higher number of positive LN was associated with lower OS (Survival decreases by 0.15 month per each positive node, p= 0.001).

Conclusions: 1) Total number of LN identified does not impact AJCC stage or OS 2) Higher number of positive LN alone is associated with worse OS In our experience, the two step LN isolation is an effective way to exceed the National Quality Forum requirement of 12 LN. However, after following our own guidelines for over ten years, our findings suggest that the second step of using carcinogens like Carnoy's solution may not be needed. Twelve lymph nodes may not be a magic number for QA and should be reconsidered.

545 Ampulla of Vater: Morphologic, Clinical, Survival, and Second Colon Primary Cancers Based on 5,625 Cases from the SEER Program

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Background: Cancers of the ampulla have been infrequently studied, especially at a population level. Herein, we report the epidemiologic and morphological characteristics of cancer of the ampulla of Vater and its relationship with primary carcinomas arising in the colon.

Design: In SEER, all patients with a diagnosis of carcinoma of the ampulla were identified between 1973 and 2005. The demographic features, five-year survival rates according to stage, grade, and histologic tumor type, distribution of histological types, and frequency of a primary colon cancer preceding or following the development of the ampullary cancer were compared.

Results: A total of 5,625 cases of ampullary cancer were identified. Ampullary cancers have increased annually since 1973. In both blacks and whites, the disease is more common in men than in women. Adenocarcinomas, NOS comprised 65% of histologic types. Five-year relative survival depends on stage of disease, grade, and histological tumor type. Papillary carcinomas and carcinomas arising in adenomas had a significantly more favorable survival than other types. 10% of patients with ampullary cancer had a preceding primary cancer in another site. Of 571,304 cases of primary colorectal cancer, 134 developed a second primary in the ampulla. Twelve patients were between 15 and 30 years of age. Of 5,625 patients with primary cancer of the ampulla, 59 had second primary cancers in the colon or rectum.

Conclusions: The histological type, grade, co-existing adenoma, and stage serve as prognostic factors. The location of either first or second primary cancers in the colon or rectum associated with cancers in the ampulla followed the frequency distribution of all primary colon and rectal cancers seen in all patients.

546 Usefulness of p16 Immunohistochemistry in the Diagnosis of Lynch's Syndrome

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Background: MLH1 inactivation may be observed in sporadic and Lynch's syndrome colorectal carcinoma (CRC). Lynch syndrome is caused by germline mutations in the mismatch repair genes. Sporadic CRC is caused by epigenetic silencing of MLH1, because of its promoter methylation. These tumours are due to hypermethylation of multiple genetic locus, one of them, *CDKN2A* (*p16*). The aim of this study is to evaluate the value of p16 immunohistochemistry in the prediction of germline *MLH1* mutation in patients with CRC that show loss of MLH1 expression.

Design: The study was performed in 89 tumours with loss of MLH1 immunohistochemical expression from patients of the Genetic Counselling in Cancer Department of HGUE and from a series of non-selected surgical CRC specimens from the EPICOLON study and the Pathology Department of the HGUA. Immunohistochemical analysis for p16 was performed on tissue microarray. The MLH1 and *CDKN2A* (p16) methylation analysis was performed by Methylight. *BRAF V600E* mutation was detected using specific TaqMan probes by real time PCR. In 54 tumours, mutation analysis of *MLH1* was performed.

Results: Loss of p16 expression was seen in 21 out of 76 valuable samples (27.5%). All tumours with loss of p16 expression showed hypermethylation of p16 (21/21, p<0.001), 95.2% (20/21, p<0.005) showed MLH1 methylation and 66.7% (14/21, p<0.005) were mutated for *BRAF V600E*. Values of diferent strategies for detecting Lynch Syndrome are shown.