no change to assessing their grossing speed. As a result of the positive feedback, the program is introducing a similar tracking form for Clinical Pathology.
Conclusions: Our results show that all participants felt greater satisfaction in identifying progress, sign-out accuracy and feedback from the staff pathologist. This implies an increase in self-reflection and therefore the potential for improved performance. We believe, in the long term, this tracking form will allow for continued resident self-reflection, inspire residents to seek out additional learning opportunities, and allow for meaningful discussion between the Program Director and resident concerning self-reflection and improvement.

527 Effectiveness of Training in Gross Image Disease Recognition and Technical Skills Using Simulation Based Medical Education (SBME)

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Background: Knowledge of gross tissue disease and proper technical gross examination skill is important for patient safety. Current residency training in gross tissue examination generally is dependent on daily practice volume and case type and learning gross skills on unusual diseases or atypical patterns of presentation is challenging. We developed and tested the effectiveness of a gross tissue disease SBME system.
Design: From institutional image files and from internet images, we developed an intestinal and an ovarian database of gross tissue cases consisting of 250 cases each. Cases represented a spectrum of common to rare disease types and of common to unusual presentations (graded on a 1(easy)-4(difficult) Likert scale). Multiple images of all major diseases were included (e.g., 32 cases of Crohn's disease). We also developed a gross tissue examination question bank that queried gross technique skills based on institutional protocols (e.g., how many sections would you take of this lesion?). We tested the gross disease identification and technique skills components on 10 and 5 residents, respectively. We used a skills-checklist to determine the number of images required to reach successful mastery of gross diagnosis (2 consecutive scores of Likert-4 cases provided randomly) and the number of images required for successful mastery of gross technique (based on all correct responses per case type).
Results: For gross technique, residents reached mastery of reporting the appropriate grossing protocol after a mean of 2.2 challenges, range 1-5, depending on experience level. For gross tissue diagnosis, residents rapidly improved in their correct identification of common diseases. PGY1 and PGY2 residents generally examined 1-2 (mean 1.5) images at Likert difficulty 1 and 2, prior to diagnosing the disease accurately. More experienced residents correctly diagnosed most diseases without prior challenges at Likert difficulty 3. All residents were challenged on rarer diseases for even Likert difficulty 1 or 2 cases.
Conclusions: In our SBME training, residents quickly and accurately learned to report grossing protocols for all case types. All residents also quickly established accuracy for diagnosing moderately difficult cases (Likert 3) of common diseases and many reached mastery level. We hypothesize that SBME training is a highly useful training tool that augments traditional training methods.

528 Emergency Frozen Section: Development of a Standardized Teaching Module for Residents

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Background: Emergency frozen section is likely the most demanding and stressful task facing a pathologist, yet this aspect is rarely systematically addressed by residency programs. Residents may go through an entire residency without experiencing after-hours frozen section due to their low frequency. Moreover, the emergent frozen sections are likely to involve a distinct set of specimens from surgical procedures for conditions not typically encountered on routine frozen sections. To fill this gap in the current curriculum of the Anatomic Pathology residency training program at the University of British Columbia, we identified thematic categories of surgical specimens submitted for emergency frozen sections at our institution and created a teaching module centered around these categories.
Design: Retrospective analysis of the emergency frozen sections performed on weekends for non-neuropathology cases at large teaching hospital between June 2010 and Aug 2012 revealed eighteen cases representing four major types of specimens with distinct clinical implications and diagnoses. Through consultation with surgeons and pathologists we developed a teaching module centered around the four themes. The module explains the typical clinical scenario, the differential diagnoses likely to be involved, and information required by the surgeon at the time of frozen section. Each module includes digitally scanned original frozen section slides (40x, Aperio scanner) made available to residents online utilizing the Aperio Spectrum server associated with the local pathology learning center. The module was presented to the residents in years 2-5 of training during a one-hour teaching session.
Results: Residents were asked to complete the initial test set of 18 cases by providing diagnoses and appropriate feedback to the surgeon. Following the test, the didactic portion was presented with four themes: acute general surgery cases, spinal cord compression, head and neck masses with acute neurologic deficits, and mediastinal masses causing SVC syndrome or cardiac symptoms. Additional related cases were presented. Objective measures of improvement in responses from pre- and post-tests as well as participants' feedback will be reported.
Conclusions: Pathology resident training in the area of emergency frozen sections is currently sporadic and random. We have developed a teaching module to help fill this gap in residency training and to develop confidence in a difficult and uncertain area of practice.

529 Cytology of the Week Cases (COWs): Impact of a Longitudinal Educational Intervention on Cytopathology RISE Scores

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Background: The ACGME program requirements for anatomic pathology require that residents examine 1,500 cytology specimens over the course of residency training. In the era of duty-hour limitations and continually expanding medical knowledge, attainment of this goal is increasingly challenging for residency programs.
Design: To increase the number of cytology specimens for residents, our program instituted a longitudinal program, cytology of the week cases (COWs), in 2010. Images of 2-5 cases with a corresponding line of clinical information are sent to residents on a weekly basis by a cytopathologist. Cases are housed on a secure department server. In addition, a direct link to the cases is on the password-protected departmental website, to enable off-site access. Residents are given one week to respond via email with diagnoses. Correct answers to the cases are emailed to residents and published on the departmental website weekly. COWs participation is entirely voluntary. Collaboration and use of ancillary materials to arrive at a diagnosis is allowed. Rise scores were de-identified for analysis.
Results: An increase of 445% in mean points attempted (PA) was seen between the first and second year of the program (14.7 to 77.7).

Table 1: Participation

Academic Year	N = Participants	Mean Points Attempted (PA)	SD	Range (pts)
2010-2011	17/23	14.7	15.2	2-64
2011-2012	23/26	77.7	44.0	4-145

Dummy variables were created for PA below mean (0-77) and above mean (78-145) participation. Multiple regression analysis of 2012 PA on the cytopathology RISE percentile scores demonstrated an estimated mean RISE percentile score 33% higher for residents with above average participation in COWs compared to those with below average participation (p=.006, t=3.03, CI=11-56%). We controlled for year of training and for residents committed to a cytology fellowship in the regression analysis. The program's RISE national percentile score in cytopathology increased by 7% from 2011 to 2012; however, the difference is not statistically significant.
Conclusions: Participation in a longitudinal COWs program correlates with an increase in RISE cytopathology scores. COWs is a feasible educational intervention from a program perspective, and residents participate voluntarily. Pathology residency training programs can utilize COWs to meet ACGME training requirements and to effectively enhance cytopathology teaching. Additionally, data collected from COWs can be used to identify global areas of diagnostic weakness among residents for future intervention in cytology and may have applications in other areas.

Endocrine

530 Pathology of Prophylactic Thyroidectomies in Patients with Germline Mutations Affecting the Extracellular Cysteine-Rich and the Intracellular Tyrosine Kinase Domains of the RET Protein

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Background: Approximately 25% of medullary thyroid carcinomas (MTC) are due to germline mutations in the *RET* proto-oncogene leading to an abnormally active transmembrane RET tyrosine kinase receptor, and are associated with either multiple endocrine neoplasia-2 (MEN2A and MEN2B) or familial MTC (FMTC). Nearly 98% of the MEN2A patients harbor a point mutation affecting the extracellular cysteine-rich domain, whereas all MEN2B patients, and some MEN2A and FMTC patients harbor mutations affecting the intracellular tyrosine kinase domain of the RET protein. In this study we compare the pathological features in prophylactic thyroidectomies for mutations affecting the extracellular cysteine-rich domain and the intracellular tyrosine kinase domain of the RET protein.
Design: We reviewed 14 prophylactic thyroidectomies with central lymph node dissection performed for known germline mutations in the *RET* proto-oncogene.
Results: *RET* proto-oncogene mutations involved codons 618 (n=2) and 634 (n=4) affecting the cysteine-rich domain (group 1), and codons 804 (n=7) and 790 (n=1) affecting the tyrosine kinase domain (group 2). The findings are summarized in the table. Five thyroids harbored a single focus of MTC (micro-MTC, size range 0.1 - 0.8 cm) limited to the thyroid gland. There were no MTC >1 cm and no vascular invasion. Central lymph node dissection, performed in all patients in group 1 (6/6) and in five patients in group 2 (5/8) were negative for metastasis.

	Cysteine-rich	Tyrosine kinase
Number of cases	6	8
Age range in years (median)	1.5-9 (1.5)	8-52 (14)
Sex	4M, 2F	6M, 2F
Micro-MTC	3/6	2/8
C-cell hyperplasia (nodular and/or diffuse)	5/6	5/8
Multifocal C-cell hyperplasia	2/6	2/8
Chronic lymphocytic thyroiditis	None	2/8

Conclusions: Patients with mutations in the cysteine-rich domain were younger. Micro-MTCs and C-cell hyperplasia were more frequent in cysteine-rich domain mutated thyroid specimens. Male sex predominated in both groups of patients with prophylactic thyroidectomies for germline mutations in the *RET* proto-oncogene.

531 BRAF V600E Mutation-Specific Monoclonal Antibody Is Predictive of *BRAF* Mutation Status Determined by Genotyping in Papillary Thyroid Carcinoma

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Background: An activating mutation in *BRAF* (V600E) is identified in approximately 45% of papillary thyroid carcinomas (PTCs) and has been reported to be associated with more aggressive tumor behavior. With the advent of new targeted therapies, *BRAF* status is becoming increasingly important in cases of radioactive iodine refractory disease. A BRAF V600E-specific monoclonal antibody has recently become commercially available. The aim of this study was to validate this antibody in a genotyped cohort of PTCs.

Design: 44 cases of resected PTC were examined. *BRAF* codon 600 was genotyped in all cases using pyrosequencing. A subset of *BRAF* wild type (WT) cases were also tested for *KRAS*, *NRAS* and *HRAS* mutations using a Sequenom platform. Twenty-two tumors had *BRAF* V600E mutation and 22 tumors were *BRAF* WT (of these 6 were *NRAS* and 5 were *HRAS* mutated). Immunohistochemistry (IHC) was performed on whole sections following pressure cooker antigen retrieval with an anti-BRAF V600E monoclonal antibody (Spring Bioscience; clone VE1; 1:50 dilution with overnight incubation). Cytoplasmic staining was scored as "positive" or "negative".

Results: Cytoplasmic staining for BRAF was observed in all 22 cases with the *BRAF* V600E mutation. Staining was negative in all 22 cases with WT *BRAF*, including all cases with *NRAS* and *HRAS* mutations. Positive cases showed a homogeneous staining pattern. Negative cases frequently showed non-specific staining of tumor cell nuclei and colloid; however, this pattern was easily distinguishable from the cytoplasmic staining seen in *BRAF*-mutant tumors.

Conclusions: IHC for BRAF V600E is predictive of *BRAF* mutation status determined by genotyping in PTC. In our study the stain showed both a sensitivity and specificity of 100%.

532 Cytoplasmic OCT4 Staining Is a Sensitive and Specific Marker of Neuroendocrine Differentiation

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Background: OCT4 is a transcription factor that has gained wide clinical usage as a diagnostic marker in the setting of germ cell tumors. A previous study by the authors described a novel cytoplasmic staining pattern in normal adrenal medullary tissue and pheochromocytomas. Ultrastructural studies performed at that time showed the antibody binding to neurosecretory granules (NSGs). From this, we hypothesized that similar staining may be detected in other neuroendocrine tissues. The goal of this study is to test this hypothesis by examining a wide array of neuroendocrine tumors for the presence of this novel cytoplasmic staining pattern.

Design: 49 cases of neuroendocrine tumors were selected from our institution's case files. These consisted of 26 cases classified as well-differentiated, 6 moderately differentiated, and 17 cases of small cell or high grade neuroendocrine carcinoma. All cases were immunostained with OCT4 and Ki-67. OCT4 reactivity was then scored for intensity (0-3+) and extent (0-3+). Ki-67 proliferation index was scored as a percentage of total tumor cells. Immunoelectronmicroscopy (IEM) was performed to determine precise location of antibody binding within cells.

Results: Immunoreactivity was seen in 24 of 26 (92%) cases of carcinoid tumors. 23 of these 24 (96%) cases showed strong reactivity (2-3+ intensity and extent). The same type of strong staining was seen in 4 of 6 (67%) moderately differentiated neuroendocrine tumors. Only 2 of 17 (12%) cases of high grade neuroendocrine carcinoma cases showed similar staining. A strong, inverse correlation was seen with OCT4 staining intensity and degree of differentiation (correlation coefficient of -0.73). The same correlation was seen with regards to OCT4 staining and Ki-67 index. IEM showed binding of OCT4 antibody to neurosecretory granules.

Conclusions: Cytoplasmic staining of OCT4 is a sensitive and specific marker of neuroendocrine differentiation. These findings expand those of our previous work and assert that this particular antibody has a high affinity for neuroendocrine tissue. This report also shows that as neuroendocrine tumors become less differentiated and lose their cytoplasm and, therefore its contents (i.e., NSGs), OCT4 staining decreases accordingly. Though further validation is necessary, OCT4 may prove to be a clinically useful immunostain in the diagnosis of neuroendocrine tumors. Furthermore, the loss or depletion of immunoreactivity in higher-grade tumors may provide an adjunct for classification in difficult cases with limited tissue available.

533 Clinical Characteristics of Cribriform-Morular Variant of Papillary Thyroid Carcinoma

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Background: The incidence of thyroid carcinomas in patients with Gardner Syndrome (GS) is uncommon. GS is an autosomal dominant disorder caused by *APC* gene mutation, leading to increased propensity of certain neoplasms. These include colonic adenomas/carcinomas, desmoid tumors, and thyroid cancers. The thyroid tumors often represent cribriform-morular variant of papillary carcinoma (C-MV). It is reported that most cases of C-MV take an indolent course. This study examines the relationship of C-MV to GS and familial adenomatous polyposis (FAP), with particular focus on the clinical characteristics of the thyroid cancers.

Design: Electronic medical records were searched for a 5 year period (2007-2012) for patients known to have GS or C-MV. Histologic and immunohistochemical studies as well as relevant serologic and molecular test reports were reviewed.

Results: Eight patients with GS and one patient with C-MV without GS were identified. Five were male and 4 female, ranging in age from 15 to 59 (average: 33). Seven patients presented with adenomatous polyposis, leading to total colectomy. Four patients also developed abdominal wall fibromatosis in addition to FAP. One patient had an initial presentation of a thyroid nodule. Three patients (all female) with thyroid lesions had C-MV, characterized by areas of cribriform architecture and squamous morules. Of these, one patient suffered a protracted course, with regional extension of the tumor, increased serologic β-hCG, and vaginal bleeding. After resection of the mass, the tumor cells were immunoreactive for β-hCG, indicating a paraneoplastic syndrome. *APC* mutation analysis was performed on the 3 patients with C-MV, 2 of whom were positive. Additionally, β-catenin immunostaining was positive in these two patients, in contrast to the one patient without *APC* mutation.

Conclusions: Most patients with GS present with polyposis and undergo total colectomy for prevention of carcinoma. In this series, patients with GS and thyroid lesions all had C-MV. In one case, C-MV was diagnosed in a patient without FAP; in this case, mutational analysis was negative, suggesting that C-MV is not restricted to patients with GS and FAP. Additionally, one case of C-MV had paraneoplastic secretion of β-hCG, a finding not reported in the literature. In most cases of C-MV, as shown here, there is a prior history of GS and/or FAP. Patients with C-MV are largely female, and the course, in general, is indolent. Rare paraneoplastic secretion may also occur. Immunoreactivity for β-catenin correlates well with mutational analysis and can be used to help identify patients with GS.

534 Hobnail Variant of Papillary Thyroid Carcinoma: Case Series with Fine-Needle Aspiration Cytology and Cell-Blocks

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Background: Recent reports indicate that papillary thyroid carcinoma (PTC) with hobnail features (HPTC) is a rare, but very aggressive variant of PTC. The cytological features of HPTCs on fine needle aspiration smears have never been described.

Design: We examined smears and cell blocks of 5 cases of HPTC to define their diagnostic cytological features. *B-RAF* mutation was studied in all cases by pyrosequencing. Histopathological and immunohistochemical data were further obtained from surgical specimens. Follow up information were obtained from medical record review.

Results: The patients (3 females and 2 males) age ranged from 27 to 86 (mean 65) years. Tumor size ranged from 2 to 9 cm (mean 4,2 cm). All aspirates were highly cellular with a bloody background and scant colloid. The cells were arranged in papillary clusters or in micropapillary groups. The proportion of isolated cells versus clusters varied from case to case. The cell population consisted of small to medium-size cells with tear-drop cytoplasm, apically placed occasionally grooved nuclei that produced a surface bulge leading to a hobnail appearance and high N/C ratio. At higher magnification, the nuclei showed variable degrees of atypia with occasional pink intranuclear holes. Nuclear stratification and atypical mitotic figures were present. *B-RAF* mutation was present in 3/5 cases. All cases showed positivity for Thyroglobulin, Thyroid transcription factor-1 (TTF-1), partial loss of E-cadherin expression and over-expressioin of p53 protein on histological sections. Four patients were alive without diseases and 1 patient showed a locoregional recurrence at 8.5 months (range: 2 to 24 months) of follow-up.

Conclusions: A combination of papillary/micropapillary and hobnail cyto-architectural patterns of growth, single tear-drop cells, high N/C ratio, severe crowding could be the most useful criteria for diagnosing HPTC on cytological smear.

535 Pediatric Thyroid Carcinomas of Follicular Cell Origin: A Clinico-Pathologic Study of 42 Cases

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Background: Pediatric thyroid carcinomas (TC) of follicular cell origin are rare. Our aims are 1) to assess the prognostic value of histologic subtyping and other morphologic parameters in pediatric TC 2) Correlate the histologic findings with the presence of metastatic disease.

Design: 42 cases of pediatric TC of follicular cell origin presenting in children $\leq$ 18 years old without history of radiation exposure were included. All tumors were subjected to a meticulous histopathologic examination.

Results: There were 31 (74%) females and 11 (26%) males with a median age of 13.4 years. The tumor histotype was distributed as follows: 17 (40%) classical papillary thyroid carcinoma (PTC), 7 (17%) encapsulated follicular variant (FV) PTC, 6 (14%) diffuse sclerosing variant (DSV) PTC, 5 (12%) tall cell variant PTC, 1 (2.5%) infiltrative FV PTC, 1 (2.5%) solid variant PTC, 1 (2.5%) unclassifiable PTC, 1 (2.5%) minimally invasive follicular carcinoma and 3 (7%) poorly differentiated (PD)TC. At presentation, cervical lymph node metastases (LNM) were found in 24 (57%) of cases and distant metastases (DM) in 8 (19%) of individuals. While age did not affect the LNM and DM rates, male patients had a higher incidence of LNM (p=0.0122) and DM (p=0.0195). DSV had a higher DM rate (4/6, 67%) than the remaining TC (4/36, 11%, p=0.0079). The LNM frequency was also more elevated in the DSV (6/6, 100%) compared to the rest of the TC (18/32, 50%; P=0.0292). Infiltrative TC had a very high LNM rate (24/26, 92%) while encapsulated non-infiltrative tumor lacked nodal metastases (0/16, p=0.0001). Distant metastases were present in 8 of 26 (31%) of infiltrative tumors while absent in encapsulated neoplasms (0/16, 0.0159).The presence of a positive margin and extensive extra-thyroid extension (ETE) strongly correlated with the presence of LNM ($p<0.01$) and DM ($p<0.01$). Primary tumor $\geq$4 cm had a higher DM rate (p=0.03). With a median follow up of 3.7 years, only 2 patients recurred: 1 infiltrative classical PTC with extensive extra-thyroid extension and metastatic PD in the LN; and 1 encapsulated FV PTC with focal angioinvasion and extensive capsular invasion. All 8 encapsulated TC without vascular invasion did not harbor metastases or recur.

Conclusions: DSV, infiltration, male gender, positive margins, extensive ETE and large tumor size are associated with the presence of DM in pediatric TC 2) Encapsulated TC without vascular invasion appear to behave in an indolent fashion in children.

536 BRAF Mutation Testing in Atypia of Undetermined Significance and Suspicious for Malignant Categories of Thyroid Fine Needle Aspirations in Clinical Practice

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Background: Genetic analyses of known mutations are helpful in thyroid fine needle aspirations (FNAC) in diagnosing suspicious cases. Herein we report our institution experience of BRAF mutation analyses in FNAC diagnosed as atypia of undetermined significance (AUS/FLUS) and suspicious for malignant/papillary carcinoma (SFM) according to Bethesda classification.

Design: FNACs in our institution are air dried and MGG stained. Cases with AUS/FLUS and SFM categories were further reviewed. After keeping one diagnostic slide for archive, the cases with extra slides with enough cellularity are tested for BRAF mutation. Enough cellularity is defined as a moderately cellular slide with >75% atypical or suspicious cells. BRAF codon 600 (600 GTG>GAG -1799T>A, V600E) mutation was searched by real time PCR (Entrogen BRAF Mutation Analysis Kit, Applied Biosystem Stepone Plus Real Time PCR).

Results: AUS/FLUS group: Fifty of 155 cases diagnosed as AUS/FLUS were included. DNA extraction was successful in 44 of 50 cases DNA. Of 44 cases 2 were positive and 42 were negative. Two BRAF positive cases had thyroidectomy and diagnosed as papillary thyroid carcinoma (PTC). In 4/42 BRAF negative cases, thyroidectomy was performed due to clinical features: 2 were hyperplastic nodules and 2 had PTC. SFM group: Thirteen of 22 cases diagnosed as SFM were included and DNA could be isolated in all of them. Six of 13 cases were positive and 7 of 13 cases were negative. Of 6 BRAF positive cases 4 underwent thyroidectomy and diagnosed as papillary thyroid carcinoma. Of 6 negative cases 1 had thyroidectomy and diagnosed as Hurthle cell carcinoma.

Conclusions: In our series BRAF positivity was 4.5% (2/44) in AUS/FLUS and 46.1% (6/13) in SFM categories. We also found that hypocellularity may be an obstacle in DNA isolation process especially in AUS/FLUS group. Using only BRAF mutation status, there were 3 underpredicted cases whereas no overprediction of malignancy was observed. Since BRAF negativity does not rule out PTC presence; it is necessary to combine Bethesda classification and mutation status with clinical and radiological suspicious features for thyroidectomy decision.

537 SDHB Mutation in High-Altitute Paraganglioma/Pheochromocytoma

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Background: Pheochromocytomas/Paragangliomas (PPG) are components of several hereditary cancer syndromes, and up to 30% may be associated with germ line mutations of genes including VHL, RET and SDH. SDHB and SDHD encode proteins that regulate hypoxia-inducible factor 1 subunit alpha (HIF1α) that helps cells adapt to hypoxia. High levels of HIF1α can suppress SDHB, suggesting a hypoxic pattern in SDHB-mutant tumors. In this study we examine clinical and pathologic features of high-altitute PPG and determine SDHB mutation status.

Design: We reviewed twenty four PPG from Mexican patients living 2,000 m above sea level (Mexico City and Puebla) procured in our institution. Immunohistochemistry (IHC) was performed using antibodies for synaptophysin, chromogranin, S100 and SDHB. All cases were tested for genetic SDHB mutation. DNA extraction was carried out and SDHB gene was amplified by PCR.

Results: The mean age was 50 years (range 13-72) with a female predominance 7:1. The most common location was head and neck (19/24, 79.2%) followed by abdominal/pelvic region (3/24, 12.5%) and adrenal (2/24, 8.3%). PPG were positive for synaptophysin and chromogranin; S100 was positive in sustentacular cells. Patients with abdominal/pelvic PPG were members of the same family and had SDHB mutation in exon 6. In 3/3 abdominal/pelvic region and one adrenal PPG a loss of SDHB protein expression was observed. Eleven out of nineteen head and neck PPG had SDHB mutations involving exon 3, 4, 5 and 8; in three of these cases double mutation was found.

Conclusions: In Mexican patients, mutation in exon 5 of SDHB is present in high-altitute PPG of the head and neck. SDHB mutation in exon 6 is seen in familial PPG.

538 BRAF Genetic Heterogeneity in Papillary Thyroid Carcinoma and Its Metastasis

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Background: BRAF V600E mutation is reported in up to 65% of papillary thyroid carcinomas (PTC). Detection of this mutation is helpful in the cytodiagnosis of indeterminate thyroid nodules and is a possible stratification tool for extent of surgical treatment as well as a potential therapeutic target in PTC. Heterogeneity for BRAF mutation in tumor samples from a patient with PTC can impact management. This pilot study was designed to assess BRAF genetic heterogeneity (GH) within primary PTCs and between paired primary and metastatic lesions.

Design: 38 patients who underwent total thyroidectomy for conventional and/or follicular variant PTC were identified in our database. They included patients with metastases to regional lymph nodes at thyroidectomy and patients with persistent metastases >5 years. Slides were reviewed, diagnoses confirmed, and patient demographics and tumor size were recorded. Formalin fixed paraffin embedded sections of the primary PTCs and their metastases were macrodissected and analyzed for BRAF V600E (1799T>A) mutation using real-time PCR. BRAF GH was assessed in 3 groups of paired tumor samples:

Group A: two separate areas of the primary tumor [28 cases (in 15 cases one of the areas analyzed was ≤0.5 cm in diameter; in the remaining 13 cases both samples were >1.5 cm)]

Group B: primary tumor vs. metastasis at thyroidectomy [16 cases]

Group C: primary tumor vs. metastasis >5 years post-thyroidectomy [9 cases].

Results: The 38 patients (24 females, 14 males) ranged from 16 to 77 years in age (median 45 yrs) at thyroidectomy. The primary tumors ranged from 0.8 to 4.9 cm in diameter (median 2 cm). The persistent metastases analyzed were excised from 5 to 26 years (median 6 yrs) after thyroidectomy. Analysis for the presence of the BRAF mutation yielded discordant results in 1 (3.6%) of the 28 cases in Group A, 0 (0%) of the 16 cases in Group B, and 2 (22.2%) of the 9 cases in Group C. In both of the discordant cases in Group C, the BRAF mutation was detected in the primary but not in the persistent metastasis excised 6 years post-thyroidectomy.

Conclusions: Our findings suggest that

-heterogeneity for BRAF mutation is infrequent within a primary PTC and BRAF mutation status can be reliably determined from analysis of a small biopsy of the tumor,

-BRAF GH is infrequent between a PTC and its concurrent metastasis but might occur more frequently with tumor persistence,

-larger studies are warranted to confirm our findings.

539 Sunitinib Targets in Pheochromocytomas and Paragangliomas

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Background: Sunitinib, a multitargeted tyrosine kinase inhibitor (TKi), has been reported as a successful treatment alternative for metastatic pheochromocytoma (PCT)/paraganglioma (PGL). To determine whether this is due to its well-known anti-angiogenic properties or to direct anti-proliferative/pro-apoptotic effects on tumour cells, we created an *in vitro* model of Sunitinib treatment using MPC 4/30 cells. Potential Sunitinib targets were also investigated in human tumors using a tissue microarray (TMA) and correlated with outcomes.

Design: MPC 4/30 cells were treated with 0uM, 2.5uM and 5.0uM Sunitinib for 72h. Treatment effect were analysed by flow cytometry (FACSCalibur) and gene expression profiling (Gene 1.0 ST Array). Results were examined using DAVID Bioinformatic Resources 6.7 and String protein association network software. IHC expression of Sunitinib targets (VEGFR1, VEGF2, PDGFRα, PDGFRβ, C-KIT) and new potential targets identified through gene expression profiling - Aurora Kinases A (ArkA) and B (ArkB) - were evaluated on a TMA from 39 PCTs and 76 PGLs. Outcome correlations were investigated by logistic regression.

Results: Cell-cycle analysis showed Sunitinib decreases MPC 4/30 proliferation (13.5% of untreated cells on M2 phase *versus* 5.5% in 5uM treated; p=0.001) and increases apoptosis (14.5% apoptotic debris in untreated cultures *versus* 24% in 2.5uM and 19.5% in 5uM treated; p non-significant). Gene expression profiling revealed Sunitinib strongly down-regulates cell cycle associated genes (37% of all genes with >2fold downregulation), with more than 50% of these affecting M phase. IHC showed VEGFR2 and PDGFRα to be overexpressed in both tumors, PDGFRβ and VEGFR1 overexpressed only in PGLs and C-KIT overexpressed only in PCTs*. ArkA was underexpressed in PGLs* and ArkB expression was negligible. Increased risk of metastasis was associated with membranous expression of PDGFRα (OR=13.712,*) and VEGFR1 (OR=8.009;*).*p<0.05

Conclusions: Sunitinib decreases cell proliferation in PCT cells mainly by targeting cell cycle, as well DNA metabolism, and cell organization genes. Membranous expression of PDGFRα and VEGFR1 correlated with increased risk of metastastic disease. In contrast with most malignancies, ArkA was underexpressed PGLs. This was unexpected given Aurora's downregulation by Sunitinib in PCT cells in vitro. The findings provide novel insights into mechanistic actions of Sunitinib and identify potential new therapeutic targets for PCTs/PGLs.

540 SDHB Negative Staining as an Indicator of Presence of SDHB, SDHC and SDHD Germ Line Mutations in Pheocromocytomas and Paragangliomas

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Background: Pheocromocytoma (PCC) and paragangliomas (PGL) can occur sporadically or as a part of several different hereditary tumor syndromes. The PGL/PCC syndrome is associated with different germline mutations in *SDHD, SDHAF2, SDHC* and *SDHB*. Negative staining of SDHB has been proposed as an indicator of the presence of *SDHB, SDHC* and *SDHD* germline mutations. Our aim was to confirm the usefulness of SDHB negative staining as a predictor of the presence of *SDHB, SDHC* and *SDHD* germline mutations in a series of PCC and PGL.

Design: Sixty-four tumors (36 adrenal PCC, 11 thoracic-abdominal PGL and 17 head and neck PGL) were assessed for SDHB immunostaining. Mutational status of *SDHD, SDHAF2, SHDC, SDHB, RET, VHL,* was assessed in normal and tumor tissue.

Results: SDHB staining was negative or granular-like in 17 cases (27%) and positive in 47 (73%). A germ line *SDHB* mutation was detected in 9 patients (7 with negative and 2 with granular-like staining). *SDHD* germline mutation was detected in 5 patients (all 5 with granular like staining). Germline mutations in *RET* (23 patients) and *VHL* (8 patients) were always associated with positive SDHB immunostaining. Nineteen patients showed absence of any germline mutation; SDHB positive stain was seen in 16 cases, while 3 of them had negative staining.

Conclusions: SDHB negative staining is a sensitive and cost effective screening tool for patients with PCC and PGL, since the vast majority of tumors with negative SDHB immunostaing were associated with germline mutations in *SDH* genes.

541 Napsin A Expression in Anaplastic, Poorly Differentiated and Micropapillary Pattern Thyroid Carcinomas

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Background: Napsin A is more sensitive and specific for pulmonary adenocarcinoma versus squamous cell carcinoma than thyroid transcription factor-1 (TTF-1). TTF-1 is also a recognized marker of thyroid carcinomas. Preliminary studies have shown that napsin A is positive in ~5% of papillary thyroid carcinomas as well. The prevalence of napsin A in anaplastic (ATC) and poorly differentiated (PDCa) thyroid carcinomas has not been thoroughly investigated. Napsin A positivity in metastatic thyroid carcinoma, especially in conjunction with TTF-1, could potentially be misdiagnosed as a lung metastasis. The aim of this study is to investigate the prevalence of napsin A expression in ATC, PDCa and the recently described micropapillary pattern (MPP) thyroid carcinoma, which show histologic similarity to a subset of lung adenocarcinomas.

Design: Immunohistochemistry for Napsin A, TTF-1 and PAX-8 was performed. Staining strength (weak, moderate or strong) and extent (1+ = 1-25%, 2+ = >25-50%, 3+ = >50-75%, 4+ = >75%) were evaluated.

Results: Twenty-six ATCs (21 primary, 4 regional lymph node metastases, 1 metastasis to larynx), as well as 16 PDCa (14 primary and 2 metastases—1 regional lymph node, 1 lung) and 2 MPP carcinomas (1 primary and 1 regional lymph node metastasis) were identified. A focal MPP component was seen in 3 PDCa and 3 ATCs. The immunohistochemistry results are summarized below:

Table 1. Immunohistochemistry results

Histologic Type	Napsin A	TTF-1	PAX-8
Anaplastic	4/26(15%)	8/26(32%)	18/26(69%)
Poorly differentiated	2/16(13%)	16/16(100%)	15/16(94%)
Micropapillary	2/2(100%)	2/2(100%)	1/2(50%)
Micropapillary component	3*/6(50%)	6/6(100%)	5/6(83%)

*All but one Napsin A positive micropapillary component were also Napsin A positive in the poorly differentiated or anaplastic component

All Napsin A positive cases were positive for TTF-1 and all but the 1 primary MPP carcinoma were also PAX-8 positive. Napsin A staining strength was moderate but extent was variable (1+ in 4 cases, 3-4+ in 5 cases).

Conclusions: A minority of ATCs and PDCas are Napsin A positive. Napsin A positivity is more common in carcinomas with a MPP component, a pattern that can also be seen in lung adenocarcinomas. PAX-8 may be diagnostically useful to distinguish these Napsin A positive thyroid carcinomas from lung adenocarcinomas, since PAX8 is typically negative in lung adenocarcinomas but positive in thyroid carcinomas. In this study, only one Napsin A positive thyroid tumor was PAX-8 negative.

542 Clear Cell Variant of Thyroid Carcinoma: A Clinicopathologic Study

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Background: Clear cell carcinoma of the thyroid is classified by the WHO as a variant of follicular or papillary thyroid carcinoma. Clear cell change in thyroid carcinoma has been reported in the literature, often in relation to the differential diagnosis of metastatic clear cell renal cell carcinoma. In modern practice, immunohistochemistry renders this distinction relatively facile. In this study, we address the clinicopathologic characteristics of primary thyroid neoplasms with cytoplasmic clear features.

Design: Our pathology department database was queried for the words "thyroid carcinoma" and "clear cell," resulting in 21 primary thyroid carcinomas from 20 patients from 1992-2012. These 21 cases represent 0.5% of the total in-house thyroid carcinomas (4330) during this time frame.

Results: Of the 21 carcinomas, 12 (57%) were follicular carcinoma (FTC), 5 were conventional papillary carcinoma (PTC), 3 were follicular variant of PTC, and 1 was poorly differentiated carcinoma (PDC). Clear cell change in all cases was focal or multifocal, never diffuse. The average tumor size was 2.8 cm among 12 female and 8 male patients with an average age of 55 years (range: 26 – 80). Five (24%) carcinomas were metastatic to regional lymph nodes (3 FTC and 2 PTC), and two (10%) were metastatic to bone, both FTC. Disease confined to the thyroid (67%) and rates of regional lymph node metastasis (24%) were both near the national averages (68% and 25%, respectively). Distant metastasis (10% in this study) was slightly higher than the 5% national average. The patient with PDC died 1 year after diagnosis, and a patient with metastatic FTC died 2 years after diagnosis. No other deaths were reported. Overall mortality was 10%, which matched national averages when adjusted for age (17% of patients aged 55-64 and 25% of patients aged 65-74).

Conclusions: Clear cell change in thyroid carcinoma is rare and found focally or multifocally within a given lesion. Our findings indicate that clear cell change is more common in FTCs, and is associated with an increased risk of distant metastasis but with a prognosis that is similar to other differentiated thyroid carcinomas.

543 Claudin Proteins Are Differentially Expressed in Gastrointestinal and Pulmonary Neuroendocrine Tumors

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Background: Claudins are members of a large family of tight junction proteins, which regulate cellular adhesion, polarity, and glandular differentiation. Dysregulation of claudin protein expression has been described in a number of malignancies, including lung and gastrointestinal carcinomas; however, expression of claudins in neuroendocrine tumors (NET) has not been addressed. Our goal was to investigate the protein expression patterns of claudins 1,3,4,7, 8 in neuroendocrine tumors from the GI tract and lung.

Design: One hundred and eight cases of low-to-intermediate grade NETs were retrieved from the archives of Rhode Island Hospital, including 70 of the GI tract (41 ileal (IC), 17 appendiceal (AC), and 12 rectal (RC)) and 38 typical pulmonary carcinoids (TPC). Paraffin embedded tissue microarrays were analyzed for IHC expression of claudins 1,3,4,7, and 8. The immunoreactivity was assessed based on a combined score of the extent and intensity on a scale of 0-3+.

Results: Normal intestinal and bronchial epithelium showed positive membranous staining for all claudins studied. In addition, alveolar cells showed immunoreactivity for claudins 3,4,7, and 8. In NETs, all claudins exhibited a membranous staining pattern independent of location. GI NETs demonstrated significantly higher frequencies of moderate to strong (2-3+) staining for all claudins as opposed to TPC ($p<0.002$). The differences between GI and pulmonary NET were most striking in claudin 8 with moderate to strong expression (2-3+) between IC and TPC (78.3% vs 16.2%, $p<0.0001$) and claudin 4 with moderate to strong expression (2-3+) among all GI NETs as compared to TPC (56.3-60.9% vs 2.6%, $p<0.0001$). In addition, within the GI NET group, claudin 8 expression was significantly more frequent in IC as opposed to AC (78.3% vs 50.0%, $p=0.03$). Claudin 3 expression was significantly higher in RC as compared to IC (69.2% vs 26.1%, $p=0.0082$).

Claudin Expression in NETs

	Ileal NET (n=41)	Appendiceal NET (n=17)	Rectal NET (n=12)	Typical Pulmonary Carcinoids (n=38)
Claudin 1	60.0%	47.1%	15.4%	5.3%
Claudin 3	26.1%	52.9%	69.2%	10.5%
Claudin 4	60.9%	56.3%	58.3%	2.6%
Claudin 7	63.0%	55.6%	77.0%	21.6%
Claudin 8	78.3%	50.0%	46.2%	16.2%

Conclusions: This study is the first to comprehensively examine expression of claudins 1,3,4,7 and 8 in the NETs of the GI tract and lung. NETs comprise a heterogenous group and exhibit site-specific differential expression of claudin proteins. Site-specific claudin expression may help us better understand the differences among biological behaviors of NETs.

544 Micronuclei and Nuclear Buds: Highlighting Overlooked Indicators of Increased Chromosomal Damage in Thyroid Lesions with Emerin Immunohistochemistry

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Background: Emerin immunohistochemistry provides detailed information about nuclear shape and irregularities in thyroid. We aimed to investigate the presence of micronuclei and nuclear buds, which are considered to be indicators of increased chromosomal damage, in well differentiated neoplastic and non-neoplastic follicular epithelial lesions of thyroid by using emerin immunohistochemistry.

Design: Tissue microarrays consisting of samples from well differentiated neoplastic (follicular/oncocytic adenoma (FA/OA), follicular/oncocytic carcinoma (FC/OC), papillary thyroid carcinoma (PTC)) and non- neoplastic (nodular hyperplasia (NH), Hashimoto's thyroiditis (HT), adenomatous nodule (AN)) epithelial lesions were stained with anti-emerin monoclonal antibody and evaluated for the presence of nuclear buds and micronuclei.

Results: Of the 340 cases examined 275 were diagnosed as neoplastic (77FA/OA, 48FC/OC, 150 PTC) and 65 as non- neoplastic (10 NH, 21 HT, 34 AN). All malignant cases (FC/OC, PTC), 19 (25%) of 77 FA/OA, 2 (10%) of HT were positive for micronuclei and nuclear buds, whereas all of the NH an AN cases were negative. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the presence of nuclear buds and micronuclei (fig.) for neoplasia and malignancy are shown in table 1.

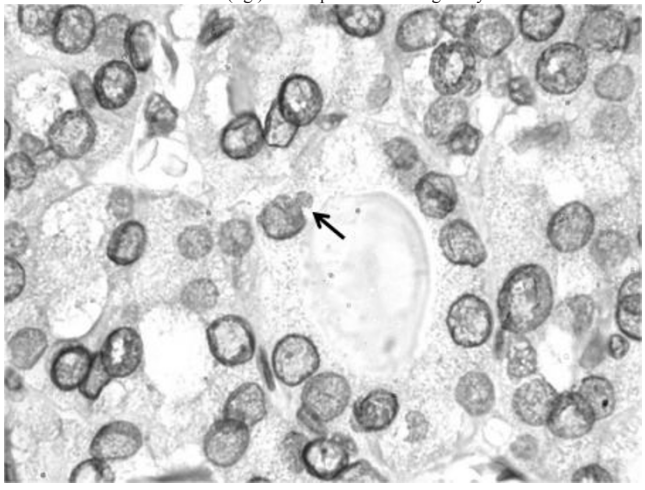

Table 1: Statistical data

Diagnostic Group	Sensitivity	Spescificity	PPV	NPV
Neoplasia	0.83	0.86	0.96	0.54
Malignancy	1.00	0.73	0.84	1.00

Conclusions: Highlighting the presence of nuclear buds and micronuclei with emerin immunohistochemistry may be a useful diagnostic tool in evaluating the extent of cells with chromosomal damage as this overlooked finding in thyroid lesions appears to be indicating malignant behavior with high sensitivity and reasonable specificity.

545 MicroRNA Expression Profiles in Poorly Differentiated Thyroid Carcinomas

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Background: Conventional and oncocytic poorly differentiated thyroid carcinomas (PD and oPD) are among the most difficult thyroid tumors to be diagnosed. In contrast to papillary thyroid carcinomas (PTC), where some miRNAs are established as reliable markers, this information is very limited for PD and oPD. The aim of this study was to characterize the miRNA profile for PD and oPD and identify miRNAs which could eventually serve as diagnostic and prognostic markers in thyroid specimens.

Design: Ninty-eight thyroid carcinomas (14 PD, 12 oPD, 44 PTC and 28 follicular thyroid tumors (FTC)) and 8 normal thyroid tissues were studied for expression of 768 miRNAs using Human Microarray Assays (Applied Biosystems) on ABI 7900. miRNAs were isolated using using RecoverAll Total Nucleic Acid kit (Ambion) from FFPE tissue specimens (n=106). The data analysis was performed with Data Assist v3.1 (Applied Biosystems) and SPSSv.17 (IBM) program.

Results: miRNA expression was different between PD and oPDs demonstrating individual clusters on the unsupervised hierarchical clustering analysis. Both, PD and oPDs showed upregulation of miR-125a-5p, miR-15a-3p, miR-182, miR-183-3p, miR-222, miR-222-5p. Downregulation of miR-130b, -139-5p, -150, -193a-5p, -219-5p, -23b, -451, -455-3p and of miR-886-3p was observed. In addition, upregulation of miR-221 and miR-885-5p was strongly detected in oPD. Only significant deregulated miRNAs in PD and oPD carcinomas are reported. The expression of these markers was compared to their expression in PTC and FTC. Kaplan-Meier analysis demonstrated a significant association with tumorrelapse for miR-139-5p, -183-3p, -221, -222, -222-5p, -23b and miR-886-3p. MiR-15a-3p, -23b and miR-885-5p were significant correlated with tumor specific death.

Conclusions: PD and oPD are an ongoing diagnostic challenge. The new identified specific deregulated miRNAs bear the potential to facilitate their diagnoses in a daily preoperative and postoperative routine setting. Furthermore, the prognostic information of these markers may help clinicians in the future to treat patients with these rare neoplasms better. Further studies are required to validate these findings.

546 Meta-Analysis of the Molecular-Expression Signatures Accuracy in Cytological Indeterminate Thyroid Nodules

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Background: Fine-needle aspiration cytology (FNA) has reliable accuracy with excellent clinical performance in the preoperative diagnosis of thyroid nodules. However, approximately 25% of nodules are classified as indeterminate. Multiple strategies have been developed to address this problem. Recently, molecular based tests have emerged as promising tools for improving diagnostic accuracy in indeterminate cases.

Design: A systematic review of the literature with meta-analysis of studies that evaluate molecular preoperative test for detection of thyroid cancer in FNA indeterminate nodules was performed. The studies search was executed in the database Medline/PubMed. Search terms used include: "cytology," "indeterminate," "molecular," "gene," "thyroid," "accuracy," "performance," and "cancer". Forty-two citations were found and reviewed, and only the original validation studies (OVS) were selected.

Results: We identified 10 OVS published between 2006 and September 2012, which compared different molecular preoperative thyroid test results in indeterminate cytology using histopathology results as final diagnosis. All the studies were analyzed as transversal or naturalistic validations. The total population evaluated was 1354 patients. Five studies evaluated gene-expression classifiers, 3 studies evaluated microRNA classifiers and 2 evaluated immunocytochemistry expression. The sensitivity was reported from 37% ($BRAF^{V600E}$ mutation study) to 100% (miRNA and immunocytochemistry studies) with a proportion of variability (R^2) of 1.47E-03. The specificity was reported from 20% (expression levels of four miRNAs miR-7, -126, -374a, and let-7g) to 100% (panel of mutations in $BRAF^{V600E}$, NRAS, HRAS, KRAS RET/PTC1, RET/PTC3, and PAX8/PPAR-gamma) with a R^2 of 0.18. A critical review revealed that 6 of the 10 studies had the potential for verification/selection bias and only 5 stated that the molecular test and histopathology were independently assessed.

Conclusions: New molecular strategies for the diagnosis of indeterminate thyroid nodules have been evaluated with variable sensibility and more consistent specificity. These new studies propose different molecular markers with better performances when used as panels. Limitations in the verification of data were found.

547 *BRAF* V600E Mutations in Thyroid Carcinomas: Is There a Role for BRAF Immunohistochemistry?

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Background: *BRAF* V600E mutations promote tumorigenesis via constitutive activation of the mitogen activated protein kinase pathway leading to dysregulated cellular proliferation and increased survival. *BRAF* V600E (T→A 1799) point mutations are seen in approximately 45% of papillary thyroid carcinomas (PTCs) and current practice algorithms endorse molecular testing for most patients with a diagnosis of PTC. In this study, we examined the utility of immunohistochemical (IHC) BRAF overexpression to identify thyroid carcinomas harboring *BRAF* V600E mutations.

Design: We selected a total of 41 cases for analysis: 31 papillary, 1 follicular (FTC), 5 medullary (MTC), and 4 anaplastic (ATC) thyroid carcinomas from 37 thyroidectomies and 4 fine needle aspiration cell blocks. IHC was performed with the Dako Autostainer, the BRAF EP152Y monoclonal antibody (MAB, Abcam, 1:20 dilution), and high pH antigen retrieval (Trilogy, Cell Marque). Tumors were considered to overexpress BRAF if greater than 10% of neoplastic cells showed 2+ or 3+ cytoplasmic staining. Baseline expression was determined using three pancreatic tumors. *BRAF* mutations were confirmed using pyrosequencing. Molecular analysis was used as the gold standard for statistical analysis.

Results: 33/41 (80%) of carcinomas overexpressed BRAF by IHC (27 PTCs, 1 FTC, 3 MTCs, and 2 ATCs). 12 PTCs (39%) and 2 ATCs (50%) harbored *BRAF* V600E mutations; all overexpressed BRAF by IHC. No *BRAF* V600E mutations were detected in the FTC or 5 MTCs. All 8 carcinomas that did not demonstrate BRAF overexpression were *BRAF* V600E negative (sensitivity 100%, NPV 100%). 19 tumors (46%) overexpressed BRAF by IHC but were *BRAF* V600E negative (specificity 29.6%, PPV 42.4%).

BRAF Analysis In 41 Thyroid Carcinomas

	BRAF IHC + / V600E +	BRAF IHC + / V600E -	BRAF IHC - / V600E +	BRAF IHC - / V600E -
PTC (n=31)	12	15	0	4
FTC (n=1)	0	1	0	0
MTC (n=5)	0	3	0	2
ATC (n=4)	2	0	0	2
Total (n=41)	14 (34%)	19 (46%)	0 (0%)	8 (19%)

Conclusions: Molecular testing for *BRAF* mutations in thyroid carcinomas remains the diagnostic gold standard. BRAF IHC provides excellent sensitivity and NPV, but the inability of the BRAF EP152Y MAB to discern endogenous wild-type BRAF expression from overexpressed BRAF secondary to genetic mutation (e.g. low specificity and PPV) limits the diagnostic utility of this antibody outside of an initial screening test. Additional research is needed to determine whether these results are applicable to BRAF IHC overexpression in other malignancies. Analysis with a mutation-specific BRAF V600E antibody is ongoing to improve the specificity and PPV.

548 Multifocal Fibrosing Thyroiditis and Its Association with Papillary Thyroid Carcinoma Using BRAF Pyrosequencing

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Background: Multifocal fibrosing thyroiditis (MFT) is characterized by numerous foci of fibrosis in a stellate configuration with fibroelastotic and fibroblastic centers entrapping epithelial structures. It is often found in thyroids with lymphocytic thyroiditis. MFT has been proposed as a risk factor for papillary thyroid carcinoma (PTC) development by initiating localized injury and stromal response, eliciting epithelial entrapment, hyperplasia and reactive atypia, proceeding to malignant transformation of follicular cells. Since activating point mutations of *BRAF* genes are found in approximately 40% PTCs; we attempted to identify whether MFT showed such molecular changes and could possibly be related to PTC.

Design: We identified 7 cases of PTC with MFT in our institutional pathology database and personal consult service of one of the authors (VAL) for the years 1999 to 2012. All available slides of each case were reviewed; areas of PTC, MFT, and normal tissue were selected for *BRAF* analysis. The areas of interest in the paraffin embedded formalin-fixed tissue were macro-dissected and DNA was extracted using institutional protocols. The extracted genomic DNA was amplified with primers which target a 122 base pair region of the *BRAF* gene, including codon 600. Using pyrosequencing (PyroMark Q24), the PCR product was evaluated by two sequencing primers for codon 600 mutations which were designed to detect the three most common *BRAF* mutations in codon 600 including V600E, V600K and V600R. We evaluated each of the specimen pyrograms for presence or absence of mutation.

Results: Each case had at least one area each of PTC, MFT, and normal thyroid tissue which was selected for macro-dissection and *BRAF* analysis. All of the MFT lesions and normal thyroid tissue were negative for *BRAF* mutations. Of the 7 PTCs analyzed, 5 (61%) were negative for *BRAF* mutations, while 2 cases were positive.

Conclusions: In our study, none of the MFT lesions harbored *BRAF* mutations whereas 39% (2 of 7) PTCs in the same gland were either positive. Hence in this small study, we found no evidence that the MFT lesion is a direct precursor to PTC. It is likely an incidental bystander in the process and a reflection of the background thyroiditis.

549 Immunohistochemical (IHC) Detection of Mutated BrafV600E along the Spectrum of Thyroid Carcinoma (TC) Progression

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Background: Recently, the mutated BRAFV600E protein has been specifically detected in papillary thyroid carcinoma (PTC) using IHC. (Am J Surg Pathol 36: 844, 2012). Questions have also been raised about whether BRAFV600E is a clonal or subclonal event in TC. Our aims were to 1) compare BRAF V600E IHC expression in PTC, poorly differentiated (PDTC) and anaplastic TC (ATC) with mutation analysis 2) study the distribution of BRAF V600E expression within TC 3) correlate the IHC findings with the cytologic phenotype of PDTC.

Design: Whole sections and tissue microarray from 31 PTC, 38 PDTC and 22 ATC were subjected to both mass spectrometry genotyping for BRAFV600E mutation as well as IHC using the antibody VE1 directed against the V600E BRAF protein.

Staining intensity was scored as 0 (no staining), 1+ (faint), 2+ (moderate) and 3+ (strong). Homogeneous expression was defined as analogous labeling intensity in ≥ 80% of the tumor cells.
Results: 15 of 31 (48%) PTC showed strong (3+) IHC staining and BRAFV600E mutation while the remaining 16 (52%) showed 0/1+ staining and no BRAF mutations. 33 of the 38 (87%) PDTC showed0/1+ staining and no BRAF mutations while 5 (13%) contained BRAF mutations with a staining intensity of 2+ (2 cases) and 3+ (3 samples). All 12 PDTC with an exclusively follicular/Hurthle phenotype lacked BRAF mutations and showed 0/1+ staining. 5 of the 24 (21%) PDTC with PTC phenotype harbored BRAF mutations with a 2+/3+ IHC labeling. All 14 ATC with a staining intensity of 3+ harbored BRAF V600E mutations while the 2 ATC with 0/1+ staining lacked BRAF mutations. Six ATC showed a staining of 2+ including 5 with a high background staining. Of those 6 cases, BRAFV600E mutation was present only in the tumor without background. In all 10 ATC with associated PTC, both components of the tumor showed BRAFV600E mutation and strong expression. Homogeneous staining was found in 15 of 16 classical PTC (94%), 34 of 35 (97%) PDTC and 18 of 21 ATC (86%).
Conclusions: 1) Absent/faint staining for the VE1 antibody correlates perfectly with lack of BRAFV600E mutation while strong staining is always synonymous of BRAFV600E mutation in PTC, PDTC and ATC. Moderate staining intensity cannot be relied upon and should lead to genotypic analysis. 2) BRAFV600E expression correlates with PDTC and ATC derived from PTC. 3) Homogenous staining occurs in the vast majority of cases indicating that BRAFV600E mutation is a clonal event in TC.

550 Pathologic and Molecular Characteristics of Papillary Thyroid Carcinoma in Pediatric Patients and Young Adults

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Background: *BRAF* V600E mutation is the most frequent genetic abnormality detected in PTC (~40-70%). However, its incidence and significance in PTC in younger patients is unknown.
Design: Since routine testing for *BRAF* V600E of PTC was started at our institution, 16 PTC were identified in pediatric patients (age ≤18 years), and 43 PTC were identified in young adults (age 19 to 29 years). The pathologic characteristics and *BRAF* V600E mutation status were reviewed.
Results: The age range of the pediatric group included patients 11 to 18 years, whereas the young adult group included patients 19-29 years. The PTC tumors in the pediatric group included 12 classic type (75%) and 4 follicular variant (25%). The PTC tumors in the young adult group included 28 classic type (65%), 7 follicular variant (16%), 3 diffuse sclerosing type (7%), 2 subcapsular sclerosing type (5%) with one oncocytic variant (2%), one Warthin-like variant(2%), and one capsular variant (2%). The pathologic results are summarized in the table. *BRAF* V600E mutation was found in 6/16 (38%) of the pediatric tumors and 24/43 (67%) of the young adult tumors.

Pathologic Characteristics in Pediatric and Young Adult PTC

Age Group	F:M Ratio	Average Tumor Size	Multifocal	LVI	Pos LN	ETE	CLT
≤18 (n=16)	2.2:1 (11:5)	1.7 cm	44%(7)	38% (6)	53%(8)	19%(3)	63%(10)
19-29 (n=43)	6.2:1 (37:6)	2 cm	44%(19)	44%(19)	60%(26)	26%(11)	37%(16)

LVI: lymphovascular invasion, Pos LN: any positive lymph nodes, ETE: Extrathyroidal extension, CLT: chronic lymphocytic thyroiditis or Hashimoto thyroiditis
Conclusions: PTC in the pediatric poulation seems to be different from PTC in young adults in terms of gender distribution and frequency of *BRAF* V600E mutation.

551 Molecular Alterations in Partially-Encapsulated/Well-Circumscribed Follicular Variant of Papillary Thyroid Carcinoma

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Background: Recent studies have described encapsulated and infiltrative forms of follicular variant of papillary thyroid carcinoma (FVPTC). Encapsulated tumors have been reported to have virtually no metastatic potential or recurrence risk and to harbor *RAS* mutations but not *BRAF* mutations. In contrast, infiltrative tumors have significant metastatic potential, a risk of recurrence, and a *BRAF* mutation frequency of approximately 25%. In our experience, a substantial number of FVPTCs are neither fully encapsulated nor infiltrative, but instead are partially-encapsulated (PE) or well-circumscribed (WC). We have previously reported that PE/WC FVPTCs behave in an indolent fashion similar to encapsulated tumors. The purpose of the current study was to evaluate the molecular alterations in PE/WC FVPTC.
Design: We identified 28 PE/WC FVPTCs resected consecutively at our institution between 2005 and 2006. The following pathologic and clinical parameters were recorded: patient age, gender, resection type, tumor size, extrathyroidal extension, margin status, lymphovascular invasion (LVI), lymph node (LN) status, radioactive iodine (RAI) treatment, and development of recurrences. All tumor slides were reviewed. DNA was extracted from formalin-fixed paraffin embedded blocks. Targeted mutation analysis of 41 genes including members of the RAS and RAF families was performed using single-base extension chemistry and mass spectrometry (Sequenom).
Results: The PE/WC tumors were from 23 women and 5 men, with a mean age at resection of 49 years (range 24-75 years). Tumor size ranged from 1 to 7.6 cm (mean 2.4 cm). Extrathyroidal extension and LVI were absent and surgical resection margins were negative in all cases. LN metastases were absent in all cases of PE/WC tumors with sampled lymph nodes. RAI was given to 14 (50%) patients. No patients developed tumor recurrences (mean follow-up time 63 months). Overall, 13 (46%) cases harbored *RAS* mutations, including 7 (25%) with *NRAS* mutations (p.Gln61Arg), and 6 (21%) with *HRAS* mutations (5 had p.Gln61Arg and 1 had a p.Gln61Lys). No tumors had *BRAF* mutations.
Conclusions: Our results confirm our previous finding that PE/WC FVPTCs pursue an indolent clinical course similar to encapsulated FVPTCs. Additionally, we found that PE/WC tumors have a similar molecular profile to encapsulated FVPTCs, with frequent *RAS* mutations (46%) and no *BRAF* mutations. These molecular results provide further evidence that PE/WC and encapsulated FVPTCs are biologically similar and should be distinguished from more aggressive infiltrative FVPTCs.

552 Morphological and Stem Cell-Like Features Predictive of Stage in Medullary Thyroid Carcinoma

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Background: Histological and biological features predictive of staging in medullary thyroid carcinomas remain unknown and can serve as the basis of a grading system in these neoplasms.
Design: We analyzed primary and secondary growth patterns (tubulo-papillary, nested-trabecular, nodular-solid, diffuse), nuclear grade (nuclear abnormalities, including chromatin, nucleolus, pleomorphism and anisokaryosis), stromal reaction, and confluent necrosis in C-cell hyperplasias (CCH, 18), and medullary thyroid carcinomas (MTC, 46) (WHO criteria). Representative samples were evaluated by quantitative RT-PCR and standard in situ techniques for stress-stem cell pathways (telomere PNA-FISH, TERT, TP63, ATF2, BMP4, PTCH1, CCND1, FN1, F13A, CXCR3, MMP10, OCT4, KITLG, MYC, JUN, and FOS), proliferation (Ki-67) and apoptosis (TUNEL assay). Appropriate controls were run. Fisher's exact tests and analysis of variance (significant if $P<0.05$) were used for comparison; significant variables were then selected for discriminant analysis with cross-validation for histological diagnosis, RET genotype, sporadic/familial neoplasms and tumor stage (extrathyroidal 4, lymph node mets 37).
Results: No extrathyroidal extension or lymph node metastasis was observed in the absence of stromal reaction (10/46), lack of necrosis (43/46) or distrophic calcification (39/46) or the presence of pushing edges (21/46) or euchromatin. These aggressive features correlated with the expression of stem cell-like features (TERT, TP63, CD133) that correlated inversely with the presence of apoptotic cells at the tumor periphery and positively with the lack of stromal reaction. TERT, TP63 and CD133 expression correlated positively with ATF2, CXCR3, MYC, FOS, CCND1 (cyclin D1), and FN1 (fibronectin). All other genes showed no statistically significant differences by histological subtypes and no significant differences were observed for each diagnostic group by the RET genotype.
Conclusions: Locally advanced staging in MTC is defined by expression of stem cell-like (PI3 kinase/AKT -cyclin D1, fibronectin- and NFkB -TERT- pathways) and morphological features (stromal reaction, necrosis, tumor edges, chromatin and dystrophic calcification) that would represent the bases for MTC grading. This grading is independent of RET genotype.

553 Combination of HMGA2 and IMP3 Relative Quantitative RT-PCR Can Improve the Diagnostic Utility in Thyroid Neoplasms

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Background: The distinction between benign and malignant thyroid tumors in some cytological and histological specimens remains challenging. High Mobility Group A2 (HMGA2) and insulin-like growth factor II mRNA binding protein-3 (IMP3) expression analyzed by relative quantitative RT-PCR (qRT-PCR) have been reported to distinguish benign from malignant thyroid tumors with high sensitivity and specificity. We evaluated the diagnostic utility of HMGA2 and IMP3 expression levels, individually and in combination, in thyroid tumors.
Design: 120 thyroid fine needle aspiration (FNA) specimens (10 hyperplastic, 4 Hashimoto's thyroiditis (HT), 20 follicular adenomas (FA), 22 Hürthle cell adenomas (HA), 24 classical papillary thyroid carcinomas (PTC), 10 follicular variant PTC (FVPTC), 17 follicular thyroid carcinomas (FTC), and 13 Hürthle cell carcinomas (HC)) and 80 corresponding formalin-fixed paraffin-embedded (FFPE) tissues (9 normal thyroids, 11 hyperplastic, 2 HT, 12 FA, 14 HA, 10 PTC, 5 FVPTC, 6 FTC, and 11 HC) were included in this study. HMGA2 and IMP3 qRT-PCR were performed on a LightCycler 480. HMGA2 and IMP3 mRNA levels were expressed as fold change compared to normal thyroid level. The qRT-PCR results were evaluated with HMGA2, IMP3 or HMGA2 + IMP3 (HMGA2 positive and /or IMP3 positive) separately. The test sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were analyzed.
Results: HMGA2 and IMP3 expression in FNAs was consistently higher in thyroid malignancies compared to benign samples in all subgroups except HA and HC. After exclusion of HA and HC, the sensitivity was 90.2% (HMGA2) and 88.2% (IMP3); the specificity was 97.1% (HMGA2) and 79.4% (IMP3). Combining HMGA2 and IMP3 qRT-PCR data generated 98% sensitivity and 96.4% NPV. QRT-PCR data showed similar results in FFPE tissues: sensitivity was 84.2% (HMGA2), 85.7% (IMP3) and 94.7% (HMGA2 + IMP3); specificity was 96.9% (HMGA2), 91.2% (IMP3) and 90.6% (HMGA2 + IMP3). Combining HMGA2 + IMP3 data also increased the sensitivity to 94.7% and NPV to 96.7%. QRT-PCR data were concordant between FNA and FFPE samples for HMGA2 (97.4%) and IMP3 (96.9%).
Conclusions: HMGA2 and IMP3 expression by qRT-PCR analysis may be a useful ancillary technique to assist in the classification of difficult thyroid specimens, excluding Hürthle cell lesions. Combination of HMGA2 and IMP3 qRT-PCR model can increase the sensitivity and NPV and may be especially useful in screening thyroid cytology specimens.

554 Achaete-Scute Homolog-1 (ASH1) as a Marker of Poorly Differentiated Neuroendocrine Carcinomas of Different Sites: A Validation Study Using Immunohistochemistry and Quantitative RT-PCR on 335 Cases

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Background: Neuroendocrine carcinomas (NECs) show overlapping morphologic and immunohistochemical features independently of the site of origin, so the identification of the primary site, when diagnosed as metastases of unknown origin, may be problematic. The distinction of NECs from differentiated neuroendocrine tumors (NETs) is generally easy on morphological ground in surgical material, but it may be difficult on small biopsy specimens. The diagnostic usefulness of different transcription factors, including TTF1 and CDX2, as site-specific markers or as discriminating markers between NECs and NETs has been studied with results not conclusive and sometimes contradictory. In this respect, the role of the transcription factor ASH1, known to be involved in neuroendocrine cell differentiation of the lung, has been poorly investigated.

Design: We investigated using immunohistochemistry and quantitative RT-PCR the expression of ASH1 in 335 neuroendocrine neoplasms of different sites to check its possible utility as a diagnostic marker. 194 NECs (34 lung, 12 head and neck, 83 digestive, 42 urogenital, and 23 skin cases) and 141 NETs (107 lung, 24 gut, and 10 pancreatic) were studied.

Results: High concordance between immunohistochemical and molecular findings was observed. ASH1 expression was identified in 28/34 (82%) lung NECs, but also in 65/160 (41%) NECs elsewhere located, including gastric, colonic, prostatic, urinary bladder and skin NECs. The sensitivity and specificity of ASH1 in identifying lung NECs were 83.2% and 59.4%, respectively. ASH1 was not detected in any gastroenteropancreatic NET but was found in a minor population of tumor cells of 11/107 (10%) lung carcinoids. The sensitivity and specificity of ASH1 in distinguishing lung NECs from NETs were 82.4% and 89.7%, respectively. The sensitivity and specificity of ASH1 in distinguishing extrapulmonary NECs from NETs were 40.6% and 100%, respectively.

Conclusions: Our data suggest that ASH1 is not a site-specific marker to identify lung NECs. However, ASH1 may be proposed as a diagnostic marker of poor differentiation and can be used to differentiate NECs from NETs in difficult cases.

555 Usefulness of Immunohistochemistry for the Detection of BRAFV600E Mutation in Papillary Thyroid Carcinoma. Comparison with Three Molecular Biology Methods (Dideoxy Sequencing, Pyrosequencing and SNaPshot)

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Background: Several molecular biology technologies are available for the detection of BRAFV600E mutation in papillary thyroid carcinoma (PTC). The use of immunohistochemistry (IHC) as an alternative approach of these technologies for detection of BRAFV600E in PTC has not been evaluated until now.

Design: BRAFV600E mutation was analyzed by dideoxy sequencing, pyrosequencing and SNaPshot methods in 198 frozen PTC samples. Detection of the BRAFV600E mutation was performed by IHC using the VE1 clone antibody and compared to results of molecular methodologies. Consensus mutation was used as a reference test to calculate each parameter. Consensus mutations were defined as those detected at two or more of the four methods.

Results: 150/198 (75%) of the conventional PTC harbored a BRAFV600E mutation when using the SNaPshot and Pyrosequencing. 141/198 (71%) of the PTC showed a BRAFV600E mutation using dideoxy sequencing. IHC VE1 assay was positive in 145/150 (96%) BRAFV600E mutated tumors.

Conclusions: IHC using the VE1 clone is a specific and sensitive method for the detection of BRAFV600E and may be an alternative to molecular biology for detection of mutations in PTC.

556 PHH3, Ki-67 and Mitotic Count Correlation in Adrenal Cortical Carcinoma

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Background: Adrenal cortical carcinoma is an aggressive, uncommon neoplasm and the diagnosis can be challenging in small lesions confined to the adrenal gland. Scoring methods utilizing criteria such as nuclear grade, mitotic count, growth pattern, necrosis and vascular/capsular invasion have been proposed to aid in the diagnosis. Ki-67 proliferation index of over 5% favors a malignant diagnosis. Phosphohistone H3 (pHH3) is an immunohistochemical marker recently used to evaluate the proliferation index of tumors such as melanoma and urologic malignancies. In this study, we evaluate the correlation between PHH3, Ki-67 and mitotic count in adrenal cortical carcinomas to assess its utility in the evaluation of these cases.

Design: A database search for cases of adrenal cortical carcinoma was performed (1991-2010). On each case, a hematoxylin and eosin (H&E) stained slide was obtained and immunohistochemical (IHC) stains for Ki-67 (clone MIB-1; 1:20 dilution, Dako M7240) and pHH3 (polyclonal; 1:25 dilution, Cell Marque 369A) were performed. Mitotic count was performed on 50 high-power fields (HPF) on the H&E slides. IHC slides were analyzed by digital image analysis software (InForm, Caliper Life Sciences) and percent nuclear positivity was recorded. Pearson test of correlation significance was performed using the R statistical package.

Results: Fifteen cases (n=15) were analyzed in this study (14 primary tumors and one metastatic tumor). Patient age ranged from 29 to 77 years (mean 51 years). The mitotic count ranged from 5 to 73 mitosis per 50 HPF (mean 26 mitosis/50 HPF). pHH3 positivity ranged from 0.1% to 4.2% (mean 1.2%) and Ki-67 positivity ranged from 0.94% to 76% (mean 13.2%). pHH3 IHC positivity was seen only in nuclei of cells in M phase of the cell cycle, while Ki-67 positivity was seen also in cells not actively undergoing mitosis. Statistical analysis revealed a significant correlation between mitosic count and pHH3 positivity (0.67; 95% CI 0.27-0.88) (p=0.006) and between pHH3 positivity and Ki-67 positivity (0.70; 95% CI 0.29-0.89) (p=0.004). No statistically significant correlation was noted between mitotic count and Ki-67 positivity (0.33; 95% CI 0.21-0.72) (p=0.223).

Conclusions: Our study shows a direct correlation between mitotic count and pHH3 positivity. Since mitotic count is one of the main criteria for diagnosing adrenal cortical carcinoma, these findings support the use of pHH3 IHC in the evaluation of adrenal cortical tumors, in particular the more challenging cases. Furthermore, the use of pHH3 IHC facilitates the mitotic count in these cases.

557 Correlation of Immnunostains for Protein Expression with Genetic Abnormality of SDHB in Paraganglioma/Pheochromcytoma

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Background: Pheochromocytomas (PCC) and paragangliomas (PGL) are rare tumors of chromaffin tissue in adrenal medulla and extraadrenal ganglia. Ten susceptibility genes are reported, accounting for ~50% of the cases. The loss-of-function mutations of any of the 3 subunits of succinate dehydrogenase (SDHD, SDHC, and SDHB) have been recently associated with PCC/PGL by potentially activating the hypoxic gene response pathway. Immunostaining analysis may be able to identify tumors with mutations of SDH complex, triage patients for genetic testing, and potentially for treatment follow-up.

Design: We selected a group of PCC/PGL patients who underwent surgical resections and genetic testing at our institution from 1999 to 2012. Immunostains of SDHB were performed on paraffin-embedded tissue sections. The staining results were first evaluated blind to the genetic testing results, and the two sets of results were compared. The patient group (n=27; 10 females and 17 males) had a wide age range (22 y/o to 77 y/o) at the first diagnosis.

Results: Among the 20 patients with no abnormality of the SDHB gene (neither mutation nor deletion of SDHB gene, and documented alternative gene abnormality, *e.g*. VHL gene), 9 were stained positive for SDHB. All 7 patients with documented SDHB gene abnormality stained negative for SDHB for a specificity of 100%. Thus the positive predictive value of positive SDHB immunostain for no genetic abnormality is also 100%.

Conclusions: Our data suggests that intact staining for SDHB indicates a lack of mutation in the SDH family. Loss of SDH in the absence of documented SDHB mutation may suggest that another member of the SDH family is mutated or lost.

558 TROP2 Expression in Various Tumors – A Potential Diagnostic Marker for Papillary Thyroid Carcinoma

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Background: TROP2, a type 1 transmembrane protein, is encoded by tumor-associated calcium signal transducer 2 (TACSTD2), a member TACSTD gene family. TROP2 overexpression has been reported in some carcinomas (CA), including colorectal, gastric, oral and pancreatic CA. More recently, it has been actively studied as a prognostic marker. TROP2 expression by immunohistochemical analysis has not been extensively evaluated. In the current study, we immunohistochemically evaluated the expression of TROP2 in a large series of carcinomas from various organs using a Ventana staining system.

Design: Immunohistochemical evaluation of TROP2 (Santa Cruz Biotechnology, Inc., sc-376181) expression on TMA sections of 1,234 cases of carcinomas from various organs was performed. The staining intensity (membranous staining) was graded as weak or strong. The distribution was recorded as negative, 1+ to 4+.

Results: TROP2 expression was not identified in neuroendocrine tumors of pancreas and lung (N=78), testicular tumors (N=103), gastric adenocarcinoma (ADC, N=21), hepatocellular CA (N=18), invasive lobular CA of breast (N=31) or gastrointestinal stromal tumor (N=36). The expression of TROP2 on tumors from kidney, bladder, thyroid, breast, gynecologic, and gastrointestinal system is summarized in Table 1.

Table 1. Summary of TROP2 expression in Various CA

Diagnosis (N)	1+ (N)	2+ (N)	3+ (N)	4+ (N)	Percentage (%)
Prostatic ADC (97)	25	29	26	7	90%
Clear Cell RCC (82)	0	2	0	0	3%
ChRCC, Oncocytoma (30)	0	1	0	0	3%
Papillary RCC (18)	1	2	1	4	44%
PTC (48)	5	10	18	10	90%
Follicular CA (37) and adenoma (51)	3	0	0	0	3%
Inv. UCA (43)	4	17	4	0	58%
LGPUCA (38)	1	2	12	23	100%
Esophageal CA (30)	4	1	1	0	20%
Colonic ADC (43)	2	0	0	0	5%
Pancreatic ADC (98)	8	10	1	1	20%
Endometial ADC (131)	28	32	15	4	60%
Endocervical ADC (25)	0	8	0	0	32%
Ovarian papillary serous CA (41)	6	9	4	0	46%
ADC of lung (44)	10	5	3	0	41%
Squamous CA of lung (49)	1	15	5	1	45%
Inv. Ductal CA of breast (110)	10	12	15	4	37%

N-number of cases; CC:renal cell CA; ChRCC:chromophobe RCC; PTC:papillary thyroid CA; Inv. UCA:invasive urothelial CA; LGPUCA:non-invasive low grade papillary urothelial carcinoma

Conclusions: Our data suggest the potential diagnostic utility of TROP2 in: 1) differentiating PTC from follicular neoplasms (FA and FC); 2) suggesting PRCC in renal tumors if positive; 3) suggesting ductal CA over lobular CA if positive; 4) if diffusely

positive in urothelial CA, likely LGPUCA. Weak or negative expression in invasive UCA suggests that TROP2 may play a role in tumor progression.

559 MicroRNA Are Differentially Expressed in Appendiceal Versus Ileal Neuroendocrine Tumors

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Background: Gastrointestinal neuroendocrine tumors (NETs) comprise a heterogenous group of neoplasms with a varying spectrum of aggressiveness. Ileal NETs have greater metastatic potential than appendiceal carcinoids. The role of microRNAs (miRNAs) in tumor growth and progression is an active area of ongoing research; however, miRNA profiles of gastrointestinal NETs have not as yet been elucidated. The goal of this study was to compare the expression of miRNAs in ileal versus appendiceal NETs.

Design: Formalin fixed paraffin embedded tissues from 4 primary ileal NETs and 4 appendiceal NETs were retrieved from the archives of Rhode Island Hospital. The tumor cells were microdissected from 10 mm sections using Arcturus Laser Capture Microdissection. MiRNA was isolated and profiled using Taqman Human miRNA Array cards containing probes for 380 human miRNAs. Bioinformatic mRNA targets of miRNA were generated by Ingenuity Pathway Analysis (IPA). The miRNA candidates were further confirmed using individual miRNA quantitative RT-PCR assays on a new set of 10 ileal and 5 appendiceal NETs. The DAVID web program was used to analyze the functionality of gene groups.

Results: A total of 31 miRNAs were differentially expressed in 4 primary ileal NETs as opposed to 4 appendiceal NETs ($P<0.05$). All these miRNAs were up-regulated in ileal NETs as compared to appendiceal NETs. In search of their targets, IPA analysis identified 55 mRNAs, each one targeted by 4 or more of 9 miRNAs: miR-194, miR-218, miR-24, miR-30c, miR-26a, miR-28-3p, miR-574-3p, miR-320d, and miR-342-3p. Eight of these 9 miRNAs (except miR-320d) were further validated using quantitative RT-PCR in the same set of tumors studied by the Array cards and in an additional 10 ileal and 5 appendiceal NETs (fold changes from 4.8 to 27.2). Bioinformatic analysis (DAVID) of the 55 mRNAs revealed gene products with functions related to cellular signaling, growth factor binding, ion transportation, and cellular biosynthetic process, and neuron morphogenesis and differentiation.

Conclusions: This study is the first to compare the miRNA profile of ileal and appendiceal NETs. We identified and validated the differential expression of eight miRNAs in ileal as opposed to appendiceal NETs. Further studies will be needed to investigate the role of these novel miRNAs in the biological behavior of ileal NETs.

560 Spindle Cell Oncocytomas and Granular Cell Tumors of the Pituitary Are Variants of Pituicytoma

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Background: Sustentacular cells of the neurohypophysis, pituicytes, are specialized glia that have five distinct ultrastructural variants: light, dark, granular, ependymal and oncocytic. Pituicytomas are rare neoplasms arising from pituicytes. Granular cell tumors of the sella are unique neoplasms of the neurohypophysis that have also been suggested to arise from pituicytes. Spindle cell oncocytomas of the pituitary are considered to arise from folliculostellate cells, sustentacular cells of the adenohypophysis. Recent data, however, suggest that while pituicytes, pituicytomas, spindle cell oncocytomas and granular cell tumors are positive for TTF-1, folliculostellate cells are negative for TTF-1. We investigated the genetic, proteomic, and ultrastructural features of these neoplasms in order to refine the classification of these neoplasms.

Design: We collected 7 spindle cell oncocytomas, 4 pituicytomas and 3 granular cell tumors. Tumors were stained for GFAP, S100, olig2, IDH1, NF, vimentin, galectin-3, CD56, chromogranin-A, EMA, CAM5.2, CD68 and bcl-2. Five nontumorous adenohypophysial and five neurohypophysial tissues were also stained for TTF-1. The status of $BRAF^{V600E}$ mutation and BRAF-KIAA fusion was investigated in 12 cases using PCR with sequencing and FISH techniques, respectively. The ultrastructural features of 2 granular cell tumors, 2 pituicytomas and 4 spindle cell oncocytomas were also reviewed.

Results: TTF-1 was negative in all adenohypophysial tissues and was positive in pituicytes. All tumors were positive for TTF-1 and were negative for CAM5.2, IDH1 and NF. Vimentin, S100, galectin-3, EMA and CD68 were variably positive in the majority of these tumors. Olig2 was only positive in one pituicytoma. GFAP expression was seen in some granular cell tumors and pituicytomas. While granular cell tumors were negative for bcl-2 and CD56, pituicytomas and spindle cell oncocytomas showed variable positivity. No $BRAF^{V600E}$ mutation or BRAF-KIAA fusion was detected. The ultrastructural features of granular cell tumors and spindle cell oncocytomas revealed similarities with granular and oncocytic pituicytes, respectively.

Conclusions: Diffuse TTF-1 expression in pituicytes, pituicytomas, spindle cell oncocytomas and granular cell tumors indicates a common pituicyte-lineage. The ultrastructural variants of pituicytes are reflected in morphological variants of tumors arising from these cells. We propose the terminology "oncocytic pituicytomas" and "granular cell pituicytomas" to refine the classification of these lesions.

561 Extra-Adrenal Pheochromocytoma/Paraganglioma – A Clinicopathological Study

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Background: Criteria predicting malignant behavior in pheochromocytomas have been described. Extra-adrenal location (paragangliomas) is one of the features that predicts aggressiveness for pheochromocytoma. However, there are no established criteria for predicting clinical behavior in extra-adrenal paraganglioma. We studied a cohort of paragangliomas from different sites, to determine any pathologic features that may correlate with clinical outcome.

Design: 28 cases of extra-adrenal paraganglioma resected at our institution from 2000 to 2012 were retrieved from our surgical pathology files and form the basis of this study. Follow-up was obtained from the clinical records and outcomes were correlated with histopathological features of the tumors. Development of metastasis or local tumor recurrence was considered as "adverse outcome".

Results: Among the 28 cases, 16 patients were female and 12 male, with a median age of 41 years [range: 20 to 75]; tumors ranged in size from 1.3 to 9 cm, with the retroperitoneum [n=7] and carotid body [n=6] being the two most common sites. Over a median clinical follow-up of 24 months [range: 1 to 156 months], 13 tumors developed metastasis/recurrences. Of these 13, one case that recurred had family history of paraganglioma and was excluded from final analysis. Among morphologic features, only microscopic tumor necrosis ($p= 0.0237$) and tumor cell spindling ($p=0.0369$) showed significant association with adverse outcomes. None of the other morphologic features [vascular invasion, capsular invasion, presence of large nests or diffuse growth (in >10% of tumor volume), high cellularity, cellular monotony, increased mitotic figures (>3/10 HPF) or profound nuclear pleomorphism/multinucleation] showed significant association with outcome.

Conclusions: Nearly half of extra-adrenal paragangliomas in this series showed aggressive clinical behavior. Like pheochromocytoma, tumor necrosis and spindling in paragangliomas are associated with adverse clinical outcome. Unlike pheochromocytoma however, other morphologic features do not seem to predict aggressive behavior.

562 Differential Expression of the Epithelial-Mesenchymal Transition Regulator ZEB1 in Thyroid Carcinomas

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Background: The Zinc-finger E-box-binding homeobox1 (ZEB1) induces epithelial-mesenchymal transition (EMT) and down-regulates E-cadherin in epithelial cells. ZEB1 is regulated by transforming growth factor beta 1 (TGF Beta 1) and other signaling proteins. We examined the expression of ZEB1, E-cadherin, TGF Beta receptor 2 (TGFBetaR2), SMAD4 and SMAD7 in benign thyroid tissues, well differentiated and anaplastic thyroid carcinomas (ATC) by immunohistochemistry (IHC).

Design: Ten ATC, 32 follicular adenomas (FA), 28 follicular carcinomas (FTC), 57 papillary thyroid carcinomas (PTC) including 29 follicular variant of PTC, 10 multinodular goiters and 10 normal thyroids were used in a formalin-fixed paraffin-embedded tissue microarray (TMA) analysis. IHC was scored based on the percentage of cells stained. The expression of ZEB1 and E-cadherin were also analyzed by quantitative reverse transcriptase- polymerase chain reaction (QRT-PCR) in 3 cell lines- including a normal thyroid cell line, PTC and ATC cell lines.

Results: Most (6/10) ATC showed strong nuclear staining for ZEB1 while 3/10 ATC cases showed only focal staining for E- cadherin. One (1/28) FTC was positive for ZEB1, while all FTC were positive for E-cadherin. ZEB1 expression in ATC was significantly different from that in FTC ($p<0.001$). All cases of PTC, FA and nonneoplastic thyroids were negative for ZEB1, but positive for E-cadherin. Q RT-PCR showed the highest levels of ZEB1 mRNA expression in the ATC while E-cadherin was highest in the normal thyroid cell line.

Conclusions: The EMT regulator ZEB1 is expressed in ATC and is associated with loss of E-cadherin supporting the role of EMT in ATC. TGFBeta R2, SMAD4 and SMAD7 are expressed in benign thyroid tissues, well differentiated and ATC indicating preservation of TGFBeta1 signaling in thyroid carcinomas during EMT.

563 The Utility of Frozen Section in Surgical Management of Patients with Prior Indeterminate Thyroid FNA

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Background: Fine needle aspiration (FNA) is a safe and widely used diagnostic tool for evaluation of thyroid nodules, with a high accuracy in detection of papillary thyroid carcinoma (PTC). FNA results that fall into the atypical (Bethesda category III) or suspicious for PTC (Bethesda category V) remain a surgical dilemma. Intraoperative frozen section (FS) is often used to help guide the surgical choice of lobectomy vs total thyroidectomy. The accuracy of FS is limited due to sampling errors and freezing artifacts. We retrospectively reviewed cases of PTC diagnosed on thyroidectomy that had concurrent FS and FNA performed. Our goal was to assess the role of FS in patients with prior inconclusive or suspicious for PTC FNA diagnoses.

Design: We reviewed final surgical pathology (SP), FS and prior FNA results in cases diagnosed as PTC on resection specimens from 2008 to 2012. All FNA diagnoses were reclassified according The Bethesda System. Discrepancies in the SP, FS and FNA diagnoses were analyzed.

Results: 56 patients were included in the study.

FS↓ \ FNA→	I Non-diagnostic	II. Benign	III. AUS	IV. Suspicious for FN	V. Suspicious for PTC	VI. Malignant	Total
Benign	1	5	5	1	1	0	13
Atypical/Inclonclusive	0	1	2	0	0	1	4
Follicular Lesion	1	3	1	3	3	1	12
Malignant	0	0	3	4	5	15	27
Total	2	9	11	8	9	17	56

In our institution FS was concordant with the SP diagnosis in 27% of AUS cases. In the suspicious for PTC category, 55% of patients underwent total thyroidectomy based on the FS diagnosis. Eight cases were misclassified on FNA as suspicious for FN, and 50% of these were called PTC on frozen section, requiring completion thyroidectomy.

Conclusions: FS has limited utility in intraoperative management of patient with prior AUS FNA. This can be explained by a 5-15% malignancy risk and that AUS represents a heterogeneous category, with subtle cytologic findings, which may not be appreciated on FS. The malignancy risk in suspicious for PTC is 60-75%, on FS only 50% of cases were correctly diagnosed. Follicular variant of PTC can be difficult to diagnose and may be misinterpreted as a FN on FNA. In our study, 50% of patients were diagnosed correctly as PTC, indicating that this category is diagnostically challenging. FS has limited utility in intraoperative management of patient with prior AUS FNA. This can be explained by a 5-15% malignancy risk and that AUS represents a heterogeneous category, with often subtle cytologic findings, which may not be appreciated on FS. While the malignancy risk in suspicious for PTC is 60-75%, on FS only 50% of cases were correctly diagnosed.

564 Whole Genome and Targeted Next Generation Sequencing in Thyroid Cancer

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Background: Thyroid cancer is the most common type of endocrine malignancy. Somatic mutations in BRAF, RAS, RET/PTC, and PAX8/PPARg are the most common genetic alterations in thyroid cancer and important in diagnostics and prognostication. However, around 30% of thyroid tumors do not harbor any known mutations and another 10% carry rare type of mutations which is difficult to detect using conventional techniques. Next generation sequencing can be used for discovery of novel genetic alterations and for cost-efficient detection of rare mutation types, and both of these approaches were evaluated in thyroid cancer in this study.

Design: Nine mutation-negative papillary thyroid carcinomas (PTC) and corresponding normal thyroid samples were studied using whole genome sequencing (WGS) or whole transcriptome sequencing (RNA-Seq) on the Illumina HiSeq2000. In addition, 60 thyroid samples including 25 PTC, 2 follicular carcinomas (FC), 6 anaplastic carcinomas (AC), 3 normal tissues and 24 FNA samples were analyzed for mutations in 739 hot spots of 46 cancer-related genes on Ion Torrent PGM (Life Technologies).

Results: WGS had an average coverage of 49 reads and yielded 3-19 nonsynonymous single nucleotide variations (SNV) and 3-35 structural variations (SV) per tumor. One metastatic FVPTC tumor revealed a fusion between *TMP3* and *NTRK1* with a novel breakpoint, and other tumors revealed several novel interchromosomal rearrangements and promising SNVs. Targeted NGS on Ion Torrent allowed to test all samples for extensive number of mutations using small amount of DNA (10 ng) in a cost-efficient way by barcoding 8-10 samples per run. The platform detected all common point mutations (BRAF, RAS) and rare mutation types including RET M918T, KRAS Q61R, PIK3CA E707K TP53 L194R, ATM V410A, and MET E168D in thyroid surgical and FNA specimens.

Conclusions: Whole genome sequencing and RNA-Seq analysis of PTC revealed a *TPM3/NTRK1* fusion with a novel breakpoint, as well as several promising novel chromosomal rearrangement and SNVs, which may allow better characterization of the biology and clinical behavior of PTC. Targeted NGS on Ion Torrent platform allows testing for a broad spectrum of mutations and may further improve the diagnostic accuracy of molecular analysis in thyroid surgical and FNA samples.

565 Application of Mitosis-Specific Antibody Phospho-Histone H3 in Neuroendocrine Tumors

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Background: Mitotic count provides an important parameter for grading and prognostication in a variety of tumors, including neuroendocrine tumors. For instance, in WHO classification, mitotic count, along with necrosis or Ki67, defines grading in neuroendocrine tumors of lung and GI tract. However, mitotic figure can be difficult to detect due to crush artifact, inappropriate fixation, presence of apoptosis and cellular degeneration, as is often the case in neuroendocrine tumors. Phospho-histone H3 (PHH3) has been reported as a useful marker to assist mitotic figure counting, and this study has investigated the utility of this marker in neuroendocrine tumors.

Design: Anti-PHH3 antibody (clone Ser28) was applied to a total of 60 resected and biopsied tumors from lung, appendix, small intestine, rectum, pancreas, thyroid and parathyroid, including carcinoid/NET, medullary carcinoma, parathyroid adenoma/carcinoma, and large cell neuroendocrine carcinoma. Anti-PHH3 and H&E were stained on consecutive sections, and mitotic figures on H&E and PHH3 stained sections were counted in 10 high power fields (x400) in the same area.

Results: Mitotic count on H&E and PHH3-staining, each designated as MC and PC, ranged from 0 to 21 and from 0 to 34, with average MC and PC of 1.725 and 4.925, respectively. Discrepancy between the two was mainly attributed to sensitivity of PHH3 staining, and poor fixation (usually in large tumor resection), degenerative change, and crush artifacts (particularly in biopsy) of the tumor.

Conclusions: PHH3 is a sensitive marker to detect mitotic figures in both resection and biopsy, with above-mentioned pitfalls, therefore mitotic count based on PHH3 staining would provide more accurate tumor grading.

566 A Study of Phox2b and Gata3 Expression in Tumors of Autonomic Nervous System Derivation

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Background: The sympathetic, parasympathetic and enteric ganglia, and adrenal medulla and extraadrenal paraganglia constitute the autonomic nervous system (ANS). Autonomic neurons and chromaffin cells are derived from a common progenitor from neural crest, and its development is controlled by a network of transcription factors (TFs), including the master regulator, Phox2b, and its downstream Gata3. Immunohistochemical expression of these two TFs has scarcely been studied in ANS-derived tumors.

Design: Anti-Phox2b and Gata-3 antibodies were applied to a total of 77 ANS tumors, including 35 paragangliomas, 21 pheochromocytomas, 9 neuroblastomas, 4 ganglioneuroblastomas, and 8 ganglioneuromas, as well as their potential morphologic mimickers, including 46 tumors of small round cell tumor group, such as olfactory neuroblastomas, rhabdomyosarcomas, germ cell tumors, Ewing sarcoma/PNET, and diffuse large B cell lymphomas, 127 neuroendocrine carcinomas of lung and GI (89 carcinoid tumors/NET, 11 large cell neuroendocrine carcinomas, and 27 small cell carcinomas), 26 Merkel cell carcinomas, 97 tumors of thyroid, parathyroid, and adrenal cortex, and 10 melanomas. The extent of staining was graded as focal (5-50%) and diffuse (50-100%).

Results: Gata3 expression was seen in 31/35 (89%) paragangliomas (25 diffuse, 6 focal), 20/21 (95%) pheochromocytomas (18 diffuse, 2 focal), and all neuroblastomas, ganglioneuroblastomas, and ganglioneuromas (all diffuse) while all other tumors were negative, except for 7 parathyroid tumors (2 carcinomas, 5 adenomas), which were all diffusely positive. Phox2b expression was seen in 14/35 (40%) paragangliomas (11 diffuse, 3 focal), none of pheochromocytomas, and all neuroblastomas, ganglioneuroblastomas, and ganglioneuromas (all diffuse except for 1 ganglioneuroma with focal staining) whereas all other tumors were negative.

Conclusions: Gata3 is a highly reliable marker for paragangliomas/pheochromocytomas and neuroblastic tumors to distinguish from their mimickers. This is an additional utility for this marker, which is used for the diagnosis of urothelial and mammary carcinomas. Phox2b is also highly specific, but its low sensitivity to paragangliomas/pheochromocytomas would limit the utility only to neuroblastic tumors.

567 Correlation between immunohistochemical Expressions of Somatostatin Receptor (SSTR)2a & mTOR and Clinical Response by Octreotide & Everolimus in Metastatic Pancreatic Neuroendocrine Tumors (PNET) in the Liver: A First Report on the Predictive Role of SSTR and mTOR in the Large Series of Referred Cases

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Background: Currently, for PNETs, targeted therapeutic approaches include somatostatin analogue(octreotide) and mTOR inhibitor(everolimus). This study is aimed at to elucidate the expression rate of SSTR2a,a target for octreotide in a large series of neuroendocrine tumors(NET) and to further elucidate the relationship between immunohistochemistry of SSTR2a & mTOR and clinical responses to octreotide & everolimus in the selected cases of metastatic PNETs in the liver. This is the first study on the predictive role of SSTR and mTOR.

Design: Total 269 referred cases of NET were immunohistochemically stained for SSTR2a(antibody supplied by EPITOMICS®). Among these, total 17 cases with metastatic PNETs in the liver were further subjected to immunohistochemical staining for phosphorylated(p)-mTOR(antibody supplied by Cell Signaling®), For SSTR2a, the interpretation was done according to Volante et al.(2007). The cytoplasmic p-mTOR was interpreted positive according to both positive areas(>1%) and intensity(1+,2+,3+). Clinical condition after the therapy was either (1)tumor shrunken,(2)stable disease(SD) or (3)tumor recurred or enlarged.

Results: Among 269 cases, SSTR2a was positive(score 2 and 3) in 61% of cases. The selected 17 PNET cases with metastases were treated with octreotide and/or everolimus according to the immunohistochemical expression of SSTR2a and p–mTOR. Among 7 cases with the tumors which were positive only for SSTR2a, clinical response was SD in 5 cases(71%). Two tumors were enlarged after the therapy. The other four cases were both positive for SSTR2a & p-mTOR, three of them were treated with both octreotide & everolimus. Two case showed SD(67%) and one recurred. Another case was treated with only everolimus and the response was SD. One case of PNET in VHL family with negative p-mTOR did not respond to everolimus.

Conclusions: Overall positive rate for SSTR2a was 61%. For the metastatic cases with positive for SSTR2a or positive for both SSTR2a & p-mTOR, clinical response with octreotide or octreotide & everolimus was 71% and 67% respectively. The p-mTOR negative VHL-related PNET was unresponsive to everolimus treatment. **This is the first report which suggests the predictive role of SSTR2a and p-mTOR detection in the targeted therapy for the metastatic PNET.**

568 BRAFV600E Mutation in Papillary Thyroid Carcinomas (PTC) Associated with Hashimoto Thyroiditis (HASHI): A Clinicopathological Correlation

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Background: The pathogenesis of PTC arising in HASHI is not well understood. HASHI has been suggested to be a premalignant counterpart of PTC. It has also been proposed that the intense lymphocytic response in HASHI may have a mitigating role in cancer progression. *BRAFV600E* mutation has emerged as a frequent genetic abnormality in PTC (upto 70% in our institution) and has been shown to be associated with aggressive pathologic features. We explore the incidence of *BRAFV600E* mutation in PTC associated with HASHI and correlate the findings with clinical and pathological features.

Design: 45 PTCs arising in the background of HASHI diagnosed between August 2011 and July 2012 that were analyzed for *BRAFV600E* mutation were correlated with their clinicopathological features. HASHI was diagnosed based on morphological criteria

that included moderate to severe chronic lymphocytic thyroiditis with germinal center formation, and follicular destruction with Hurthle cell metaplasia. *BRAFV600E* mutation was detected by single strand conformational polymorphism.

Results: Patients included 40 women and 5 men aged 18 to 85 years (median 42). Tumor size varied from 0.4 cm to 5 cm (median 1.1 cm). The histologic subtypes included 29 classic variants (63%), 11 follicular variants (24%), the rest included subcapsular sclerosing type and tall cell variants of PTC. Lymph nodes were examined in 42 patients (central neck dissections with additional lateral neck dissection in 5 patients). 19 of 42 cases (45%) showed metastatic disease, 14(33%) had pN1a stage disease and 5(12%) had pN1b stage disease. 6 tumors(13%) had extrathyroidal extension. *BRAFV600E* mutation was identified in 21 tumors (47%). Table 1 summarizes the clinocopathological characteristic of PTCs arising in HASHI. Median age and tumor size were significantly lower in the *BRAFV600E* mutation positive group ($p<0.05$).

Table 1

Clinicopathological features	BRAFV600E Positive (n=21; 47%)	BRAFV600E Negative (n=24; 53%)
Median age (range)	37 (18-59)	44 (21-85)
Gender (F:M)	18:3	22:2
Median size (range)	0.8 (0.5-3.2)	1.7 (0.4-5)
Extrathyroidal extension	2	4
Lymph nodes metastasis		
pN1a	9	5
pN1b	3	2

Conclusions: *BRAFV600E* [/italic]mutation in PTC associated with HASHI seems to be less frequent than in all other PTCs at our institution (47% vs 70%). *BRAFV600E* mutation positive PTCs with HASHI affected younger patients and were of smaller size, features generally associated with better outcome, compared to mutation negative PTCs with HASHI.

569 TGFß1 and Not TGFß2 Is Upregulated in Hashimoto Thyroiditis-Related Papillary Thyroid Carcinoma

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Background: TGFβ is a cytokine with 3 isoforms. It is known to function as both a tumor suppressor and tumor promoter, yet there is limited information about what factors are involved in the functional switch and when this switch occurs. Recent discoveries in immunology indicate that TGFβ is one of the key cytokines required for the differentiation of Type 17 T helper cells (TH17), which have a dominant role in mediating chronic inflammation. Interleukin 17 (IL-17), the signature cytokine of TH17 cells, has been shown to be present in the tumor microenvironment of various cancers. Depending on the cancer in question, IL-17 may be either protective or non-protective. Recent data also suggests that patients with Hashimoto thyroiditis (HT) have an increase in both pro-inflammatory cytokines and TH17 lymphocytes in the serum and thyroid tissue. As HT may be a risk factor for papillary thyroid cancer (PTC), the aim of this study was to evaluate the expression levels of key molecules related to the TH17 cells, including TGFβ, in patients with HT and PTC.

Design: Fresh snap frozen tissue samples from 47 patients (n=12 PTC with HT, n=12 PTC without HT, n=11 benign thyroid tissue with HT and n=12 benign thyroid tissue without HT) were processed and RNA extracted to generate cDNA. Quantitative PCR (qPCR) was used to determine expression levels for IL-17, IL-17F, Il-6, IL-22, RORC, TGFβ-1, TGFβ-2, IL-1β, Foxp3 and IL-23p19.

Results: In the PTC with HT group there was significant increase in the expression of TGFβ1 and Foxp3 versus the other samples ($p<0.05$). Additionally, TGFβ1 was significantly increased in the PTC samples as a whole and IL-23p19 was found to be significantly increased in the HT group.

Conclusions: Upregulation of TGFβ, particularly TGFβ1, may play an important role in the development of PTC in patients with HT, possibly by promoting differentiation of TH17 cells, with downstream immunomodulatory effects. The relationship between TGFβ upregulation and BRAF V600E mutation in these patients is currently unknown and is a direction of future studies.

570 Circulating Tumor Cells as Diagnostic Marker of Adrenocortical Carcinoma: Preliminary Results of a Single Center Study

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Background: Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy which gains access to the circulation very early during disease progression. Circulating tumor cells (CTC) detection may have enormous potential in establishing a diagnosis of malignancy, estimating prognosis and monitoring disease progression. To our knowledge, CTC detection rate in ACC patients is presently unknown.

Design: CTC analysis was carried out in 11 ACC and 10 adrenocortical adenoma (ACA) patients. Blood samples obtained before and after surgery were filtered on Screencell devices (Screencell®), polycarbonate membranes with 8 µm pores which isolate CTC by size. Filters and sections of the corresponding primary tumors were assessed immunohistochemically for adrenocortical markers (i.e. MART-1, alpha-inhibin and synaptophysin) and proliferation status (Ki67).

Results: CTC were seen in all ACC but not in ACA samples. Immunocytochemical analysis with MART-1, alpha-inhibin and synaptophysin identified circulating adrenocortical cells as weak to moderately stained cells. CTC numbers were significantly lower in the samples obtained after surgery (mean CTC number/blood ml±SD: before, 3.80±4.4 and after, 0.64±0.62, P=0.007). A statistically significant linear relationship was found between the number of CTC recovered after surgery and the tumor diameter (R=0.867, R^2=0.752, P=0.001) and Ki67 expression (R=0.795, R^2=0.632, P=0.007).

Conclusions: We identified CTC in the peripheral blood of ACC but not of ACA patients. This suggests that CTC may represent a promising diagnostic marker of malignancy. Considering that there is a reduction in the number of CTC isolated after surgery, CTC detection could also assist in estimating prognosis and monitoring treatment success in ACC patients.

571 A Review of Cystic Lesions of the Adrenal Gland: Our Experience over the Last 20 Years

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Background: Cystic lesions of the adrenal gland are uncommon, often presenting with non-specific clinical and radiologic findings, and are thus underrecognized. They are occasionally associated with malignant neoplasms, which can greatly mimic benign lesions and carry detrimental clinical consequences if misdiagnosed. A review is desired on the demographic, clinical, radiologic, gross and microscopic pathologic features, as well as the differential diagnoses of cystic adrenal lesions. Here we present our 20-year experience with these lesions at a large academic medical center.

Design: An extensive database search (1992-2012) for primary cystic adrenal lesions was performed. Macroscopic descriptions, available histologic and immunostain slides, and available radiologic records were reviewed for all included cases.

Results: Among over 4,500 adrenal gland specimens microscopically examined, 31 cases of adrenal lesions with predominant cystic components were identified in 30 patients, summarized in Table 1. The patients ranged in age from 34 to 86 years (median, 55.5) with a male to female ratio of 13:17. Radiologic studies and gross examination correlated well: 20 (64%) of these lesions were almost entirely cystic, 8 (26%) exhibited accompanying solid components, and the remaining 3 (10%) had significant cystic degeneration. In addition, hemorrhage (26 cases, 84%) and encapsulation (24 cases, 77%) appeared to be non-specific radiologic/gross features shared across histologic subtypes. Microscopic review identified 2 cases (6%) as malignant neoplasms (1 adrenocortical carcinoma, 1 epithelioid angiosarcoma); the remaining 29 (94%) were benign lesions. Radiologic impression and histopathologic diagnosis were concordant in 11 of the 15 cases (73%) for which radiologic records were available.

Conclusions: This study represents the second largest case series to date on cystic adrenal lesions, summarizing their most updated clinicopathologic data, and presents a comprehensive review on their differential diagnoses.

Table 1

Subtypes of adrenal cysts	Subtotal	Benign	Malignant
Pseudocysts (no inner wall lining)	12 (39%)	12	0
Epithelial-derived	17 (56%)	16 (pheochromocytomas)	1 (adrenocortical carcinoma)
Endothelial-derived	2 (6%)	1	1 (angiosarcoma)
Total	31 (100%)	29 (93.6%)	2 (6.4%)

572 DNA Methylation Profiling Identifies Different Prognostic Clusters of Pancreatic NeuroEndocrine Tumors

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Background: Pancreatic NeuroEndocrine Tumors (PanNETs) are rare and heterogeneous neoplasms accounting for about 1-2% of pancreatic tumors. To date, very few studies have been published about DNA hypermethylation in such tumors with a limited number of genes analyzed and there is no information about distinct hypermethylation profiles of PanNETs.

Design: We investigated the epigenetic profile of 58 morphologically and immunohistochemically well characterized PanNETs analyzing the hypermethylation status of 33 tumor suppressor genes using MS-MLPA technique and bisulphite pyrosequencing. The aims of the study were to evaluate the frequency of aberrant DNA methylation in PanNETs and the occurrence of specific methylation profiles with respect to the morphofunctional and clinical profile of each tumor. Unsupervised hierarchical cluster analysis was performed using Jaccard index as dissimilarity measure.

Results: A subset of 17 PanNETs (29%) showed high levels of aberrant DNA methylation (more than 25% of the promoters examined) and this phenotype was positively correlated with nonfunctioning profile (p=0.05), advanced ENETS stage (p=0.02), and poor prognosis (p=0.02). Unsupervised hierarchical clustering provided the best performance in subtype classification of PanNETs distinguishing three specific methylation profiles that were associated with prognosis in both univariable (p=0.008) and multivariable analysis (p=0.05). Cluster 1 and cluster 2 (13 and 25 patients, respectively) showed very low levels of gene methylation and were associated with good prognosis. By contrast, cluster 3 (20 patients) was positively correlated with poor prognosis compared to cluster 1 and cluster 2 and showed significant higher levels of methylation in the following genes: DAPK1, TIMP3, PAX5, HIC1, IGSF4, PYCARD, ESR1, VHL, RARB, WT1 ($p<1^{-4}$).

Conclusions: PanNETs showing a widespread DNA methylation are a distinct clinico-pathological entity characterized by an aggressive tumor behavior. PanNETs with specific epigenetic profiles are strongly associated with poor prognosis, suggesting that the study of DNA methylation may be a promising tool to identify molecular markers of potential diagnostic and prognostic values.

573 Distinct Thyroid Pathology Findings from Patients with Li-Fraumeni Syndrome

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Background: Li-Fraumeni syndrome (LFS) is a classic cancer predisposition syndrome that is secondary to germline *p53* mutations. The most common tumors associated with LFS are soft tissue sarcomas, premenopausal breast cancers, and adrenal cortical carcinomas (ACC). Thyroid pathology findings from patients with LFS have not been reported.

Design: We identified thyroidectomy specimens from 4 patients previously diagnosed with LFS. All findings from the preceding fine needle aspiration (FNA) biopsies were reviewed and all H&E slides of the thyroidectomy specimens were evaluated. Clinical history was obtained from electronic medical records.

Results: The thyroidectomies were from 1 man (30 years-old) and 3 women (31, 33 and 52 years-old). Two patients had a history of a soft tissue sarcoma, 2 patients had a history of breast carcinoma, and 1 patient had a history of ACC. Three of the thyroids showed severe cytologic atypia. In 1 case the atypia was present within adenomatous change of nodular hyperplasia, in 2 cases it was present within adenomatous nodules (including oncocytic nodules), and in 1 case it was present within a minimally invasive follicular thyroid carcinoma. One case had a history of prior head and neck irradiation; however, the atypia in this case was beyond that seen with radiation, and in all cases was beyond typical "endocrine atypia". The severe cytologic atypia was observed in the prior FNA biopsies, and in one case prompted concern that the nodule could represent a metastasis of the patient's prior ACC. One case lacked nodules with severe cytologic atypia. Instead, this case had multifocal follicular variant of papillary thyroid carcinoma (PTC), with a prior FNA that was positive for PTC.

Conclusions: This is the first report of histologic thyroid findings from patients with LFS. The common thyroid pathology findings are bilateral involvement of both lobes by multiple nodules. Furthermore, the most striking feature was the profound cytologic atypia that was present within thyroid nodules in these cases. While this atypia, in the context of an underlying germline *p53* mutation, raised concern for an aggressive malignancy, the clinical significance of this severe atypia is at this time uncertain. Thyroid involvement may be considered as an associated event in patients with LFS and possibly included as one of the familial thyroid syndromes.

574 Anti-Tn and Anti-Sialyl-Tn Antibodies in the Evaluation of Thyroid Neoplasms and Normal Thyroid Tissue

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Background: Investigators have long attempted to identify cancer-associated antigens. Consequently, antibodies have been created to aid in diagnosis. Recent studies suggest that alterations in post-translational modifications to Tn and sialyl-Tn (sTn) carbohydrates may occur following mutations in Cosmc, a key regulator of these modifications. These aberrant modifications could serve as unique targets of neoplastic disease. Tn, normally immunologically inaccessible in adult human tissue, has been shown to be expressed in endometrial, breast, ovarian, lung, colon, pancreatic, and urothelial carcinomas. The purpose of this study is to determine Tn and sTn expression in neoplastic and nonneoplastic thyroid tissue, in which determination of neoplastic vs. nonneoplastic is challenging, especially in cytology and surgical specimens where the differential includes follicular adenoma and carcinoma.

Design: Tissue microarrays (TMAs) of thyroid neoplasms and adjacent normal thyroid were created. Neoplasms included papillary thyroid carcinoma, follicular carcinoma, follicular adenoma, anaplastic carcinoma, medullary carcinoma, Hurthle cell neoplasms, and multinodular goiters. TMAs were immunostained with Tn and sTn monoclonal antibodies identified by glycan microarray at Emory University. Expression for both antigens was cytoplasmic. An immunostain intensity of 2 (scale:0-3) and a percentage of positive-staining cells of >5% were considered positive.

Results: See table.

Tn and sTn Expression in Normal and Neoplastic Thyroid Tissue

Tissue type (no.)	Tn positive no. (%)	P-value*	sTn Positive no. (%)	P-value*
Normal (140)	0 (0)	N/A	0 (0)	N/A
Papillary thyroid carcinoma (94)	3 (3)	0.64	0 (0)	N/A
Follicular carcinoma (52)	0 (0)	N/A	0 (0)	N/A
Follicular adenoma (8)	0 (0)	N/A	0 (0)	N/A
Anaplastic carcinoma (14)	4 (29)	<0.0001	0 (0)	N/A
Medullary carcinoma (2)	0 (0)	N/A	0 (0)	N/A
Hurthle cell neoplasm (25)	0 (0)	N/A	1 (4)	0.151
Multinodular goiter (38)	0 (0)	N/A	1 (3)	0.212

* Tn and sTn expression compared between normal tissue and each respective neoplastic subtype. For neoplasms that did not express antigen, no p-value could be calculated.

Conclusions: While none of the normal thyroid tissue expressed Tn or sTn, neither did most of the neoplastic thyroid tissue. The notable exception is Tn expression in 29% of anaplastic thyroid carcinomas. Thus, although Tn and sTn expression may be of benefit in identifying and characterizing various other carcinomas, only in the thyroid anaplastic subset does Tn appear clinically promising, for diagnosis and therapy.

575 Analysis of BRAF^{V600E} Mutation Status in Papillary Thyroid Carcinoma with Single or Multiple Tumor Nodules

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Background: The advent of molecular studies has refined the approach to the diagnosis, prognosis and treatment of cancers. In papillary thyroid carcinoma (PTC), BRAF mutation is reported to be the most prevalent genetic alteration particularly the BRAF^{V600E} that has been correlated with more aggressive behavior. Patients diagnosed with PTC often present with multifocal tumors with a predominant nodule measuring >1cm or multiple microcarcinomas. It has been previously proposed that this is the result of intraglandular metastasis arising from a single tumor. However, recent molecular studies support the independent clonal origin of these tumors. Furthermore, the uncertainty of how microcarcinomas behave has also been questioned with the reported prevalence of BRAF^{V600E} mutation in these tumors. The purpose of this study is to determine the BRAF^{V600E} status in multifocal nodules when this mutation in the predominant nodule is absent.

Design: A retrospective review of the BRAF^{V600E} mutation of cases with a diagnosis of PTC between 08/2011 and 09/2012 was performed. All multicentric tumors with absent BRAF^{V600E} mutation in the dominant nodule were reviewed and an additional tumor focus was submitted for BRAF assay with standard diagnostic allelle-specific PCR.

Results: A total of 64 cases were included in this study. Thirty-three (33) cases had single nodules, among which 16 had BRAF^{V600E} mutation. Among the 31 cases with multiple tumor foci, 17 had BRAF^{V600E} mutation in the predominant nodule. The remaining 14 cases without BRAF^{V600E} mutation in the predominant nodule, 3 additional tumor foci were found to have this mutation.

Table 1. Clinicopathological features and BRAFV600E mutation status in single and multifocal PTC

				Mean size, cm (range)			
PTC variant	No. of cases	Gender, F	Mean age, years (range)	Single tumor n=33	Multifocal n=31		BRAF v600E mutation by variant
					Dominant tumor	Additional foci	
Classic	33	27	46.8(27-73)	1.39(0.6-3.2)	1.96(0.8-6)	0.55(0.1-1.6)	25
Follicular	27	23	48.6(23-70)	1.84(0.6-4.6)	2.67(0.1-5.5)	0.38(0.1-1.1)	8
*Others	4	3	46.0(24-71)	2.25(0.6-6.5)	N/A	N/A	3
BRAF v600E mutation by tumor focality							
Positive (%)				16(48.5)	17(54.8)	3(21.4)	
Negative				17	14	11	

N/A=not applicable; *Others=Warthin-like, sclerosing and mixed variant

Conclusions: The heterogeneity of papillary thyroid carcinoma is evident in this study. BRAF ^{v600E} mutation may not always be present in the dominant nodule. It is important to further test additional foci, including microcarcinomas due to the evidence that PTC's with BRAF mutations have worse clinical prognosis.

576 Interobserver Agreement in Interpreting Thyroid FNA with a Diagnosis of Atypia/Follicular Lesion of Undetermined Significance (FLUS/AUS)

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Background: The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) was developed to refine definitions and improve clinical communication and management. However, the diagnostic category of atypia/follicular lesion of undetermined significance (FLUS/AUS) remains heterogenous in terms of usage and clinical outcome. Because of the "gray zone" that exists in the interpretation of thyroid FNA demonstrated minor architectural and/or cytologic atypia, this study was undertaken to evaluate the degree of interobserver agreement in the evaluation of thyroid FNAs originally interpreted as FLUS/AUS.

Design: Twenty-three thyroid FNAs including 18 cases originally diagnosed as FLUS/AUS, 2 as negative for malignant cells, 2 as positive for malignancy, and 2 as follicular neoplasms were selected. Two representatives slides from each case were circulated to 13 board certified or eligible cytopathologists; all were from academic institutions with 6 were from the same institution. Each reviewer was asked to evaluate each cases using the BSRTC. The kappa statistics was calculated.

Results: Only 2 cases (22%) were in complete agreement: one originally interpreted as negative and one as positive for malignancy; both cases were confirmed on histology. There was a majority agreement (among 10 or more) in 6 cases: one originally diagnosed as positive, one negative, and 4 FLUS/AUS. Both positive and negative cases were confirmed on histology; among the 4 FLUS/AUS, 2 were found to be negative and 2 follicular adenoma on histology. For the entire group of reviewers, the mean kappa statistic was 0.34±0.13. The mean kappa statistic was 0.42±0.7 and 0.29±0.14 among reviewers of the same institution and among reviewers from different institutions. The difference was statistically significant.

Conclusions: The interobserver agreement for thyroid FNA cases originally classified as FLUS/AUS was fair among academic cytopathologists. It appeared that the interobserver agreement was better among cytopathologists who were from the same institution.

577 Development of a Multi-Gene qPCR Assay for Adrenocortical Tumor Diagnosis and Prognosis

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Background: As incidentally detected adrenal masses are increasing, the correct diagnosis of adrenocortical tumors, particularly differentiating adrenocortical carcinomas (ACC) from adrenocortical adenomas (ACA), is of growing importance. Differentiating ACAs and ACCs can be challenging, and although overall survival of patients with ACC is poor, the course can be variable. Hence, there is a need for novel biomarkers to classify ambiguous ACTs and predict ACC behavior.

Design: We developed a multi-gene qPCR panel optimized for RNA from formalin fixed paraffin embedded (FFPE) tissues, consisting of 33 assays targeting 28 genes of interest (two assays targeted *IGF2*) and 4 housekeeping genes. Total RNA was isolated from 3x10um FFPE sections from 96 cases (5 normal adrenal cortex [NAC], 24 ACAs

and 67 ACCs). Reverse transcription and qPCR was applied to these cases, with two cases assessed in duplicate (total qPCR n=98). Target gene expression was normalized to three housekeeping genes showing robust amplification.
Results: A median of 7.0 ug RNA was isolated per case (range: 1.0-37.5ug). Eighty two of 98 (84%) samples had sufficient amplifiable RNA for analysis. Samples with sufficient amplifiable RNA were from significantly more recent blocks than samples with insufficient RNA (mean 7 years vs. 12 years, p=0.0002). Of blocks less than 10 years old, 59/64 (92%) had sufficient amplifiable RNA for analysis. To assess the reproducibility of the reverse transcription and qPCR, RNA from two samples (ACC_64 and ACA_18) was subjected to duplicate reverse transcription and qPCR; for each sample, the duplicate sample showed the highest correlation of normalized target gene expression when compared to the 81 other samples (ACC_64_1 and ACC_64_2, r=0.992; ACA_18_1 and ACA_18_2, r=0.997). Normalized expression of *IGF2* by the two assays was highly correlated across the 82 samples (r=0.963), supporting the robustness of the qPCR assay design. Unsupervised centroid linkage hierarchical clustering using normalized expression of the target gene assays robustly separated NAC, ACAs and ACCs (one low grade ACC clustered with ACAs and NACs). Expression of cell cycle genes was highly correlated across samples, and a cluster of ACCs with low proliferation and long over-all survival was present.
Conclusions: We have developed a robust, highly reproducible multi-gene qPCR panel for FFPE tissue which robustly discriminates ACAs and ACCs. Optimized diagnostic and prognostic models will be presented, including comparisons to standard clinicopathological parameters and clinical outcome.

578 Interobserver Variability in the Assessment of Extrathyroidal Extension in Papillary Thyroid Microcarcinoma

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Background: Extrathyroidal extension (ETE) in papillary thyroid microcarcinoma (PTMC) is associated with local recurrence and central (Level VI) and lateral (Levels II-V) lymph node metastasis. Presence of minimal ETE upstages the PTMC from T1a to T3, adversely affecting prognosis and necessitating treatment with postoperative radioiodine therapy. However, histopathologic assessment of ETE may be difficult due to lack of a well-demarcated thyroid capsule. Moreover, skeletal muscle may be present within thyroid parenchyma, especially in the isthmus as an embryologic remnant. The aim of this study was to assess the inter-observer variability in the assessment of ETE in PTMC using the AJCC criteria.
Design: Two endocrine pathologists independently assessed the presence of ETE in 25 randomly selected PTMCs that were located close to the thyroid capsule. One to three (1-3) representative slides were reviewed for each PTMC tumor. Minimal ETE was assessed using the AJCC staging manual (7th edition) definition, which is tumor extension either in the perithyroidal soft tissue and/or skeletal muscle. Interobserver agreement (Kappa statistics) was calculated Graph Pad software.
Results: There was "moderate" (kappa = 0.453) agreement between the two pathologists for the presence or absence of ETE. Both pathologists agreed for the presence of ETE in 14/25 (56%) tumors and absence of ETE in 5/25 (20%) cases. There was complete disagreement between the two pathologists with respect to 6/25 (24%) cases. Of these six tumors, the first observer reported ETE in 4/6 tumors whereas the second pathologist reported no ETE in those 4/6 tumors. In these six cases, both observers agreed for the involvement of thyroid capsule by the tumor but did not agree for the tumor extension beyond the capsule. There was no disagreement when ETE was diagnosed based on skeletal muscle involvement by the tumor (n=3). Furthermore, there was no discordance with respect to thyroid capsular involvement between the two pathologists in any case.
Conclusions: There is significant interobserver variability with the current AJCC criteria for assessment of ETE in microPTCs. The use of more objective criteria for defining extrathyroidal extension may help improving the consistency in assessing ETE.

579 Subcapsular Sclerosing Variant (SSV) of Papillary Thyroid Microcarcinoma (PTMC): Clinicopathologic and Molecular Features

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Background: Peripheral location of the papillary thyroid microcarcinoma (PTMC) has been suggested to be a high-risk histologic feature that correlates with aggressive behavior. Conventionally PTMC are not further subtyped. Here we define a distinct subtype of PTMC, the subcapsular sclerosing variant (SSV) along with its clinicopathological and molecular features.
Design: We identified PTMC diagnosed during 2010-2012 that met the following criteria: peripheral location (capsular or subcapsular), abutting thyroid capsule along at least 20% of the tumor's circumference, prominent tumor-associated stromal fibrosis, sclerosis and/or desmoplasia), and infiltrative tumor borders. The clinicopathological features were reviewed and *BRAF*^{V600E} mutational analysis was performed by single strand conformational polymorphism.
Results: The patients (n=39) included 31 women and 8 men (F: M- 4:1), aged 23 to 75 years (mean 50). The tumor size ranged from 2 mm to 10 mm (mean 7 mm). Thirty-four (87%) tumors harbored the *BRAF*^{V600E} mutation. Twelve SSV (31%) showed minimal extrathyroidal extension (ETE). Lymph node metastasis (LNM) was present in 30% (n=10/33) of tumors. However, in node negative patients, the tumor was upgraded from T1 to T3 based on ETE in 22% (n=5/23). Area of contact of tumor (in percentage) with thyroid capsule ranged from 20% to 90%. Interestingly, incidence of ETE increased from 18% (n=5/27) to 58% (n= 7/12) when the area of contact increased from <50% to ³50% respectively.
Conclusions: Subcapsular sclerosing variant of PTMC has a distinctive morphology, and location within the thyroid. They are associated with minimal microscopic ETE, lymph node metastasis, and with *BRAF*^{V600E} mutation in a significant proportion of cases. Identification and segregation of this subset from other PTMC may be helpful in exploring long-term disease outcome.

580 BRAF Mutation Status Has a Limited Role in Predicting Long-Term Persistence in Papillary Thyroid Carcinoma (PTC)

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Background: Papillary thyroid carcinoma (PTC) accounts for >90% of thyroid cancers in the US. Despite treatment, about 25% of these differentiated cancers persist or recur underscoring the need to identify this subset of tumors at presentation and develop effective targeted therapies. BRAF mutation, detected in up to 65% of PTC, has been associated with aggressive tumor behavior but its role as a prognostic indicator in PTC is not clear. This study compares the frequency of BRAF mutation in persistent PTC to that in PTC with negative long-term follow-up.
Design: 38 patients who underwent total thyroidectomy for conventional and/or follicular variant PTC and had been closely followed for >5 years were identified in our tumor registry. 13 of these patients had persistent metastatic PTC (PPTC) and 25 had negative follow-up (no clinical, biochemical, or imaging evidence of tumor; NPTC). Patient demographics and tumor features (size, focality, extrathyroidal extension, regional lymph node status, margin status) were recorded. After slides were reviewed and diagnoses confirmed, macrodissected formalin fixed paraffin embedded sections of each tumor were analyzed for BRAF V600E (1799T>A) mutation using real-time PCR. Presence of BRAF mutation was correlated with tumor follow-up using Fisher's two-tailed test with p<0.05 as significant.
Results: The PPTC group (8 females, 5 males) ranged from 20 to 75 years in age (median 49 yrs) at thyroidectomy with cytologically and/or histologically confirmed metastases 5 to 26 years (median 7.0 yrs) later. The NPTC group (20 females, 5 males) ranged from 24 to 75 years in age (median 39 yrs) at thyroidectomy with 8 to 21 years (median 17 yrs) of negative follow-up. The median tumor size was 2 cm (range 0.8 to 4.5 cm) in the PPTC group and 1.5 cm (range 0.5 to 3.5 cm) in the NPTC group. At thyroidectomy, positive regional lymph nodes and positive resection margins were each more frequently observed in the PPTC than in the NPTC group (p=0.0001 and p=0.0007, respectively). Comparison of the two groups showed no significant difference in tumor focality (uni vs, multi) or in presence of extrathyroidal extension. BRAF mutation was detected in 11 (84.6%) of the PPTC and in 19 (76.0%) of the NPTC; p=0.69.
Conclusions: No significant difference was observed in the frequency of BRAF V600E mutation detected in the PPTC and NPTC groups, suggesting that BRAF mutation has a limited role in predicting long-term perisistence in PTC. Prospective studies are needed to confirm our findings.

581 Adrenocortical Carcinomas: A Clinicopathological Analysis of 77 Cases

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Background: Adrenocortical carcinomas are rare malignant tumors of the adrenal cortex with an incompletely understood pathogenesis and aggressive clinical behavior. Their low incidence has prevented large scale research into these tumors and uniform treatment strategies are still lacking. We report the clinicopathological features of 77 primary adrenocortical carcinomas, in one of the largest series of these tumors to date.
Design: Seventy-seven cases of primary adrenocortical carcinomas from the surgical pathology files of the MD Anderson Cancer Center and the Medical College of Wisconsin were analyzed and clinical information including presentation, treatment and follow up was obtained from the referring pathologists or institutional clinical files.
Results: The patients were 52 females and 25 males with an age range from 17 to 76 years (mean 46.8). Hormone-related symptoms were present in 23 patients. The tumor weight ranged from 26 to 3260g (mean 457.1). Thirty-eight tumors were right sided and 39 were left sided. Histologically, 30 tumors were of the conventional type, 30 tumors showed oncocytic features, 7 tumors were classified as myxoid, 7 as rhabdoid and 3 as sarcomatoid adrenocortical carcinoma. Ten patients received adjuvant chemotherapy. Clinical follow-up available for 56 patients showed that 23 patients were alive with a follow-up period from 1-259 months (mean 141.6) and 33 patients had died 4 to 439 months after diagnosis (mean survival 43.4).
Conclusions: Adrenocortical carcinomas are tumors predominantly affecting patients in the 5th decade of life with a female predilection. They can show a wide range of morphologic variability with conventional and oncocytic types being the most common. Standard chemotherapy as adjuvant therapy for adrenocortical carcinomas is still lacking. These tumors run an aggressive clinical course with a mean survival of 43.4 months. Larger studies are needed to guide future therapies and improve patient outcome.

582 Adrenocortical Carcinomas: An Immunohistochemical Analysis of 40 Cases

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Background: Adrenocortical carcinomas are uncommon tumors of the adrenal cortex that are known to pursue an aggressive clinical course with frequent recurrence and early metastasis. The distinction of these tumors from other, often metastatic neoplasms may sometimes prove difficult due to overlapping clinical, morphological

and even immunophenotypical features. To this end, we performed a comprehensive immunohistochemical analysis using traditional and novel markers in 40 cases of adrenocortical carcinoma.
Design: Forty cases of resected adrenocortical carcinoma were reviewed and representative whole tissue sections were selected for immunohistochemical studies using antibodies directed against high molecular weight cytokeratin (HMWCK), low molecular weight cytokeratin (CAM5.2), inhibin-α, melan A, chromogranin A, synaptophysin, calretinin, steroid receptor coactivator-1 (SRC1), Pax8 and Ki67. The percentage of positive tumor cells as well as the intensity of staining were evaluated and scored; for Ki67 the percentage of positive tumor cells was recorded.
Results: Positive staining was observed for SRC1 (39/40; 97.5%), inhibin-α (37/40; 92.5%), calretinin (32/40; 80%), synaptophysin (29/40; 72.5%) and melan A (26/40; 65%). CAM5.2 was expressed in 9/40 tumors (22.5%). Rare cases showed positivity for chromogranin A (2/40; 5%) and Pax8 (1/40; 2.5%). None of the cases showed any reactivity with HMWCK. The Ki67 proliferative index ranged from <5 to 20%.
Conclusions: There are no specific markers to reliably distinguish adrenocortical carcinomas from other primary or metastatic neoplasms. However, a combination of immunohistochemical stains in a panel consisting of SRC1, inhibin-α, calretinin and HMWCK may be of aid in the differential diagnosis of these tumors. In addition, Pax8 is only rarely positive in adrenocortical carcinomas which is a useful tool in their separation from renal neoplasms.

583 *PAX8/PPARγ* Rearrangements of Thyroid Tumors: Assessment of Distribution Frequency, Histopathological Features and Cytological-Histopathological Correlations

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Background: *PAX8/PPARγ* rearrangements have been found in follicular thyroid carcinoma (FTC), follicular variant papillary thyroid carcinoma (FVPTC), and follicular adenoma. However, large-scale data have not been reported regarding its prevalence, cytological-histopathological correlation, and histopathological features. We reported the retrospective findings of thyroid tumors for positive *PAX8/PPARγ* rearrangements.
Design: We reviewed all cases for *PAX8/PPARγ* rearrangement of at our institution in the interval of 2007 to 2012. Its distribution frequency, histopathological features and cytological-histopathological correlations were assessed. Tumor size, vascular and capsular invasion, resection margins, extra-thyroid extension, encapsulation, thickness of capsule, solid growth, metastatic status of regional lymph nodes was also recorded.
Results: During a 6-year period of 2007-2012, 16 cases of *PAX8/PPARγ* rearrangement were identified. In cytology FNA aspirations, this rearrangement was found in 9 (64.3%) follicular lesion/neoplasm (FNL), 2 (14.3%) oncocytic (Hürthle cell) lesion/neoplasm (OHNL), 2 (14.3%) FLUS, 1 (7.1%) negative. 14 *PAX8-PPARγ* rearrangement-positive thyroid cancers included 9 FVPTC (64.3%), 3 FTC (21.4%), 2 PTC solid variant (14.3%). Among 9 FVPTC, the cytology FNA diagnosis were 5 FNL, 1 OHNL, 1 FLUS, 1 negative and 1 not-done. Two of 3 FTC were FNL. All 2 PTC solid variant were FNL. Average tumor size of TFC, FVPTC and PTC solid variant was 3.4, 3.7, 4.0 cm respectively. Variable solid growth pattern was present in both FTC (0-40%) and FVPTC (0-30%). All 3 FTC, 9 FVPTC and 2 PTC solid variant were encapsulated and none of them showed extrathyroid extension, positive lymph nodes and positive margins. Vascular invasion of FTC, FVPTC, PTC solid variant was 66% (2/3), 33% (3/9), 50% (1/2) respectively. The capsular invasion of them was 100% (3/3), 56% (5/9) and 50% (1/2) respectively.
Conclusions: Our data indicated that: 1) Most thyroid tumors with *PAX8/PPARγ* rearrangement are encapsulated FVPTC, followed by FC and PTC solid variant; 2) *PAX8/PPARγ* was not present in other subtypes such as infiltrative/nonencapsulated FVPTC; 3) *PAX8/PPARγ*-positive encapsulated FVPTC had features of high frequency of capsular invasion, vascular invasion, thick capsule, presence of variable solid growth, and absence of LN and extrathyroid extension.

584 The Utility of Cyclin D1 in Distinguishing Encapsulated Follicular Variant of Papillary Thyroid Carcinoma from Follicular Adenoma

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Background: Distinguishing encapsulated follicular variant of papillary carcinoma (E-FVPC) from follicular adenoma (FA) depends on the classic nuclear features of papillary carcinoma. These features are subjective and dependant on fixation and tissue processing. There is significant interobserver variability among pathologists distinguishing these lesions. In this study, we investigated whether cyclin D1 IHC has a diagnostic value for discriminating E-FVPC from FA.
Design: 23 E-FVPC and 18 FA were stained by IHC for Cyclin D1. All cases of carcinoma were reviewed by two pathologists. Two FA were excluded due to presence of atypical features suspicious for E-FVPC. Staining intensity was graded as negative (0), weak (1+), moderate (2+) or strong (3+). Stain distribution was negative (0%); 1+ (<25%); 2+ (25-75%) or 3+ (>75%) of tumor cells. Mann-Whitney U test was used for statistical analysis.
Results: E-FVPC expressed Cyclin D-1 strongly in 22/23 (96%) of cases (3+ in 70%, 2+ in 26%). Cyclin D1 distribution was 3+ in 74% and 2+ in 22% of E-FVPC. One case (4%) of E-FVPC showed only 1+ intensity and 1+ distribution of cyclin D1. Cyclin D-1 was expressed strongly in 9/18 (50%) of FA (3+ in 11%, 2+ in 39%). Four of the FA with 3+ and 2+ intensity had oncocytic metaplasia and one FA with strong intensity (5.5%) had a background of Hashimoto thyroiditis. Cyclin D1 distribution was diffuse in 8/18 (44%) FA (3+ in 22%, 2+ 22%). 9/18 (50%) FA showed a 1+ staining distribution. A statistically significant association was found between tumor type and cyclin D1 staining distribution and intensity. There were fewer cyclin D1-positive FA than E-FVPC (39% vs. 96%, respectively; p<0.001). Stain distribution was greater in E-FVPC than in FA (96% vs. 44%, respectively; p=0.001).
Conclusions: Cyclin D-1 is a useful marker in distinguishing E-FVPC from FA when there is strong and diffuse staining. Almost all E-FVPC show strong, diffuse staining, however, this marker is not helpful if the adenoma shows oncocytic metaplasia/ changes or there is Hashimoto's thyroiditis. Cases diagnosed as FA may require a review by a second pathologist to exclude the possibility of E-FVPC.

Gastrointestinal

585 Pathway-Dependent Roles of ARID1A Expression Loss in Gastric Cancer: Relationships with Epstein-Barr Virus Infection and Microsatellite Instability

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Background: The AT-rich interactive domain 1A gene (ARID1A), which encodes one of the subunits in the Switch/Sucrose Nonfermentable (SWI/SNF) chromatin remodeling complex, is occasionally mutated and is responsible for loss of protein expression in gastric carcinoma (GC), particularly with Epstein–Barr virus (EBV) infection and microsatellite instability-high (MSI-H) phenotype. However, the clinicopathological significance of ARID1A loss and the relationship with EBV infection or MSI-H were unknown.
Design: We applied immunohistochemistry of ARID1A to the tissue microarray of 857 GCs, including 67 EBV(+) and 136 MLH1-lost (corresponding to MSI-H phenotype) GCs. Whole sections of some GC cases were also stained to assess the distribution of ARID1A-lost carcinoma cells. In order to compare with gastric carcinoma, eight nasopharyngeal carcinomas, 15 lymphomas with EBV infection, and 173 colorectal carcinomas were also examined.
Results: Loss of ARID1A expression was significantly more frequent in EBV(+) (23/67; 34%) and MLH1-lost (40/136; 29%) GCs than in EBV(-)MLH1-preserved (32/657; 5%) GCs (P<0.01). Loss of ARID1A correlated with larger tumor size, advanced invasion depth, lymph node metastasis and poor prognosis in EBV(-)MLH1-preserved GC. A correlation was found only with tumor size and diffuse-type histology in MLH1-lost GC, and no correlation was observed in EBV(+) GC. Loss of ARID1A expression in EBV(+) GC was highly frequent in the early stage of GC. In whole section staining, all of 14 EBV(+) GCs with ARID1A loss were totally negative for ARID1A in the entire lesion. However, 7 of 17 MLH1-lost GCs with ARID1A loss showed regional expression loss (p=0.02). EBV(+) nasopharyngeal carcinomas and lymphomas failed to show loss of ARID1A. In 173 colorectal carcinomas, only four cases showed ARID1A-loss, and these were also MLH1-lost.
Conclusions: ARID1A expression loss may be an early change in carcinogenesis of EBV(+) GC. It specifically occurs in gastric epithelial cells but not in EBV-infection in nasal epithelial cells or lymphocytes. Loss of ARID1A is also frequent in MLH1-lost GC, but it is a late stage event. ARID1A-loss is infrequent, but is related with poor prognosis in EBV-negative and MLH1-preservedGC. ARID1A expression loss has different and pathway-dependent roles in GC.

586 Immunohistochemistry Using a BRAF V600E Mutation-Specific Monoclonal Antibody Is Not a Useful Surrogate for Genotyping in Colorectal Adenocarcinoma

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Background: Chemotherapy combined with anti-EGFR monoclonal antibodies prolongs survival in patients with advanced colorectal carcinoma (CRC). *BRAF* V600E and *KRAS* activating mutations are identified in around 20% and 50% of CRC, respectively, leading to constitutive EGFR pathway activation and lack of response to anti-EGFR therapy. Therefore, predictive mutation screening is routinely performed in patients with metastatic CRC. *BRAF* testing is also performed on CRC that demonstrate high-level microsatellite instability, since the presence of a *BRAF* mutation indicates the tumor is sporadic (i.e., not associated with Lynch syndrome). A novel BRAF V600E-specific monoclonal antibody has recently become commercially available. The aim of this study was to determine whether immunohistochemistry (IHC) for BRAF V600E can predict *BRAF* mutations in CRC.
Design: Whole tissue sections from 52 genotyped cases of CRC (46 primary tumors and 6 metastases) were examined. *BRAF* codon 600 and *KRAS* codons 12 and 13 were genotyped by pyrosequencing in a CLIA-certified laboratory. Cases included 17 tumors with *BRAF* V600E, 18 with *KRAS* mutations, and 17 wild-type for *BRAF* and *KRAS*. IHC was performed following pressure cooker antigen retrieval with an anti-BRAF V600E monoclonal antibody (Spring Bioscience; clone VE1; 1:50 dilution with overnight incubation). Cytoplasmic staining was scored as negative, weak, moderate or strong, with a known *BRAF* V600E-mutant melanoma serving as a positive control. Two authors scored the slides, with discrepancies resolved across the microscope.
Results: Cytoplasmic staining was observed in 71% of tumors with *BRAF* V600E mutation. Staining was moderate or strong in 50% of the positive cases (35% of *BRAF*-mutant cases overall). Weak cytoplasmic staining was detected in 17% of *KRAS*-mutant cases and 35% of wild-type cases. Non-specific nuclear staining in both tumor cells and normal colonic epithelium was a common finding. The sensitivity and specificity of BRAF V600E IHC for *BRAF* V600E mutation is 71% and 74%; if only moderate or strong staining is considered positive, the specificity is 100%, but the sensitivity is only 35%.
Conclusions: Overall, BRAF V600E mutation-specific IHC is not specific for *BRAF* mutations in CRC. While moderate or strong cytoplasmic staining is specific for *BRAF*