system and provide objective outcome measures applicable to the new ACGME accreditation system. For this approach to be successful correct web tools selection is critical to facilitate student contribution, participation and confidence.

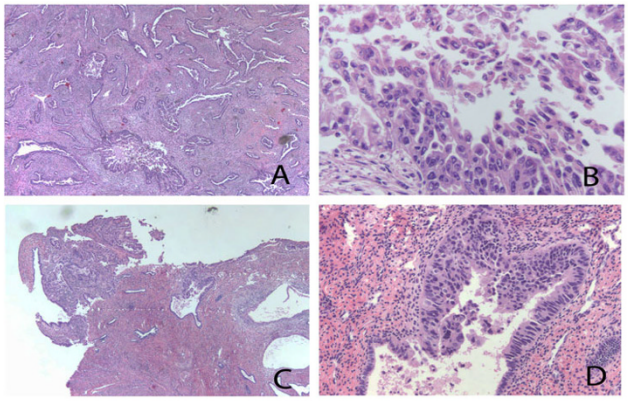
511 Assessing Resident Frozen Section Diagnostic Skills for Endometrial Cancer

William Selove, Thomas Stockl, Dina Kandil, Leslie Bradford, Yuxin Liu. University of Massachusetts Medical School, Worcester, MA; UMass Memorial Hospital, Worcester, MA.

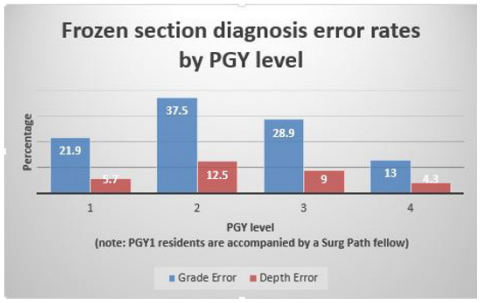
Background: The development of frozen section diagnostic skills is a critical part of pathology residency training. Our study aims to assess trainee performance on endometrial cancer frozen sections and to identify common errors while providing recommendations for improvement.

Design: Twenty-two residents at our institute (ranging from PGY1 to PGY5) performed a total of 260 endometrial cancer frozen sections from 2011 to 2014. Their independent frozen section diagnoses, including tumor type, grade, and myometrial invasion, were compared with the final pathology reports.

Results: Endometrial cancer type and grade were accurately diagnosed by residents on frozen sections in 194 of 260 cases (75%). The misdiagnosed cases resulted from undercalling endometrioid tumor grade in 43 cases (17%), overcalling in 6 (2%), and missing Type II tumor component in 17 (6%), including 7 serous carcinoma, 5 clear cell carcinoma, and 5 carcinosarcoma. (Shown in figure 1, A/B clear cell carcinoma; C/D Serous carcinoma both misdiagnosed as Grade 1 Endometrioid carcinoma on frozen section.)



The depth of myometrial invasion was accurately reported in 240 cases (92%) while undercalled in 20 cases (8%). No errors were made in the gross inspection of cervical involvement of the tumor. Frozen section error rate decreased with years of training (PGY1 accompanied with PGY5 24; PGY2 44%; PGY3 37%; and PGY4 17%). Overall, resident frozen section errors would have led to 24 (9%) of patients receiving sub-optimal staging procedures.



Conclusions: Our trainees demonstrated moderate performance on endometrial cancer frozen sections with an overall accuracy of 75%. Their frozen section skills gradually developed throughout the residency. Additional training should be devoted to training residents to recognize high grade components and type II tumor on frozen section.

512 Teaching Strategy for Colonic Polyps

Emma Whitcomb, Lei Zhao, Lindsay Alpert, Heewon Kwak, Lindsay Yassan, Mei Lin Bissonnette, Nhu Thuy Can, Bonnie Choy, Alexander Gallan, Daniel Johnson, Selene Koo, Rebecca Wolsky, Jerry Wong, Shu-Yuan Xiao, John Hart. University of Chicago, Chicago, IL.

Background: Pathology residents often find distinguishing between types of colon polyps challenging, particularly serrated lesions. In this study, the accuracy and interobserver reproducibility of resident colon polyp diagnoses, including hyperplasitc polyp (HP), colonic adenoma (CA), sessile serrated polyp (SSP), sessile serrated polyp with adenomatous change (SSPA), traditional serrated adenoma (TSA) and prolapse polyp (PP), was measured before and after a 1 hour lecture on colon polyp diagnosis to assess the effectiveness of this teaching strategy.

Design: Two sets of 40 colon polyps were compiled for which there was independent diagnostic agreement between 2 GI pathologists. 13 pathology residents of all levels of training were given the first set and asked to make a diagnosis from the following 6 options: HP, CA, SSP, SSPA, TSA, and PP. Residents then attended a 1 hour lecture focusing on key diagnostic features for each of the 6 diagnoses. Following the lecture, residents were given the second set of polyps to diagnose. Accuracy of diagnosis when compared to the expert diagnosis and interobserver variability using kappa statistics were measured before and after the lecture.

Results:

Table 1. Accuracy of diagnosis before and after lecture

Expert Diagnosis	Pre-lecture % Correct	Post-lecture % Correct
Overall	68	69
HP	89	64
CA	80	81
SSP	63	81
SSPA	44	57
TSA	46	52
PP	82	74
HP and PP	85	67
CA, SSP, SSPA and TSA	60	70

Table 2. Interobserver reproducibility before and after lecture

	Pre-lecture Interobserver Reproducibility (κ)	Post-lecture Interobserver Reproducibility (κ)
Overall	0.46	0.51
HP	0.44	0.39
CA	0.57	0.61
SSP	0.42	0.46
SSPA	0.37	0.52
TSA	0.17	0.41
PP	0.66	0.46
HP and PP	0.44	0.33
CA, SSP, SSPA and TSA	0.56	0.68

Conclusions: There was little change in the overall accuracy of diagnosis and only slightly improved interobserver reproducibility following the lecture. However, when grouping premalignant lesions (CA, SSP, SSPA, and TSA) there was more significant improvement. These results suggest a lecture format may not be the best teaching strategy for this diagnostic challenge, but may have some benefit in the diagnosis of actionable entities. A computerized teaching module with a quiz format is currently being explored.

Endocrine Pathology

513 Low Frequency of TERT Promoter Mutation in a Series of 529 FNAs

Fulvio Basolo, Agnese Proietti, Elisabetta Macerola, Riccardo Giannini. University of Pisa, Pisa, Italy; Azienda Ospedaliera-Universitaria Pisana, Pisa, Italy.

Background: The preoperative diagnosis of thyroid nodules mainly depends upon fine needle aspiration (FNA) cytology. However, 20% to 30% of cases cannot be ruled out for cancer. New approaches to diagnosis of cancer in FNA thyroid nodules are based on molecular analysis. Promoter mutations in the gene for telomerase reverse transcriptase (*TERT*) have been recently identified in thyroid cancers and shown to be important in their pathogenesis. *TERT* (C228T) was prevalent in poorly/undifferentiated thyroid cancer, as well as *BRAF* V600E mutation-positive PTC.

Design: We collected 529 thyroid FNAs from June 2013 to June 2014. Cytological diagnoses were performed according with the SIAPEC-IAP guidelines. 93 patients underwent thyroidectomy. Clinical history and thyroid ultrasound reports were matched to thyroid FNA cytology and surgical pathology report. Molecular testing for *BRAF*, *Ras* (*NRas* cod 61, *HRas* cod 61 and *KRas* cod 12/13) and *TERT* promoter mutations was performed.

Results: Of 529 thyroid FNA 23 (4.3%) were inadequate (TIR1), 368 (68.9%) were benign nodules, 92 (17.4%) indeterminate lesions (TIR3) of which 64 (12.1%) TIR3A and 28 (5.3%) TIR3B. 8 were (1.5%) suspicious nodules, and 39 (7.4%) were malignant cases. *BRAF* mutations were found in 1 TIR1 (4.3%); 1 TIR2 (0.3%); 1 (10.1%) TIR3B; 2 TIR4 (25%) and 22 TIR5 (56.4%). *Ras* mutation was found in 1 (4.3%) inadequate sample, in 19 (5.2%) benign nodules, in 11 (12%) indeterminate lesions and in 2 (25%) suspicious cases. No *Ras* mutations were found in malignant nodules. One (12.5%) case of TIR4 and 2 (5.1%) of TIR5 were *TERT* promoter mutated. Comparing with histological diagnoses we found that *BRAF* V600E mutation was present only in papillary thyroid carcinomas (PTC). *Ras* alterations were found in 3 follicular variant PTC (FVPTC), one follicular carcinomas (FTC) and 2 microfollicular adenomas. *TERT* C228T mutation was found in one anaplastic thyroid cancer which was also positive for *Ras* mutation and two classical PTCs both of them *BRAF* V600E. Overall frequency of *TERT* promoter mutation was 0,75% (4 out of 529).

Conclusions: We confirmed that *TERT* promoter mutations were associated with poor prognosis PTCs and anaplastic cancer. Undoubtedly, it will provide prognostic and therapeutic implications. The frequency of *TERT* promoter mutation in our series is very

low (0.75%). Thus, according to our results, testing of *TERT* promoter mutations on FNA will not considerably enhance the current status of molecular testing of thyroid nodules.

514 Somatostatin Receptor Subtype 2A Is Frequently Expressed By Poorly Differentiated Neuroendocrine Carcinomas: A Potential Novel Therapeutic Target

Andrew Bellizzi, Thomas Czeczok, Emily McMullen. University of Iowa Hospitals and Clinics, Iowa City, IA.

Background: Somatostatin, an inhibitory peptide produced in the brain and by D cells throughout the gastrointestinal tract, signals through somatostatin receptors (SSTRs). SSTR subtype 2A (SSTR2A) is expressed by 60-90% of well-differentiated neuroendocrine tumors (WDNETs), the basis of therapy with the somatostatin analogue octreotide and somatostatin receptor scintigraphy. Poorly differentiated neuroendocrine carcinomas (PDNECs) share a neuroendocrine phenotype but are biologically aggressive. Cisplatin-etoposide is a therapeutic mainstay in metastatic PDNECs, but relapse is invariable. We recently validated SSTR2A immunohistochemistry (IHC) in our laboratory as a WDNET predictive marker. Whether SSTR2A is expressed by PDNECs is not established; if it is expressed, it would represent a potential novel therapeutic target.
Design: SSTR2A IHC (clone UMB-1, Abcam) was performed on tissue microarrays of 113 WDNETs (54 pancreas, 59 jejunoileum) and 88 PDNECs (43 small cell carcinoma, 45 Merkel cell carcinoma). Tumors were arrayed in triplicate. Extent (%) and intensity (0, 1+, 2+, 3+) of membranous staining were assessed and an H-score (extent x intensity) calculated. An average H-score >20 was considered positive (based on published data relating IHC and in vitro SSTR autoradiography).
Results: SSTR2A was expressed by the vast majority of pancreatic (85.2%) and jejunoileal (96.6%) WDNETs, with expression diffuse and strong. SSTR2A-positivity was observed in 35% of PDNECs, in which it was rather intense in small cell carcinoma (average H-score 163) relative to Merkel cell carcinoma (average H-score 87). Detailed expression data are presented in the Tables.

SSTR2A Expression in WDNETs	% Positive	Average H-score (if positive)
Pancreas (n=54)	85.2	266
Jejunoileum (n=59)	96.6	224

SSTR2A Expression in PDNECs	% Positive	Average H-score (if positive)
Small Cell Carcinoma (n=43)	32.6	163
Merkel Cell Carcinoma (n=45)	37.8	87

Conclusions: As expected, gastroenteropancreatic WDNETs were found to express high-levels of SSTR2A. Surprisingly, SSTR2A expression of moderate extent and intensity was noted in 35% of PDNECs. This could form the basis of a new paradigm in the treatment of metastatic PDNECs in which patients with SSTR2A-expressing tumors could be placed on octreotide maintenance upon the completion of cisplatin-etoposide and/or patients with treatment-resistant metastatic disease could be candidates for peptide receptor radionuclide therapy.

515 Interlaboratory Variation Regarding MIB1-Staining in Well Differentiated Pancreatic Neuroendocrine Tumors

Annika Blank, Laura Wehweck, Ilaria Marinoni, Laura Boos, Frank Bergmann, Anja Schmitt, Aurel Perren. University of Bern, Bern, Switzerland; University of Heidelberg, Heidelberg, Germany.

Background: In neuroendocrine tumors (NET) grading and staging are the only routinely performed procedures to judge prognosis. Proliferation index using MIB1 staining has been introduced to assess grading. There are vivid discussions on cut-off definitions, automated counting and inter-observer variability. However, no data exist regarding inter-lab reproducibility for low proliferation indices which are of outstanding importance to discriminate between G1 and G2 NETs.
Design: We performed MIB1 staining in 3 different University Hospital based pathology laboratories on a TMA of a well characterized patient cohort, containing pancreatic NET of 61 patients. To calculate the proliferation index, number of positive tumor nuclei was divided by the total number of tumor nuclei. Labelling index was compared to mitotic counts in whole tissue sections and to clinical outcome. Linear regression analysis and ROC analysis were performed.
Results: Inter-lab differences regarding intensity and number of positive cells in MIB1 staining were significant. ROC-analysis found three different "optimal" cut offs for three different institutes (2.1%, 0.79% and 0.4%). Every calculated cut off stratified the patient cohort to a significant extent for the underlying staining (p=0.0009; 0.0010 and 0.0020) but showed no or lesser significance when applied to the other stainings. Especially low proliferating tumors were affected by those differences.
Conclusions: Significant and relevant inter-lab differences for MIB1 exist. Since the MIB1 proliferation index influences grading, local cut-offs or external standardization should urgently be introduced to achieve reliability and reproducibility.

516 Papillary Thyroid Carcinomas – Adverse Prognostic Factors

Laura Boos, Anja Schmitt, Holger Moch, Paul Komminoth, Aurel Perren, Matthias Dettmer. Institute of Pathology University, Bern, Switzerland; Institute of Pathology University Hospital, Zurich, Switzerland; Institute of Pathology Triemli Hospital, Zurich, Switzerland.

Background: Papillary thyroid carcinomas (PTC) have an excellent 5-10 year survival rate. Most of the patients can be cured by surgery and subsequent treatment with radioactive iodine (RAI). However, up to now it is very difficult to predict which patients will have to be followed more closely because of an increased risk of a tumor relapse.
Design: We evaluated different immunohistochemical markers in 57 patients with PTC and adverse clinical outcome (ACO), which was defined by more than one tumor relapse after receiving the initial RAI, and compared them with a control group (CG) of 68 PTC. The tumors were re-evaluated by two pathologists and the tumor type was determined according to the 2004 WHO classification. A tissue microarray was constructed and stained with HBME-1, Galectin-3, Thyreoglobulin, p27, PTEN, VEGF-A and the Androgen Receptor (AR).
Results: No significant differences were noted between the two groups concerning the expression of HBME-1 and the AR. In contrast, a loss of Thyreoglobulin, p27, PTEN and VEGF-A and a strong Galectin 3 expression is significantly associated with an ACO on univariate and multivariate analysis.
Conclusions: The markers HBME-1 and the AR do not have a prognostic significance. A loss of Thyreoglobulin, p27, PTEN and VEGF-A and a strong Galectin 3 expression is associated with an ACO. Further studies must show which genetic alterations are behind these observations and whether patients with such tumors may benefit from an additional therapy.

517 Vascular Invasion, Not Capsular Invasion, Predicts Local Recurrence and Metastasis in Well-Differentiated Follicular Thyroid Carcinoma

Nhu Thuy Can, Annemieke van Zante, Hooman Khaki, Elham Khanafshar. University of California, San Francisco, CA.

Background: The current treatment paradigm for follicular thyroid carcinoma includes completion thyroidectomy, radioiodine ablation and life-long suppressive doses of thyroxine. Considering the low malignant potential of well-differentiated follicular thyroid carcinoma, this approach may be overly aggressive. Four patterns of capsular invasion and four patterns of vascular invasion have been defined by Dr. John K.C. Chan in *Diagnostic Histopathology of Tumors*, Christopher D.M. Fletcher, Ed. We undertook a study of these rigorously-defined histologic patterns of invasion and outcomes in well-differentiated follicular thyroid carcinoma.
Design: The UCSF Pathology database was searched for all cases of well-differentiated follicular carcinoma diagnosed between 1997 and 2007. Slides were available for 95 specimens and were reviewed by an endocrine pathologist. When a discrepancy between the original diagnosis and the reviewer existed, a third pathologist was enlisted. Cases were classified as either follicular carcinoma or Hürthle cell carcinoma and further classified as minimally or widely invasive as defined by John K.C. Chan. The presence or absence of invasion as well as the pattern of invasion was assessed. The association between pathologic features and recurrence was evaluated by Fisher's exact test for categorical data and by Wilcoxon rank sum test for continuous data.
Results: The median length of follow-up was 4 years. Six cases had documented evidence of local recurrence and/or metastasis (6%). Recurrence was not associated with age, sex, Hürthle cell histology, extent of invasion, or the presence of any of four patterns of capsular invasion. Recurrence was significantly associated with size of tumor (p=0.08) and the presence of vascular invasion (p=0.03). Three of four defined patterns of vascular invasion were each significantly associated with recurrence, however no significant association was seen between the finding of endothelialized tumor that penetrates the fibrous capsule and protrudes into the lumen of a vessel (p=0.40).
Conclusions: Similar to prior studies, our data suggests that the presence of vascular invasion is significantly associated with recurrence in follicular thyroid carcinoma. In contrast to prior studies, recurrence was not significantly associated with capsular invasion, even when widespread. Patients diagnosed with well-differentiated follicular thyroid carcinoma may consider a less aggressive treatment regimen when vascular invasion is absent.

518 Amiodarone-Induced Thyrotoxicosis: A Study of 20 Cases, With Validation of Histologic Criteria

Mariana Canepa, Jinesh Patel, Deborah Chute, Charles Sturgis, Andrea Arrossi, Aaron Hoschar, Sanjay Mukhopadhyay. Cleveland Clinic, Cleveland, OH.

Background: Amiodarone-induced thyrotoxicosis (AIT) is a rare complication of amiodarone therapy, occasionally requiring thyroidectomy. The histologic features have been described in only a few cases, and have not been validated. The aim of this study was to validate histologic criteria for AIT based on a large series of resected thyroids.
Design: Twenty (20) cases of AIT and 31 controls (25 nodular hyperplasia, 6 palpation thyroiditis) were retrieved and several histologic features evaluated. Cases were defined as AIT if hyperthyroidism developed after initiating amiodarone therapy, and other causes of thyrotoxicosis were excluded clinically. Histologic features with discriminatory value were presented to 5 pathologists with head and neck subspecialty interest, along with a training set of 5 AIT cases and photomicrographs. These pathologists evaluated 20 cases blinded to diagnosis, case mix and medication history, and classified each case as "AIT" or "not AIT". The cases included 5 AIT (different from the training set) and 15 thyroids from patients not on amiodarone, including 10 nodular hyperplasia, 3 Graves', and 2 others; 6/15 were from hyperthyroid patients. The accuracy of histologic criteria for AIT was evaluated.
Results: The most consistent histologic features in AIT were distended follicles (100%), flat follicular cells (100%), increased foamy macrophages within follicles

(100%) and follicles with wavy-to-flat contours (75%), without nodules (10%), prominent hemosiderin (0%), scalloped colloid (0%), thyroiditis (30%, minimal), or diffuse hyperplasia (0%). In the validation set, diagnostic accuracy ranged from 75% (15/20 correct) to 95% (19/20 correct). Most (4/5) pathologists were ≥90% accurate. All 5 AIT cases were correctly identified by all 5 pathologists. One thyroid resected 4 years after cessation of amiodarone therapy was correctly identified as "not AIT" by all pathologists. Two non-AIT cases were each mis-classified by 3/5 observers as AIT, including a case of radioactive iodine-refractory Graves' disease and a completion thyroidectomy from a patient with papillary carcinoma. The sensitivity of histologic criteria for AIT was 100%; specificity varied from 67-93%.
Conclusions: Our histologic criteria for AIT, derived from the largest series of AIT reported thus far, are highly sensitive and fairly specific. Thyroids from patients not on amiodarone can occasionally mimic AIT (radioactive iodine-refractory Graves' disease, prior thyroid surgery). We show for the first time that the histologic features of AIT may regress several years after cessation of amiodarone therapy.

519 Clear Cell Thyroid Carcinoma: A Clinicopathologic and Molecular Study

Nicole Cipriani, Dora Dias-Santagata, William Faquin, Peter Sadow. University of Chicago, Chicago, IL; Massachusetts General Hospital, Boston, MA.
Background: Clear cell carcinoma of the thyroid is classified by the WHO as a variant of follicular (FTC) or papillary (PTC) thyroid carcinoma. Clear cell change (CCC) 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 and molecular characteristics of primary thyroid neoplasms with cytoplasmic clear features.
Design: Our pathology archives were queried for "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. H&E slides were reviewed, and SNaPshot multi-gene mutational analysis as well as a translocation panel were successfully performed on 15 cases.
Results: Average tumor size was 2.8 cm among 12 female and 8 male patients with an average age of 55 years (range: 26-80). 12 (57%) were FTC, 5 were conventional PTC, 2 were follicular variant (FV) of PTC, and 2 were poorly differentiated carcinoma (PDC). CCC in all cases was focal or multifocal, never diffuse. 4 of 9 evaluated FTC had RAS mutation (2 NRAS, 2 HRAS), 2 had PAX8-PPARγ translocation (1 with concurrent p53 mutation); 1 of 5 evaluated PTC had NRAS mutation, 1 had EML4-ALK translocation; 1 of 2 evaluated FV-PTC had TGF-MET translocation; 1 evaluated PDC had no mutations. 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. 1 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: CCC in thyroid carcinoma is rare, is more common in FTC than PTC, is found focally or multifocally within a given lesion, and in 33% of cases (5/15) is associated with RAS mutation. Although CCC is found in tumors classified as PTC and FTC, the molecular changes and biological behavior resemble FTC, and clear cell carcinomas should be regarded as a variant of FTC.

520 MYC Analysis By Fluorescent In Situ Hybridization and Immunohistochemistry in Primary Adrenal Angiosarcoma (PAA)

Kristine Cornejo, Lloyd Hutchinson, Maryann St. Cyr, Vania Nose, A John Iafrate, Peter Sadow. University of Massachusetts Medical School, Worcester, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA.
Background: Primary adrenal angiosarcomas (PAA) are rare with <30 cases reported in the literature. MYC protein expression and gene amplification has recently been detected in secondary angiosarcoma (AS), and a small subset of primary AS. The aim of this study was to report the clinicopathologic features of PAA and examine these rare tumors for MYC amplification and protein expression in a small case series.
Design: A total of 4 cases (resection, n=4), were obtained from the surgical pathology files of a large Medical Center between 1999-2013 with IRB approval. Three of the 4 cases had available material for immunohistochemical and molecular studies. All 3 cases were investigated for MYC gene abnormalities using interphase fluorescent in situ hybridization (FISH) and were examined by immunohistochemistry (IHC) for MYC protein expression.
Results: Tumors were in 3 females and 1 male with a mean age of 69 (53-75) years. Tumors ranged from 8.5-15 (mean 11.5) cm and classified as epithelioid in morphology. All tumors had prominent necrosis and the mitotic count ranged from 4-41/10 high-power fields (HPF) (mean 20/10 HPF, X400). Immunohistochemically, tumor cells were positive for CD31 (100%), CD34 (25%) and cytokeratin (100%). Follow-up information was available for all patients, with a mean of 10.8 (3-19) months. Three patients died of disease, 3 had distant metastases, and 1 patient was alive with disease. A positive immunohistochemical stain for MYC (nuclear staining) was identified in all 3 cases (100%) tested. Two cases (67%) showed polysomy of chromosome 8 without MYC amplification. A MYC translocation was not observed (0%).
Conclusions: PAA demonstrate epithelioid morphology with histologically malignant features such as tumor necrosis and high mitotic rates and are associated with a poor prognosis. We confirmed MYC protein expression in PAA, of which 2 of the 3 IHC-positive cases demonstrated a copy number gain in chromosome 8 which may explain this finding. One IHC-positive case was not associated with a chromosome 8/MYC gene abnormality. The discordant findings may be related to an alternative pathway, such as an epigenetic alteration, although further studies are needed. In the context of new targeted therapies, MYC-positivity in PAA may be clinically valuable in treating patients with these aggressive neoplasms.

521 Effect of Erlotinib on Proliferation and Steriodogenesis in Primary Cultures of Adrenocortical Carcinoma

Salvador Diaz-Cano, Pauline Szyszka, Gregory Weitsman, Dorota Dudka, Peter King, Marta Korbonits, Ashley Grossman, Klaus-Martin Schulte, Gabrielle Galata, David Taylor, Norman Taylor, Simon Aylwin, Krzysztof Sworczak, Stefan Bornstein, Tony Ng, Dorota Dworakowska. King's College Hospital, London, United Kingdom; King's College London, London, United Kingdom; Barts and the London Hospital, London, United Kingdom; University of Oxford, Oxford, United Kingdom; Medical University of Gdansk, Gdansk, Poland.
Background: Adrenocortical cancer (ACC) is a rare malignant endocrine tumor. Previously we found that erlotinib (inhibitor of EGFR) had an additive cytotoxic effect to standard therapy with mitotane in H295R cell line, especially after EGF stimulation. Primary cultures of ACC have been proven to be challenging to establish and maintain for further experiments, especially for assessing their steroid profile.
Design: The aim of this study was to test the effect in the use of Erlotinib on primary ACC cultures. We successfully cultured 3 ACCs. In 2 ACC cases radical surgery was a treatment of choice, whereas in the last case - surgery was after neo-adjuvant chemotherapy. Because last tumour was very heterogeneous, we cultured 2 samples from macroscopically different areas. Cell proliferation rate and the effect of the drugs were assessed by AlamarBlue assay. We used LCMS/MS to assess tumour steroids production from primary cultures derived from metastatic ACC cases.
Results: Treatment with mitotane resulted in the decrease of cell proliferation (9%±2%) in all cases at 10uM and total death at 50μM.
In one of radically operated ACCs, erlotinib (10μM) decreased proliferation by 24%, whereas in another it was not effective. Surprisingly, erlotinib has opposite effect on cell proliferation in two samples from metastatic ACC, however its combination with low dose of mitotane (10μM) was sufficient to induce total death. Furthermore the basal steroid production was different in those samples and it changed in a different manner after treatment with mitotane and/or erlotinib (with or without EGF stimulation).
Conclusions: Erlotinib can have an anti-proliferative effect in primary ACC cultures and can reduce mitotane effective concentration. For cases pre-treated with neo-adjuvant therapy, erlotinib may cause opposite responses in different parts of the same tumour; hence its clinical use needs further careful consideration. Different responses in steroidogenesis from different parts of the same ACC tumour has not been previously described underlines ACC heterogeneity.
Acknowledgments: This project was co-financed by the Foundation for Polish Science and European Union within the grant "Bridge" (POMOST/2012-5/3) to Dr Dorota Dworakowska.

522 Clinical and Immunohistochemical Features of Pancreatic Neuroendocrine Neoplasms

Michael DiMaio, Allison Zemek, John Allen, Hemamalini Vairamuthu, Raymond Balise, Pamela Kunz, Teri Longacre. Stanford University, Stanford, CA; University of Miami, Miller School of Medicine, Miami, FL.
Background: Pancreatic neuroendocrine tumorsare classified into three grades, using mitotic counts and Ki-67 indices (WHO 2010). It has been suggested that immunohistochemical expression of p16/p53 and loss of chromogranin may provide additional prognostic information.
Design: Pancreatic neuroendocrine tumors were identified in a retrospective search of the surgical pathology database of a large academic medical center from 1996-2013, and compiled into a tissue microarray. H&E and Ki-67 stained sections were examined to classify the lesions as well-differentiated neuroendocrine tumors, WHO grades 1 and 2 (WDNET); versus poorly-differentiated neuroendocrine carcinomas, WHO grade 3 (PDNEC). Immunohistochemistry for p16, p53 and chromogranin was performed on each tumor. Kaplan-Meier analysis was used to calculate overall survival (OS). Cox proportional hazards models were constructed to assess significance of differentiation, p16, p53 and chromogranin with survival.
Results: 168 pancreatic neuroendocrine tumorswere identified. Median age was 56 years, 92 (55%) were male, 160 (95%) were WDNET, 8 (5%) were PDNEC. The majority of cases were p16/p53 negative and chromogranin positive. The 5-year OS rate for the entire cohort was 82%. Of the PDNEC, p16/p53 positive cases had worse OS. A univariate Cox regression analysis demonstrated that PDNEC was associated with an increased hazard of death with a HR of 27.1 ($p<0.0001$), as was p53 expression with a HR of 50.6 ($p<.0001$). However, only PDNEC maintained this association in a bivariate Cox analysis ($p<0.001$).
Conclusions: In this population-based collection of pancreatic neuroendocrine tumors, patients were predominantly young with WDNET. As expected, PDNEC had worse OS compared to WDNET. Of PDNEC, p16/p53 positive cases had poorer OS compared to p16/p53 negatives cases. Both PDNEC and p53 expression were associated with increased hazard of death in univariate Cox regression analysis. In bivariate Cox analysis, only PDNEC maintained this association. Our analysis was limited by low case numbers for PDNEC and p16/p53 expression. Further studies are needed to determine whether p16, p53 and chromogranin need to be incorporated into the WHO grading system for pancreatic neuroendocrine tumors.

523 Fine Needle Aspiration Diagnoses of Non-Infiltrative, Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma

Michael Drage, Brooke Howitt, Jeffrey Krane, Justine Barletta. Brigham & Women's Hospital, Boston, MA.

Background: Recent studies have demonstrated that encapsulated or partially-encapsulated/well-circumscribed (non-infiltrative) follicular variant of papillary thyroid carcinoma (FVPTC) in the absence of capsular penetration or lymphovascular invasion (non-invasive) has virtually no metastatic potential or risk of recurrence. As the indolent behavior of these non-infiltrative, non-invasive FVPTCs is increasingly recognized, pathologists have begun to question whether the term carcinoma is warranted. If terminology for these tumors changes, this would impact the rate of malignancy of fine needle aspiration (FNA) diagnostic categories. However, the preceding spectrum of FNA diagnoses for these tumors has not been established. Therefore, the aim of this study was to determine the preceding FNA diagnoses of non-infiltrative, non-invasive FVPTC.

Design: We identified 72 consecutively resected non-infiltrative, non-invasive FVPTCs. The diagnoses of the preceding FNAs according to The Bethesda System for Reporting Thyroid Cytopathology were recorded, and the preceding FNA diagnosis associated with the highest risk of malignancy was identified for each case.

Results: Our cohort included 72 tumors from 56 (78%) women and 16 (22%) men, with a mean age of 53 years at diagnosis (range 29-82 years). Fifty-one (71%) had one preceding FNA, 20 (28%) had two preceding FNAs, and 1 (1%) had three preceding FNAs. The preceding FNA diagnosis associated with the highest risk of malignancy was malignant (positive for PTC) in 5 (6.9%), suspicious for PTC (SUS) in 35 (48.6%), suspicious for follicular neoplasm (FOL) in 7 (9.7%), atypia/follicular lesion of undetermined significance (AUS/FLUS) in 13 (18.1%), benign in 9 (12.5%), and nondiagnostic in 3 (4.2%).

Conclusions: While non-infiltrative, non-invasive FVPTCs often have subtle or focal nuclear features of PTC, in our study a significant number (55.5%) were associated with a preceding FNA diagnosis of SUS or malignant. If the terminology for non-infiltrative, non-invasive FVPTCs were to change and they were no longer considered carcinoma, this would not only impact the rate of malignancy for AUS and FOL, but also the SUS and even the malignant category.

524 BRAFV600E Status in Solitary and Multiple Papillary Thyroid Carcinoma and in Their Lymph-Node and Distant Metastases: An Immunochemical and Genotypic Retrospective Study of 363 Consecutive Patients

Caroline Eymerit-Morin, Camille Buffet, Geraldine Cancel-Tassin, Mouawad Roger, Cecile Gaffory, Annette Lesot, Gilles Le Naour, Genevieve Herve, Johanna Wassermann, David Khayat, Christophe Tresallet, Laurence Leenhardt, Fabrice Menegaux, Marie-Christine Rousselet, Frederique Capron, Frederique Tissier. CHU, Angers, France; HUPS, AP-HP, UPMC, Sorbonne Universités, Paris, France; HUPS, Paris, France; UPMC, Laboratoire A.V.E.C, HUPS, Paris, France.

Background: BRAFV600E mutation is the most common genetic alteration in papillary thyroid cancer (PTC). The presence of this mutation is significantly associated with increased cancer-related events among patients with PTC. A few studies suggest that BRAFV600E immunoshistochemistry (IHC) staining might be a promising tool for patient stratification.

Design: The genotypic and IHC BRAFV600E status was evaluated in 363 patients histologically diagnosed with PTC between July 2012 and July 2013. Of them, 338 were diagnosed with a primary PTC including 96 patients with synchronous lymph node metastases (SLNM), and 25 with metachronous metastasis (MM). The expression of the mutated BRAFV600E was performed in all cases of PTC on whole slides and on TMA using a mutation-specific antibody VE1. The IHC analyses were interpreted independently by 2 pathologists blinded to the genotyping results. The sensitivity and specificity were compared with genotyping method using the Competitive Allele Specific hydrolysis probes (TaqMan) PCR technology on FFPE tumor samples.

Results: A total of 885 whole slides by IHC and 198 tumor samples by genotyping were analyzed. The sensitivity and specificity were 99% and 93% for monoclonal VE1 compared to genotyping. There were 4 discordant cases compared to genotyping and 6 cases had an equivocal staining by IHC. Interobserver agreement for IHC detection was almost perfect for both whole slides (κ= 0.84) and TMA (κ= 0.87). Concordance between the staining (TMA & whole sections) and genotyping was substantial (κ=0.76;κ=0.72). BRAFV600E mutation was associated with the classic ([147/190] 77% vs. 58%; p<0.001), and the oncocytic variant ([63/83] 76%; p=0.004) of PTC. This mutation was also associated with SLNM (86%; p<0.001), MM (76%; p=0.005), extrathyroidal extension (75%; p<0.001) and multifocality (p=0.001). An inverse correlation was observed with the follicular variant ([151/337] 45%; p<0.001) or diffuse sclerosing ([1/9] 11%; p=0.02).

Conclusions: Our study showed that IHC is an accurate method for the detection of the BRAFV600E mutation. Thereafter, molecular techniques could be used in ambiguous, equivocal or non-contributive cases.

525 BRAFV600E Mutation Influence in Microscopic Papillary Thyroid Carcinoma – A 10-Year Follow-Up

Armando Gamboa-Dominguez, Mariana Tenorio-Serralta, Daniel Montante Montes de Oca. National Institute of Medical Sciences and Nutrition, Mexico City, Tlalpan, DF, Mexico.

Background: BRAF mutations are observed in 49% PTC and associated with adverse outcome in short term studies. Objective: identify the influence of BRAFV600E mutation in papillary microcarcinoma with more than 10y follow-up.

Design: A retrospective review of PTC in a third level hospital including demographic, clinical, surgical and morphological data. Representative slides were submitted to immunohistochemistry with BRAFV600E mutation specific antibody (clone VE1), and the stain evaluated as percentage/intensity of positive cells. Macrodissection was perfomed in all available tumors for direct sequencing. ROC curves to identify a threshold of VE1 reactivity were performed.

Results: 1342 thyroid specimens were evaluated and 511 PTC identified. 59(4.39%) were microcarcinoma with a male:female ratio of 1:14, multifocality in 51%. Median tumor size was 7mm and cervical metastases observed in 42%. Subtotal thyroidectomies were achieved in 50 cases. All but four patients receiven 131I. Tumor contact with thyroid pseudocapsule was observed in 13 patients. BRAFV600E reactivity range from 3-100% of tumor cells and from 1+ to 3+ intensity. Atrofic zones in Hashimoto´s thyroiditis depicted positive cells in 8 glands. Once sequenced, 56% of tumors had BRAFV600E mutation. More than 50% of stained tumor cells correlated with the presence of BRAFV600E mutation. After 10y follow-up, 55 patients had uneventful course; three presented local recurrences and one patient mediastinal metastases. All four had BRAFV600E mutation and tumors peripherally located. Adverse outcome was observed in microcarcinomas in contact with the thyroid pseudocapsule (p=0.001), with extra thyroidal disease at initial presentation (p=0.02) and, with BRAFV600E mutation.

Conclusions: Reactivity with the BRAFV600E mutation specific antibody should be carefully evaluated in papillary microcarcinoma; after sequencing, less than 50% of positive cells in a given tumor does not correlated with BRAFV600E mutation. This mutation was observed in 56% of microcarcinomas and a few of them with adverse outcome.

526 Prognostic Impact of Extent of Vascular Invasion in Low-Grade Encapsulated Follicular Cell Derived Thyroid Carcinomas: A Clinico-Pathologic Study of 276 Cases

Ronald Ghossein, Bin Xu, Laura Wang, R Michael Tuttle, Ian Ganly. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Continuous controversy surrounds the predictive value of vascular invasion (VI) and especially its extent in low grade encapsulated follicular cell derived thyroid carcinomas (LGEFC). In addition, some guidelines advocate conservative therapy in LGEFC with focal VI. There is therefore a need to assess the survival rates of LGEFC patients with various degrees of VI in order to better stratify patients for completion thyroidectomy and adjuvant radioactive iodine (RAI) therapy. Furthermore, the prognostic effect of VI within the different current histotypes of LGEFT is not well known and needs to be investigated.

Design: Two hundred seventy six patients with LGEFC from a tertiary care center were subjected to a meticulous histopathologic analysis. They were classified as encapsulated papillary thyroid carcinoma (EPTC), encapsulated follicular carcinoma (EFC) and encapsulated Hurthle cell carcinoma (EHCC). Poorly differentiated and anaplastic carcinoma were excluded.

Results: Of the 276 patients, 24 (9%) had extensive VI (>=4 foci) and 28 (10%) displayed focal (<4 foci) VI. EHCC and EFC showed a much higher rate of extensive VI (EVI) than EPTC (p< 0.001). EVI correlated with extensive capsular invasion (p<0.01) and extensive extra-thyroid extension (p=0.02). Median follow up (FU) was 6.4 years. All 14 tumors with adverse behavior harbored distant metastases (DM) (of which 9 had DM at presentation). 10 (42%) of 24 patients with EVI had poor outcome. All 3 patients without EVI who had aggressive carcinomas harbored distant metastasis (DM) at presentation. EVI was an independent predictor of poor disease free survival (p=0.01). Excluding cases with DM at presentation, only patients with EVI recurred (P < 0.001) and all relapsed cases were EHCC (p < 0.001). With a median FU of 4.4 years, none of 17 patients with focal VI and no RAI therapy recurred despite a median age of 52 years and a median tumor size of 3 cm.

Conclusions: 1) EVI is an independent predictor of disease free survival in LGEFC and should prompt aggressive therapy. 2) EHCC with EVI have a particularly high risk of recurrence 3) When DM are not found at presentation, patients with focal VI seem to be at a very low risk of recurrence even if not treated with RAI. In cases of LGEFC with focal VI and absence of other aggressive features, it remains to be confirmed whether or not such patients should receive RAI therapy.

527 TROP-2: Diagnostic Utility in Separating Benign and Malignant Thyroid Nodules

Reuben Jacob, Cynthia Cohen, Momin Siddiqui. Emory University Hospital, Atlanta, GA.

Background: TROP-2 is a cell surface protein that is selectively overexpressed in a variety of epithelial cancers, such as papillary thyroid carcinoma (PTC). However there is minimal expression in normal tissue. TROP-2 has recently been studied as a potential Immunohistochemical (IHC) marker for PTC. In this study, we expanded on this work by considering its expression in follicular variant of PTC (FVPTC), follicular carcinoma (FC), anaplastic carcinoma (AC), medullary carcinoma (MC), follicular adenoma (FA) and benign thyroid nodules (BTN).

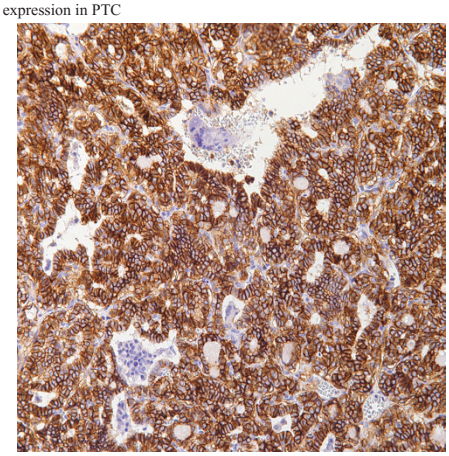
Design: IHC for TROP-2 expression was performed on 331 normal thyroid/benign and malignant tumor tissue sections in tissue microarray (TMA), consisting of PTC (60), FVPTC (48), FC (27), AC (14), MC (2), FA (8) and BTN (172). Membranous staining in greater than 5% of tumor cells was considered positive.

Results:

TROP-2 expression in 331 tissue sections in TMA.		
Diagnosis (n)	Positive	Negative
PTC (60)	54 (90%)	6
FVPTC (48)	9 (18.8%)	39
FC (27)	1 (3.7%)	26
AC (14)	3 (21.4%)	11
MC (2)	0	2
FA (8)	0	8
BTN (172)	0	172

Statistical analysis of TROP-2 expression.				
Diagnosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PTC	90	95.2	80.6	97.7
FVPTC	18.8	79.5	13.4	85.2
FC	3.7	78.3	1.49	90.2
AC	21.4	79.8	4.48	95.8

TROP-2 IHC expression in PTC



Conclusions: TROP-2 is highly sensitive (90%) and specific (95.2%) for the identification of PTC. TROP-2 does not appear to be an optimal marker for FVPTC, FC, AC and MC. This study clearly identifies a strong role for TROP-2 in separating PTC from benign nodules (FA and BTN).

528 Clinical and Pathologic Predictors of Lymph Node Metastasis and Recurrence in Papillary Thyroid Microcarcinoma

Daniel Johnson, Allison Cavallo, Saaduddin Siddiqui, Peter Angelos, Raymon Grogan, Edwin Kaplan, Richard DeMay, Tatjana Antic, Nicole Cipriani. University of Chicago Medical Center, Chicago, IL.

Background: The incidence of thyroid cancer has nearly tripled over the past 35 years, possibly accounted for by the increased incidence of papillary thyroid microcarcinomas (PTMC, £1 cm). Prognosis of PTMC is very good (15-year disease specific survival up to 99% and recurrence rates as low as 5%). Treatment varies by institution and may include lymph node dissection, as lymph node metastasis has been shown to decrease overall survival. Clinical and pathologic features predictive of lymph node metastasis or recurrence may help guide treatment. Predictive features have varied amongst published studies. Accordingly, further investigation is needed.

Design: We retrospectively identified patients diagnosed with PTMC (2000-2011) and gathered clinical and pathologic data onage, race, primary tumor size, multifocality, thyroiditis, extrathyroidal extension (ETE), surgical margins, clinical suspicion (FNA suspicious or positive for malignancy), lymph node metastasis, treatment, recurrence, and survival. A total of 273 patients were included. Statistical analyses were performed in STATA.

Results: 163 patients (60%) underwent central and/or lateral neck dissection. 38 of these (23%) had lymph node metastasis (LNM): 20 central LNM (CLNM); 14 lateral LNM (LLNM); 4 both. Multivariate logistic regression showed multifocality (OR=3.251, 95% CI=1.380-7.660, p=0.007) and ETE (OR=6.046, 95% CI=1.834-19.938, p=0.003) were significant risk factors for having any lymph node metastasis. Age ≤45 (OR=4.559, 95% CI=1.358-15.306), multifocality (OR=3.567, 95% CI=1.055-12.056) and ETE (OR=4.933, 95% CI=1.102-22.081) significantly increased the risk of CLNM while only ETE (OR=16.048, 95% CI=1.902-135.405) significantly increased the risk of LLNM. Neither tumor size nor clinical suspicion predicted CLNM or LLNM. 6 of 202 patients (3%) with follow-up data had locoregional recurrence. All 6 had tumors ^{3}0.5 cm, LNM at diagnosis, and at least one additional high-risk feature (ETE or multifocality). There were no deaths due to PTMC.

Conclusions: In the current study, 23% of PTMC cases had LNM. ETE and multifocality were major predictors of LNM while size and pre-operative suspicion were not. Aggressive management of PTMCs can be considered in patients with high-risk features (ETE, multifocality, age ≤45) regardless of size and pre-operative suspicion for malignancy.

529 Does Thyroid Nodule Size Affect the Accuracy of Cytology?

Daniel Johnson, Allison Cavallo, Saaduddin Siddiqui, Raymon Grogan, Peter Angelos, Edwin Kaplan, Richard DeMay, Tatjana Antic, Nicole Cipriani. University of Chicago Medical Center, Chicago, IL.

Background: The incidence of thyroid cancer has nearly tripled over the past 35 years and thyroid nodules are estimated to exist in nearly 50% of the adult population based on autopsy studies. Fine needle aspiration (FNA)-based cytologic diagnoses often guide surgical intervention. Recent studies have argued that large thyroid nodules have a greater rate of malignancy but are not associated with a decrease in accuracy of cytology (Kamran, et al 2013). Others have observed up to 10% "false-negative" rates (malignant histology of a nodule with benign cytology) in nodules ≥4cm, leading to recommendation of surgery as management of these nodules (Wharry, et al 2013). Given the critical importance of cytologic diagnosis in clinical management, we attempt to investigate whether thyroid nodule size impacts the accuracy of FNA.

Design: We identified 541 nodules with both cytologic and histologic diagnoses (2011-2013) in our pathology archives. Nodules were correlated using location, size, ultrasound characteristics, and gross examination at resection. We categorized FNA diagnoses into 4 groups [non-diagnostic (Bethesda I), benign (Bethesda II), indeterminate (Bethesda III-IV), and suspicious or positive for malignancy (Bethesda V-VI)] and histologic diagnoses as either benign or malignant.

Results:

Table: Percent malignancy stratified by ultrasound size and Bethesda category

Bethesda	0-4 cm	≥ 4 cm
I	5/21 (23.8%)	1/3 (33.3%)
II	3/118 (2.5%)	2/36 (8.6%)
III-IV	47/171 (27.5%)	7/30 (23.3%)
V-VI	136/149 (91.3%)	11/14 (78.6%)
ALL	191/459 (41.6%)	21/83 (25.3%)

Conclusions: The "false-negative" rate of benign FNA diagnoses in nodules >4cm (5.6%) was slightly less than that reported by Wharry, et al (10%), and was slightly higher than that of smaller nodules (2.6%). Both malignant nodules >4cm were follicular variant of papillary carcinoma. One nodule ≤4cm was follicular carcinoma and 2 were papillary carcinoma. Interestingly, the overall rate of malignancy in nodules >4cm was substantially less than in those ≤4cm (25.3 vs. 41.7%, respectively). Additionally, nodules >6cm, had a "false-negative" rate of 0% (n=9). We believe that increased nodule size does not have a direct effect on FNA accuracy, however, increasing nodule size may lead to inadequate sampling of follicular variants of papillary carcinoma, in which cytologic atypia may be patchy.

530 Prevalence of RAS Mutations in Well-Circumscribed Follicular Variant of Papillary Thyroid Carcinoma and Concurrent Benign Nodules

Daniel Johnson, Larissa Furtado, Nicole Cipriani. University of Chicago Medical Center, Chicago, IL.

Background: Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy and accounts for ~80% of all thyroid cancers in the United States. Follicular variant of PTC (FVPTC) can be clinically and genetically divided into an infiltrative type (with BRAF mutation and risk for nodal metastases) and a well-circumscribed or encapsulated type (with RAS mutation and indolent behavior if non-infiltrative). We aim to analyze benign thyroid nodules with bland or minimally atypical nuclear features arising in the context of well-circumscribed FVPTC.

Design: 14 patients (10 with FVPTC and benign nodules; 4 with only benign bland or minimally atypical nodules) were identified. DNA was successfully isolated from 23 nodules and mutational analysis for 45 commonly-mutated genes (including *BRAF*, *HRAS*, *KRAS*, *NRAS*) was performed (AmpliSeq).

Results: 6 of 9 FVPTCs had *RAS* mutations (3 *NRAS*, 2 *HRAS*, 1 *KRAS*). Identical mutations were found in concurrent benign nodules in 4 patients. 3 FVPTC had variants of unknown significance (VUS):1) *KDR* mutation in FVPTC and two concurrent benign nodules in the same patient; 2) *PI3KCA* mutation in FVPTC and a *PTEN* mutaton in a concurrent benign nodule in the same patient; 3) concomitant *VHL* and *ATM* mutations in FVPTC. Additional *RAS* mutations were found in benign nodules in 3 patients.

Genetics	Bland	Atypia	FVPTC
Normal	2	2	0
VUS	2	1	3
RAS mutation	4	3	6

Conclusions: In well-circumcsribed FVPTC, *RAS* mutations were a frequent occurrence (6 of 9, 67%); *BRAF* mutations were not identified. Similar *RAS* mutations were found in 7 of 14 (50%) of benign nodules with and without minimal nuclear atypia. These findings suggest that FVPTC may lie along a spectrum with benign and minimally atypical nodules, and, as recent literature has suggested, may behave in an indolent manner if non-infiltrative. Follicular-patterned thyroid nodules may perhaps best be classified based on the presence or absence of infiltrative growth rather than on nuclear features, as they demonstrate genetic profiles and clinical behavior similar to benign thyroid nodules.

531 Value of Core-Needle Biopsy for the Preoperative Diagnosis of Follicular Neoplasm in Thyroid Nodule Screening

Chan Kwon Jung, Sung Hak Lee, So Lyung Jung, Min-Hee Kim, Ja Seong Bae, Dong Jun Lim, Youn Soo Lee. The Catholic University of Korea, Seoul St. Mary's Hospital, Seoul, Korea.

Background: Ultrasound-guided core-needle biopsy (CNB) has increasingly been used as an alternative to fine needle aspiration cytology (FNAC) for the preoperative diagnosis of thyroid nodules. CNB has been reported to have a lower non-diagnostic rate and a higher diagnostic accuracy for malignancy than FNAC. But, there has been concern about the over-diagnosis of follicular neoplasm (FN) in thyroid nodule screening by CNB, leading to unnecessary surgery. We recently published the new diagnostic criteria for FN on the CNB specimen. This study aimed to evaluate the diagnostic role of CNB for FN and revalidate the usefulness of our diagnostic method.

Design: We retrospectively reviewed prospectively collected data on 172 CNBs and 117 FNACs diagnosed with FN/suspicious for FN. The diagnosis was confirmed histologically in all cases after surgical resection.

Results: A total of 289 specimens included 229 conventional and 60 Hürthle cell type cases. There was no statistical difference in malignancy rates of FN/suspicious for FN between CNB and FNAC (43.0% versus 48.7%, respectively, p=0.340). Encapsulated follicular variant of papillary carcinoma (EFVPC) were the most common subtype in malignant tumors in both groups. After excluding non-invasive EFVPC, the rates of relatively more aggressive malignancies were similar in both groups (22.7% and 27.4% in CNB and FNAC, respectively, p=0.365).

Conclusions: The diagnosis of FN was straightforward in CNB specimens with application of our histological criteria. CNB has increased the diagnostic yield and sensitivity for the diagnosis of FN in the thyroid nodule screening, but the risk of malignancy in patients with a diagnosis of FN was similar in both CNB and FNAC groups.

532 Cytokeratin Immunoreactivity in Paragangliomas: A Comparative Study of Spinal and Extra-Spinal Paragangliomas

Seema Khutti, Christy Valles, Jason Burghardt, Richard Cartun, Srinivas Mandavilli. Hartford Hospital, Hartford, CT.

Background: The diagnosis of paraganglioma can be challenging and requires an immunohistochemical (IHC) work-up to exclude metastatic neuroendocrine tumors and carcinomas. An IHC panel in such scenarios would include synaptophysin, chromogranin, S-100 and cytokeratin (CK). CK is typically negative in paragangliomas, but CK expression has been described anecdotally in spinal paragangliomas which can be a pitfall in excluding metastatic carcinoma and neuroendocrine tumors. The aim of this study was to evaluate CK expression in paragangliomas and compare profiles of spinal with extra-spinal paragangliomas.

Design: 11 cases of paragangliomas were retrieved including 5 cases of spinal and 6 cases of extra-spinal paragangliomas. The following CK antibodies were applied to all cases: Pan-cytokeratins (clones AE1/AE3 and MNF116/Dako), CK-8/18 (clone 5D3/ Leica), and CK-5/14 (clones EP1601Y/LL002/Cell Marque). Previously performed IHC tests for synaptophysin,chromogranin and S-100 were also reviewed.

Results: All 5 spinal paragangliomas showed diffuse, strong reactivity for MNF116 (CK-5/6/8/17/19) and 5D3 (CK-8/18), and were negative for AE1/AE3 (pan CK) and EP1601/LL002 (CK-5/14). All 6 extra-spinal paragangliomas were negative for all CK antibodies. All cases showed diffuse, strong reactivity for synaptophysin, chromogranin. S-100 highlighted sustentacular cells: focally in spinal paragangliomas and more diffusely in extra-spinal paragangliomas.

Table 1:Cytokeratin reactivites in spinal paragangliomas

Sr .No	Site	CKMNF116	CK5D3	AE1/AE3	CK5/14
1	L3	P	P	N	N
2	L2-L4	P	P	N	N
3	L3-L4	P	P	N	N
4	L2-L3	P	P	N	N
5	L3	P	P	N	N

(P=strong and diffuse staining, N=Negative).

Conclusions: 1.Spinal paragangliomas express low-molecular weight cytokeratins (CK-8 and possibly CK-18) which are targets of CKMNF116 and 5D3. This can be a pitfall since CK reactivity expression in paragangliomas is not well-described or recognized by pathologists.

2.Laboratories using Pan-CK AE1/AE3 may not see this result due to the poor efficiency of AE1/AE3 in identifying CK-8 in formalin-fixed tissue. Hence, AE1/AE3 should be the preferred "pancytokeratin" antibody in an IHC panel to distinguish spinal paraganglioma from carcinomas and neuroendocrine tumors

3.Extra-spinal paragangliomas of a variety of locations are negative for all four CK antibodies used in this study and it is unclear why such differences exist in CK expression between spinal and extra-spinal paragangliomas.

533 Clinical Impact of the Minor Proportion of Poorly Differentiated Component in Thyroid Carcinoma

Tetsuo Kondo, Tadao Nakazawa, Junko Akaishi, Kunio Mochizuki, Naoki Oishi, Koichi Itoh, Ryohei Katoh. University of Yamanashi, Chuo, Yamanashi, Japan; Ito Hopital, Shibuya, Tokyo, Japan.

Background: Poorly differentiated carcinoma (PDC) is a rare histological subtype in thyroid carcinoma. It is composed of solid, trabecular and/or insular architecture (so-called poorly differentiated component) and lack of typical nuclear features of papillary carcinoma. In general, poorly differentiated area constitutes a majority of the tumor. However, poorly differentiated component could be present as a minor component in differentiated thyroid carcinoma, and its clinical importance is still to be elucidated.

Design: The purpose of the current study is to clarify the significance of the proportion of poorly differentiated component in thyroid carcinoma. We retrieved 73 cases of thyroid carcinoma that have poorly differentiated component in Ito Hospital (Japan) during 2005 to 2011 years. Based on WHO 2014 criteria and Turin proposal, these tumors were classified into PDCs (24 cases), solid variant follicular carcinomas and solid variant papillary carcinomas. PDCs were further divided the tumors with 17 cases of major PDC component (over 50%) and 7 cases of minor PDC component (under 50%). Differentiated component in the tumors of minor PDC component were follicular carcinoma in all cases.

Results: We compared the clininopathological factors between major PDCs and minor PDCs. Mean age were 56 y.o (29-78 y.o.) and 64 y.o.(30-72 y.o), in respectively Sex ratio (male: female) were 1:3.3 and 1:15. All of major PDCs were preoperative diagnosed as "malignant" or "suspicious of malignant" category by fine needle aspiration, but none of minor PDCs. Tumor sizes were 47mm (12-80mm) and 61mm (45-100mm). Extrathyroidal invasion were 24% (4/17) and 14% (1/7). Lymph node metastases were 29% (5/17) and 29% (5/7). Distant metastases were found 53% (9/17) and 71% (5/7). Three cases of major PDC and one case of minor PDC were dead by the disease.

Conclusions: We found that the thyroid carcinoma with minor component of poorly differentiated area has a potential of aggressive behavior. Even if PDC is minor component, we should judge as high aggressive tumor especially when these components reached the criteria of Turin proposal.

534 Immunohistochemical Expression of NKX2.2 in 1434 Human Tumors

Haiyan Liu, Fan Lin. Geisinger Medical Center, Danville, PA.

Background: NKX2.2 is a homeodomain transcription factor associated with neurodevelopmental processes, which has been recently reported as a useful marker for diagnosis of Ewing sarcoma. NKX2.2 regulates development of gut serotonin cells and is claimed to be a marker for gastrointestinal neuroendocrine tumors (NETs). Expression of NKX2.2 in tumors from other organs is limited. In this study, we conducted an immunohistochemical (IHC) analysis of NKX2.2 expression in 1434 tumors from various organs to further explore its diagnostic utility.

Design: IHC analysis of NKX2.2 (74.5A5, DSHB) expression was conducted on tissue microarray sections of 1434 tumors (including 198 NETs) from various organs. The nuclear staining was interpreted as negative, 1+ to 4+.

Results: The tumor types and staining results are summarized in the Table. Of the 198 NETs, colorectal NETs showed the highest expression of NKX2.2 (72%); NETs of the pancreas, stomach and small intestine expressed NKX2.2 in slightly over 50% of cases, while the majority of pulmonary neuroendocrine carcinoma (NE CA) and cutaneous NETs lacked NKX2.2 expression. Other non-NETs, except one endometrial adenocarcinoma (ADC) and one lung ADC, were negative for NKX2.2.

NKX2.2 Expression in 1434 Tumors

Tumor	N. of Positive/Total	Percentage
NE CA of Lung	1/40	2.5%
Pancreatic NET	25/47	53%
Colorectal NET	26/36	72%
Stomach/small bowel NET	10/37	51%
Skin NET	0/38	0%
Pituitary adenoma	2/29	7%
Endometrial CA	3/268	1%
Endocervical ADC	0/39	0%
PSCa and CCCa	0/110	0%
Lung ADC	1/206	0.5%
Prostatic ADC	0/22	0%
Urothelial CA	0/50	0%
Renal cell CA	0/120	0%
Breast CA	0/102	0%
Pancreatic ADC	0/56	0%
Esophageal ADC	0/48	0%
Colorectal ADC	0/86	0%
GIST	0/34	0%
Mesothelioma	0/17	0%
SqCC of ENT	0/74	0%
Hepatocellular CA	0/36	0%
Melanoma	0/93	0%

PSCa:papillary serous CA; CCCa:clear cell CA; GIST:gastrointestinal stromal tumor.

Conclusions: Our data suggest that NKX2.2 is a highly specific and relatively sensitive immunomarker for NETs, especially for gastrointestinal (including pancreatic) primary. NKX2.2 can potentially serve as a neuroendocrine marker in daily practice.

535 Glucose Transporter-1 (GLUT-1) Expression and Its Association With Epithelial-Mesenchymal Transition (EMT) in Thyroid Tumor Progression

Rakesh Mandal, Heather Hardin, Darya Buehler, Wei Huang, Herbert Chen, Tomas Prolla, Ricardo Lloyd. University of Wisconsin, Madison, WI.

Background: Malignant cells have a reduced ability to use oxidative metabolism and are dependent on an increase in glycolysis. They show an increased rate of glucose transport across the plasma membrane. Thyroid cancers have an increased ability to transport glucose compared to normal thyroid tissue which is regulated by GLUT-1 and other glucose transporters. EMT is associated with thyroid cancer progression and increased expression of specific protein markers including the transcription factor Slug and loss of E-Cadherin. We examined the relationship between GLUT-1 expression and EMT during tumor progression in a group of thyroid tumors.

Design: A tissue microarray (TMA) with normal thyroid (NT, n=10), nodular goiters (NG, n=10), follicular adenomas (FA, n=32), follicular thyroid carcinomas (FTC, n=32), papillary thyroid carcinomas (PTC, n=28), follicular variant of PTC (FVPTC, n=29) and anaplastic thyroid carcinomas (ATC, n=10) were analyzed by immunohistochemistry (IHC) on a TMA with antibodies to GLUT-1(Novus, 1:100). E-Cadherin (Cell Signaling, 1:400) and Slug (Cell Signaling, 1:100) using formalin-fixed paraffin-embedded tissues (FFPE). IHC was scored based on the percentage and intensity of staining. qRT-PCR was also done for GLUT-1 using FFPE tissue sections.

Results: GLUT-1 showed distinct cell membrane staining. The highest level of GLUT-1 was detected in ATC (100%), with a smaller percentage of cells staining positively in FVPTC (13%), PTC (14 %), FTC (7 %), and FA (6%). E-Cadherin was expressed in all NT, FA, FTC, PTC and FVPTC (100%), but was seen only focally in a few ATC (30%). In contrast Slug, which is a marker of EMT, was highly expressed in ATC (80%), but not in any of the other tumors or NT. GLUT-1 mRNA expression in a subset of the FFPE tissue sections supported the IHC findings with the highest levels of GLUT-1 in the ATCs.

Conclusions: GLUT-1 is upregulated during tumor progression and may play a major role in glucose uptake in thyroid cancer cells. The increase in Slug expression and loss of E-Cadherin suggest that there are concurrent changes in glucose transport during EMT and thyroid tumor progression.

536 Clusterin in Neuroendocrine Epithelial Neoplasms: Absence of Expression in a Well-Differentiated Tumor Suggests a Jejunoileal Origin

Emily McMullen, Jason Hornick, Andrew Bellizzi. University of Iowa Hospitals and Clinics, Iowa City, IA; Brigham & Women's Hospital, Boston, MA.

Background: Clusterin, an apoptosis-associated glycoprotein, is a diagnostic marker of several tumors including anaplastic large cell lymphoma and tenosynovial giant cell tumor. A recent study has suggested it is highly expressed by neuroendocrine tumors (WDNETs) arising at most anatomic sites, with the exception of jejunoileal tumors, and that it is similarly not expressed by neuroendocrine carcinomas (PDNECs). We sought to validate this result in a large cohort of WDNETs and PDNECs.

Design: Clusterin immunohistochemistry was performed on tissue microarrays of 255 WDNETs [45 lung, 4 stomach, 8 duodenum, 75 pancreas (62 primary, 13 metastatic), 107 jejunoileum (69 primary, 38 metastatic), 16 appendix] and 88 PDNECs (43 small cell carcinoma, 45 Merkel cell carcinoma). Tumors were arrayed in triplicate. Extent (%) and intensity (0, 1+, 2+, 3+) of staining were assessed and an H-score (extent x intensity) calculated. An average H-score >5 was considered positive.

Results: Clusterin expression was noted in 82.4% of 148 non-jejunoileal WDNETs (average H-score 183) and only 8.4% of 107 jejunoileal WDNETs (average H-score 31), as well as 19.3% of PDNECs (average H-score 36). Detailed expression data are presented in the Tables.

Clusterin Expression in WDNETs	% Positive	Average H-score (if positive)
Lung (n=45)	73.3	141
Stomach (n=4)	50	165
Duodenum (n=8)	62.5	224
Pancreas Primary (n=62)	88.7	215
Pancreas Metastasis (n=13)	100	215
Jejunoileum Primary (n=69)	5.8	11
Jejunoileum Metastasis (n=38)	13.2	50
Appendix (n=16)	93.8	138

Clusterin Expression in PDNECs	% Positive	Average H-score (if positive)
Small Cell Carcinoma (n=43)	21	51
Merkel Cell Carcinoma (n=45)	18	22

Conclusions: Clusterin is frequently and strongly expressed by WDNETs of diverse anatomic sites, with the exception of jejunoileal tumors, in which it is only rarely and weakly expressed. It is occasionally, weakly expressed by PDNECs. Most metastatic WDNETs of occult origin arise in the pancreas (in which clusterin is especially strongly expressed) or the jejunoileum. As the midgut WDNET marker CDX2 is only expressed by 85% of metastases, **absence** of clusterin expression in such a tumor could represent a key diagnostic adjunct in suggesting a jejunoileal origin.

537 GRIA2 Expression in Neuroendocrine Epithelial Neoplasms

Emily McMullen, Jason Hornick, Andrew Bellizzi. University of Iowa Hospitals and Clinics, Iowa City, IA; Brigham & Women's Hospital, Boston, MA.

Background: Glutamate receptor, ionotropic, AMPA2 (GRIA2), an excitatory neurotransmitter receptor subunit, is highly expressed in the brain. GRIA2 has emerged as a diagnostic marker of solitary fibrous tumor, which we have on-boarded in our immunohistochemistry (IHC) laboratory. A previous gene expression profiling experiment identified GRIA2 as highly expressed by jejunoileal neuroendocrine tumors (WDNETs). We hypothesized that GRIA2 might represent a general neuroendocrine marker (e.g., synaptophysin, chromogranin) or be expressed in a site of origin-restricted manner (e.g., TTF-1, lung; CDX2, midgut; Islet 1, pancreas).

Design: GRIA2 IHC was performed on tissue microarrays of 257 WDNETs (46 lung, 4 stomach, 8 duodenum, 62 pancreas primary, 13 pancreas metastatic, 69 jejunoileum primary, 39 jejunoileum metastatic, 16 appendix) and 88 poorly differentiated neuroendocrine carcinomas (PDNECs; 43 small cell carcinoma, 45 Merkel cell carcinoma), using a rabbit monoclonal antibody (EP929Y, Abcam). Tumors were arrayed in triplicate. Extent (%) and intensity (0, 1+, 2+, 3+) of staining were assessed and an H-score (extent x intensity) calculated. An average H-score >5 was considered positive.

Results: GRIA2 expression was noted in 14.4% (37/257) of WDNETs and 2.3% (2/88) of PDNECs. Although GRIA2-positivity was most frequent in jejunoileal primaries, it was also seen at other sites in the midgut and foregut. Interestingly, GRIA2 expression was downregulated in jejunoileal metastases (2.6%, H-score 17) versus primaries (26.2%, H-score 89). In 9 of 28 (32%) matched jejunoileal primary-metastasis pairs, GRIA2 was expressed in the primary and not the metastasis, while in the remaining 19 GRIA2 was expressed by neither. Detailed expression data are presented in the Tables.

GRIA2 Expression in WDNETs	% Positive	Average H-score (if positive)
Lung	23.9	67
Stomach	0	NA
Duodenum	12.5	255
Pancreas Primary	4.8	46
Pancreas Metastasis	0	NA
Jejunoileum Primary	26.2	89
Jejunoileum Metastasis	2.6	17
Appendix	18.8	113

GRIA2 Expression in PDNECs	% Positive	Average H-score (if positive)
Small Cell Carcinoma	0	NA
Merkel Cell Carcinoma	4	45

Conclusions: GRIA2 is expressed by a significant minority of WDNETs, regardless of site of origin. In contrast, GRIA2 is only very rarely expressed by PDNECs. GRIA2 appears downregulated in metastatic jejunoileal WDNETs, suggesting a possible role in tumor progression.

538 Primary and Recurrent/Metastatic Adrenal Cortical Carcinoma: An Immunohistochemical Study Evaluating Ki-67, p53 and B-Catenin as Predictors of Metastatic Potential

Vikas Mehta, Sahussapont Sirintrapun, Yingbei Chen, Hikmat Al-Ahmadie, Samson Fine, Satish Tickoo, Victor Reuter, Anuradha Gopalan. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Adrenal cortical carcinoma (ACC) is a rare, aggressive cancer which often presents at an advanced stage with distant metastases. Published reports suggest that Ki-67 index and *TP53/CTNNB1* mutations are associated with worse outcome in ACC. We performed immunohistochemical (IHC) evaluation of Ki-67, B-catenin (BCAT) and p53 in primary ACC (PRIM) and for a subgroup, their paired recurrence/metastasis (MET), and correlated expression with outcome data to assess whether they were predictive of metastatic potential.

Design: 37 PRIM were included, of which 17 had matched MET. IHC for Ki-67, p53 and BCAT was performed on triplicate cores from all cases arrayed on a TMA. Ki-67 was recorded as a continuous variable from 0-100%. BCAT was scored as nuclear (N), nuclear & cytoplasmic (N+C), cytoplasmic only (C) and absent. Nuclear p53 staining was scored as 0(0-5%), 1(6-25%), 2(26-50%) and 3(51-100%). Follow-up data was available in 29 cases.

Results: Based on the outcome over an average follow-up of 46 months (range, 1-132), the cohort was divided into 2 groups; group 1, who developed metastasis (23) and group 2, who did not (14). The IHC findings are as follows:

IHC marker	Group 1	Group 2	p-value
Ki-67, range (mean)	3-80 (24%)	1-45 (15%)	0.26
TP53 (score of 2/3)	7(30%)	4(28%)	0.56
Nuclear BCAT	4(17%)	2(14)	0.41

MET sites included liver (9), lung (2), colon (1), retroperitoneal (RP) lymph nodes (4) and pelvic/ RP soft tissue (3). In 6 patients >1 MET sample was available for evaluation. In the subset of group 1 with matched PRIM/MET (n=17), mean Ki-67% in the PRIM was 23 (range 3-80) and 50 (range 10-90) in the MET (p=0.002), while p53 and BCAT staining did not shown any significant difference. 14 pairs showed

classical ACC morphology; 1 pair was an oncocytic ACC; 2 cases showed heterogeneous morphology in the PRIM while the MET showed uniform histologic features resembling classical ACC.

Conclusions: ♦ Ki-67 index was not associated with metastasis in ACC in our cohort, unlike that some studies have suggested.

♦ Similarly, although at the molecular level *TP53* and *CTNNB1* mutations have been reported to show worse outcome, nuclear immunostaining for p53 and B-catenin were not associated with metastasis in this cohort.

♦ Nuclear p53/BCAT expression was higher/more frequent in metastatic ACC as compared to the matched primaries. Further investigations on a bigger dataset would possibly help to clarify the significance of these findings.

539 Clinicopathologic Features of Pituitary Acidophil Stem Cell Adenomas

Ozgur Mete, Karen Gomez-Hernandez, Shereen Ezzat, Sylvia Asa. University Health Network, University of Toronto, Toronto, ON, Canada.

Background: Acidophil stem cell adenoma (ASCA) is a rare histologic subtype of prolactin (PRL)-producing adenoma. We intestigated the clinicopathological features of these tumors to define their characteristics.

Design: A retrospective review of institutional pathology records from 2001 to 2014 identified 18 ASCAs from a cohort of 863 adenomas (2%). Clinical, biochemical and radiologic features, immunohistochemical and ultrastructural characteristics of these tumors were reviewed.

Results: This cohort included 10 females and 8 males. The mean age at diagnosis was 30.9 (range, 11-71) years. Eight patients referred for pathology consultation only had incomplete endocrine data. Of the remaining 10, 3 had acromegaly and hyperprolactinemia, and 7 had hyperprolactinemia alone. PRL levels ranged from 133.1 to 800 µg/L. All tumors were macroadenomas with a mean size of 2.48 cm (range, 1.4-5 cm). Imaging details were unavailable for 8 patients; among the other 10, combined infrasellar and parasellar growth was identified in 2, and infrasellar and/or parasellar growth with suprasellar extension was identified in 4 cases. Only 3 tumors had suprasellar extension alone, and 1 tumor had no extrasellar extension. All patients with follow-up data had residual/recurrent disease. All tumors had oncocytic morphology; all were positive for Pit-1, estrogen receptor alpha and PRL, and negative for thyrotropin. PRL staining was diffusely cytoplasmic in all but 2 cases that exhibited paranuclear globular positivity. Focal growth hormone reactivity was noted in 10 tumors. Three tumors were negative with the CAM5.2 antibody, 12 had fibrous bodies (prominent in 2; scattered in 10) and 3 had a perinuclear keratin pattern. The mean Ki-67 labeling index was 8.14% (range, 1-20%). Ten tumors subjected to ultrastructural examination showed monomorphous oncocytic cells with numerous large and/or dilated mitochondria and variable fibrous bodies; nuclear spheridia were seen in 1 case.

Conclusions: The distinction of ASCAs from other PRL-producing adenomas is clinically important given their aggressive behavior. With the exception of oncocytic change, ASCAs resemble silent subytpe III adenomas that are actually poorly differentiated plurihormonal adenomas of Pit-1 lineage; both tumor types are characterized by infra- and para-sellar extension and recurrence. We propose the classification of these tumors as "oncocytic poorly differentiated Pit-1 lineage adenomas".

540 Pituitary Silent Subtype III Adenomas Are Not Always Silent and Represents Pit-1 Lineage Adenomas

Ozgur Mete, Karen Gomez Hernandez, Shereen Ezzat, Sylvia Asa. University Health Network and University of Toronto, Toronto, ON, Canada.

Background: Originally classified as the third variant of silent corticotroph adenoma, silent subtype III adenomas (SSIIIAs) represent a distinct histologic variant of pituitary adenomas. We reviewed the clinicopathological characteristics of this rare tumor to justify the need for a change in the terminology.

Design: A retrospective review of institutional pathology records from 2001 to mid-2014 revealed 26 SSIIIAs from a total of 863 adenomas (~3%). Clinical, biochemical, radiological, immunohistochemical and ultrastructural features of these tumors were reviewed.

Results: This cohort included 15 females and 11 males. The mean age at diagnosis 44.34 (range, 11-75) years. Eight patients had incomplete endocrine data. Of the remaining 18, 1 had mild acromegaly, 1 had clinical and biochemical evidence of prolactin (PRL) excess leading amenorrhea-galactorrhea syndrome, 3 presented with clinical and biochemical evidence of hyperthyroidism characterized by increased thyrotropin (TSH) and free thyroxine, 5 had mildly elevated PRL levels (range, 18.2-91.1µg/l) consistent with stalk compression, and 8 had no evidence of hormone excess. All tumors were macroadenomas with a mean size of 2.66 cm (range, 1.2-6.1cm). Radiologic imaging details were unavailabe for 9 cases; among the other 17, infrasellar and/or parasellar growth was identified in 11 (9 with variable suprasellar growth), and 6 had only suprasellar growth. 12 of 16 patients with follow-up data had residual/recurrent disease. All tumors were positive for Pit-1. While 3 cases expressed only Pit-1, others revealed variable positivity for growth hormone (GH), PRL, TSH, alpha-subunit, and estrogen receptor. Four cases were negative for keratins with the CAM5.2 antibody, 14 and 8 showed perinuclear and diffuse keratin positivity respectively. Scattered fibrous bodies were noted in 3 cases. The mean MIB-1 labeling index was 4.2% (range, 1-9%). 12 cases with available ultrastructural analysis showed large polygonal or elongated cells with nuclear spheridia.

Conclusions: SSIIIAs are aggressive monomorphous plurihormonal Pit-1 lineage adenomas which may be associated with hyperprolactinemia, acromegaly or hyperthyroidism. These findings argue against the use of the nomenclature "silent" for these tumors. Instead, we propose the use of "poorly differentiated Pit-1 lineage adenoma" for SSIIIAs.

541 The Value of Biomarkers in the Classification of Pituitary Adenomas

Ozgur Mete, Amber Cintosun, Irwin Pressman, Sylvia Asa. University Health Network, University of Toronto, Toronto, ON, Canada; Carleton University, Toronto, ON, Canada.

Background: The classification of pituitary adenomas is recognized to predict targeted treatments and prognostic information. We reviewed a large series of pituitary adenomas to determine the role of transcription factors and biomarkers in the accurate classification of these tumors.

Design: Retrospective analysis of 863 pituitary adenomas in a series of 1020 transsphenoidal resection specimens from 2001 to 2014 included demographical data and immunohistochemical features including transcription factors (Pit-1, ER, SF-1), hormones and other biomarkers (CAM5.2, MIB-1, p27, FGFR4) analyzed using SPSS.

Results: 52.7% of patients were females and the average age was 48.9 years. 72 (8%) tumors had an incomplete workup. Gonadotroph adenomas (GAs, 336; 39%) were the most common histological subtype followed by corticotroph (154; 18%), GH-producing (146; 17%), PRL-producing (76; 9%), Null cell (45; 5.2%), Silent subtype III (25; 2.9%), TSH-producing (7; 0.8%), and unusual plurihormonal (2; 0.2%) adenomas. Densely (53; 36%) and sparsely (52; 36%) granulated somatotroph adenomas co-dominated GH-producing tumors. Sparsely granulated lactotroph adenoma (51;67%) was the most common subtype of PRL-producing tumor. Both FSH (sensitivity:47%; specificity:100%) and LH (sensitivity:21%; specificity:100%) immunohistochemistry have limited value in the diagnosis of GAs. Positivity for SF-1 (sensitivity:82%; specificity:100%), was the most valuable marker of GA, with ER (sensitivity:79%; specificity:100%), alpha-subunit (sensitivity:64%; specificity:100%), and alpha-HCG (sensitivity:40%; specificity:100%) providing additional value. Prominent fibrous bodies were exclusively identified in sparsely granulated somatotroph adenomas. Corticotroph adenomas showed significant loss of p27. FGFR4 expression was highest in unusual plurihormonal adenomas with decreasing expression in null cell, gonadotroph, sparsely granulated corticotroph, thyrotroph, and silent subtype III adenomas. Invasive tumors with a MIB-1 ³3% (so-called atypical adenomas) comprised 53% of this cohort, and were most often acidophil stem cell, densely granulated lactotroph, sparsely granulated corticotroph, and silent subtype III adenomas.

Conclusions: Our results justify the application of immunohistochemistry for transcription factors and other biomarkers in addition to hormones for accurate classification of pituitary adenomas. The term "atypical adenoma" does not seem to reflect any biological superiority to aggressive histologic subtypes determined by the accurate classification.

542 Clinico-Pathologic and Survival Analysis of 211 Gastroenteropancreatic G3 Neuroendocrine Carcinomas (GEP-NECs)

Massimo Milione, Alessio Pellegrinelli, Patrick Maisonneuve, Francesca Spada, Paola Spaggiari, Luca Albarello, Massimo Barberis, Alessandro Vanoli, Perfetti Vittorio, Buzzoni Roberto, Sara Pusceddu, Laura Concas, Barbara Martinelli, Ester Antelmi, Carlo Carnaghi, Marco Manzoni, Nicola Fazio, Fausto Sessa, Enrico Solcia, Carlo Capella, Stefano La Rosa. IRCCS Foundation National Cancer Institute, Milan, Italy; European Institute of oncology, IEO, Milan, Italy; Cancer Center Humanitas, Milan, Italy; San Raffaele Hospital Milan, Milan, Italy; IRCCS San Matteo, Pavia, Italy; Ospedale di Circolo and University of Insubria, Varese, Italy.

Background: The 2010 WHO classification defined GEP-NECs as large or small cell cancers with a Ki-67 labeling index (Ki-67)>20% and/or a Mitotic Index (MI)>20 mitoses x10 HPF, while Mixed AdenoNeuroendocrine Carcinomas (MANECs) are mixed exocrine-neuroendocrine neoplasms. NECs are generally associated with a poor prognosis, although recent published findings have suggested that there is a fraction of patients with GEP-NEC/MANECs showing a better than expected survival rate. The present study correlates the prognosis of patients with GEP-NECs or MANECs with proliferation and other clinico-pathologic features.

Design: Two hundred eleven NECs or MANECs diagnosed between 1995 and 2011 in 6 referral Italian Centers were included into the study. Specimens from all patients were revalued by 9 expert pathologists and stratified into the following subgroups: (i) NECs vs. MANECs; (ii) 'low proliferative' (LP) NECs (i.e. mitotic count ≤20 and Ki-67 between 20% and 55%) vs. 'highly proliferative' (HP) NECs (mitotic count >20 and Ki-67>55%) vs. MANECs.

Results: There were 136 NEC patients, 42 with LP-NECs and 94 with HP-NECs. The remaining 75 neoplasms were MANECs. The median follow-up time of the study was 13 months. Median overall survival (MOS) was 12 months in NECs and 18 months in MANECs (p=0.10). However, when NECs were further stratified into LP and HP subgroups, a different MOS was found. In detail, MOS was 29 months in LP-NECs, 7 months in HP-NECs and 18 months in MANECs (p=0.002). At 6 months, the survival rate was 90% in the LP-NEC group, 53% in the HP-NEC patients and 83% in MANECs. Corresponding figures at 12 months were 87%, 39% and 65%, respectively; those at 36 months were 31%, 14% and 24%, respectively. CD117 expression and vascular invasion were also statistically associated with a poor prognosis.

Conclusions: MI≤20 and a Ki67 index between 20% and 55% identified a subgroup of NEC patients with better prognosis than that of MANECs or NECs showing MI>20 and Ki-67>55%.

543 Proteomic Profiling of Adrenocortical Carcinoma

Gabriella Nesi, Raffaella Santi, Andrea Galli, Gianna Baroni, Monica Pepi, Massimo Mannelli, Michaela Luconi. University of Florence, Florence, Italy.

Background: Adrenocortical carcinoma (ACC) is a rare malignancy with dismal prognosis and lack of effective therapeutic options.

Design: In order to evaluate a differential protein expression profile between ACCs and normal adrenal tissue a proteomic approach by means of the two-dimensional difference in-gel electrophoresis (DIGE) technique was carried out.

Results: Mass spectrometry associated with DIGE analysis of ACC (n=10) and normal adrenal (n=8) samples identified 27 proteins, which were differentially expressed (fold variation <-2 or >2, p<0.05) in neoplastic compared to normal adrenal tissue. At DIGE analysis, these proteins were overexpressed in ACC, except for thiosulfate sulfurtransferase that was downregulated. Aldehyde dehydrogenase 6 A1 (ALDH6A1), transferrin, fascin-1, lamin A/C, adenylate cyclase-associated protein 1 (CAP1) and adrenodoxin reductase (ADX-Red) overexpression was then validated by Western Blotting (fold increase±SE 7.5±1.4, 3.6±1.2, 2.9±0.2, 2.6±2.1, 1.9±1.4, 1.6±0.8, p<0.05, respectively). Immunohistochemistry for ALDH6A1, transferrin and fascin-1, performed on ACC and normal adrenal FFPE samples, showed strong positive staining in ACCs and absence of immunoreactivity in normal adrenal cortex. Lamin A/C, CAP1 and ADX were diffusely expressed by neoplastic cells with normal adrenal tissue staining less intensely.
Conclusions: These preliminary findings indicate a different proteomic profile in ACCs compared to normal adrenals. The overexpressed proteins could represent novel biomarkers to improve ACC management.

544 BRAF V600E Mutation Status in Anaplastic Thyroid Carcinoma

Daisuke Nonaka, Sarah Rushton, George Burghel, Andrew Wallace. The Christie Hospital, Manchester, United Kingdom; University of Manchester, Manchester, United Kingdom; Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.
Background: Anaplastic thyroid carcinoma (ATC) is an uncommon thyroid cancer, and the majority arises as a result of anaplastic transformation (dedifferentiation) of a preexisting well-differentiated tumor of various types, therefore, it constitutes a molecularly heterogeneous group, shared by a universally aggressive clinical course. A number of studies have shown that BRAF V600E mutant-specific antibody VE1 can identify BRAF V600E mutants in a spectrum of cancers, including thyroid cancers. This study focuses on the utility of this antibody for BRAF V600E screening in ATCs.
Design: Thirty nine ATCs (31 resections, 8 biopsies) were studied using both DNA BRAF testing (pyrosequencing) and the VE1 antibody. When the tumor also contained a differentiated element such as papillary thyroid carcinoma (PTC) within the same mass, each component was separately evaluated for VE1 expression, and tested for PCR testing by macrodissecting the respective component. The intensity of VE1 expression was graded from 0 (negative) to 3+ (strong).
Results: Among the 39 tumors, 13 tumors were associated with differentiated carcinoma, including 2 poorly differentiated carcinomas (PCAs), 2 combined follicular carcinomas & PCAs, 2 Hurthle cell carcinomas, and 7 PTCs (3 tall cell variant/TC, 2 follicular variant, 1 solid variant, and 1 conventional type). BRAF V600E was identified in four ATCs (10.3%), three of which were associated with TC PTC. The TC component was individually analysed, showing BRAF V600E. Immunohistochemically all four ATCs were moderately to strongly positive for VE1 so were all three TC PTC components. Additionally, seven ATCs showed 1+ reaction, and they corresponded to wild type BRAF.
Conclusions: VE1 immunohistochemistry is a specific and sensitive method for the detection of BRAF when moderate to strong reaction is regarded as positive. In BRAF V600E mutated tumors, both ATC and PTC component were equally positive for VE1, confirming the conventional notion that some of the ATCs are transformed (dedifferentiated) from differentiated thyroid tumor. VE1 may be useful not only for screening BRAF V600E mutation status in ATCs but also for confirming the diagnosis of ATCs, in conjunction with clinical information and other immunohistochemistry stains, when dealing with undifferentiated tumor in the thyroid region.

545 Clinico-Pathologic Study of Goblet Cell Carcinoid of the Appendix

Daisuke Nonaka, Angela Lamarca, Paul Fulford, Juan Valle, Bipasha Chakrabarty. The Christie Hospital, Manchester, United Kingdom; University of Manchester, Manchester, United Kingdom.
Background: Goblet cell carcinoids (GCCs) of the appendix are rare tumors with dual neuroendocrine and mucinous differentiation, and can undergo high grade transformation. Tang LH, et al, proposed classification into 3 groups; typical GCC (Group A) and adenocarcinoma ex-GCC, which was further subdivided into signet ring cell type (Group B) and poorly differentiated adenocarcinoma type (Group C), and prognosis was most favorable in Group A, followed by B and C. We carried out a retrospective study of 69 GCCs to find prognostic values of a variety of clinical and pathological features, with special emphasis on the prognostic relevance of histologic subclassification.
Design: Sixty nine GCCs were reviewed, and the tumor was subtyped using the criteria proposed by Tang L et al. When a tumor contained more than one subtype, the more unfavorable subtype, when accounting for more than 10%, was chosen. Correlations between survival and the following clinico-pathological parameters were investigated: age, sex, symptoms, size, stage, perineural invasion, venous invasion, lymphovascular invasion, type of surgery, resection margin status, and Tang classification (3-tier: A, B, C; 2-tier: A, B/C).
Results: Age at diagnosis ranged25-75years (mean, 56.8), with M:F=1:1.57. There were 33 cases in Group A, 31 in B, and 5 in C. Follow-up ranged 3.8-218.9 months (median 30.5). Median overall survival was 52.1months. By univariate analysis, Tang classification (both 3 and 2 tiers, p<0.001), TNM staging, perineural invasion, and type of surgery correlated with disease specific survival, and by multivariate analysis, Tang classification with 2-tier system (p=0.047) and TNM staging (p=0.018) correlated with survival but not Tang classification with 3-tier system.
Conclusions: Our results indicate that both Tang classification and staging provide important prognostic information, therefore, subtype of GCC should be documented in pathology report and incorporated in clinical management. Although Tang 3 tier system correlated with survival only by univariate analysis but not multivariate analysis, Groups B and C, which represent high grade spectrum of GCC, are morphologically distinct, therefore, separate subcategory may still be justified.

546 BRAF and TERT Promoter Mutations in Papillary Thyroid Carcinoma of Young Age

Naoki Oishi, Tetsuo Kondo, Tadao Nakazawa, Kunio Mochizuki, Kazunari Kasai, Tomohiro Inoue, Ippei Tahara, Jieying Wang, Tomonori Yabuta, Mitsuyoshi Hirokawa, Akira Miyauchi, Ryohei Katoh. University of Yamanashi, Chuo, Yamanashi, Japan; Kuma Hospital, Kobe, Hyogo, Japan.
Background: Papillary thyroid carcinoma (PTC) affecting young age is rare but clinicopathologically distinct from adult PTC. Despite its aggressive nature, the outcome of young PTC is favorable. Moreover, after Fukushima Daiichi nuclear disaster there has been increasing concern about the risk for PTC in Japanese juvenile. However, molecular pathogenesis of young PTC has not been fully elucidated.
Design: PTC patient aged 20 or under was defined as young PTC. Total 81 cases of Japanese young PTCs operated from 1991 to 2013 were enrolled. As adult control group, 83 PTCs aged over 20 were also examined. *BRAF*^{V600E} and *TERT* promoter mutations were analyzed by allele-specific PCR and Sanger sequencing, respectively. Correlations between the mutations and clinicopathological characteristics were evaluated.
Results: Of 81 cases with young PTC, 81.5% (66/81), 9.9% (8/81) and 4.9% (4/81) were classified as classical PTC, solid variant/solid follicular variant (SV/SFV) and diffuse sclerosing variant (DSV), respectively. As compared with adults, young PTCs exhibited significantly larger tumor size ($p < .01$) and more frequent lymph node metastasis ($p < .01$). *BRAF*^{V600E} mutation frequency in young PTCs was significantly lower than adults, 37.0% (30/81) vs. 81.9% (68/83) ($p < .0001$). The entire mutant cases of young PTC were confined in classical PTCs, and none of SV/SFV and DSV exhibited the mutation. In both young and adult PTCs, there was no association between *BRAF*^{V600E} and aggressive clinicopathological features. Furthermore, the *BRAF* mutation frequency in young PTCs exhibited no significant alternations over 23 years. Whereas *TERT* mutations were identified in 12.0% (10/83) of adults, none of young PTCs exhibited the mutation ($p < .001$).

	Young PTCs (aged 20 or under)	Adult PTCs (aged over 20)	p-value
BRAFV600E			
Positive	30/81 (37.0%)	68/83 (81.9%)	
Negative	51/81 (63.0%)	15/83 (18.1%)	
TERT promoter mutation			
C250T	0/81 (0.0%)	2/77 (2.6%)	
C228T	0/81 (0.0%)	8/77 (10.4%)	
Wild type	81/81 (100%)	67/77 (87.0%)	

Conclusions: This is the largest molecular study on young PTCs. PTCs of young age are characterized by more aggressive clinicopathological features, lower frequency of *BRAF*^{V600E} mutation and complete absence of *TERT* promoter mutation. The lack of *TERT* promoter mutation rather than infrequent *BRAF* mutation may explain the favorable prognosis of young PTCs.

547 High Immunohistochemical Expression of Heat Shock Protein90 in the Metastatic NET/NEC in the Liver: A Next Generation Target?

Robert Osamura, Midori Matsuda, Chie Inomoto, Hiroshi Kajiwara. International University of Health and Welfare, Tokyo, Japan; Tokai University School of Medicine, Isehara, Japan.
Background: Heat shock protein (HSP) 90 is a molecular chaperone that plays an indispensable role by regulating its target proteins and cell proliferation. High HSP90 expression has been reported to be associated with poor prognosis in gastric cancer. And HSP90 inhibitors have been on clinical trial as anti-cancer therapy. It has been also shown that anti-HSP90 molecules exhibit anti-proliferative effects on NEC cell lines. This study is aimed at to elucidate the immunohistochemical expression of HSP90 in the metastatic NET/NEC in the liver in order to clarify the possible role of HSP90 protein as a new therapeutic target. The results of HSP90 staining were compared with those of somatostatin receptor(SSTR)2, SSTR5 and phosphorylated(p)-mTOR.
Design: Among the referred cases, we extracted 12 cases of metastatic NET G1(4cases), NET G2(1 case) and NEC(7 cases) in the liver (primary sites; pancreas 5 case, rectum 3 cases, stomach 2 cases, duodenum 1 case, gallbladder 1 case). Formalin-fixed paraffin embedded (FFPE) tissue sections were subjected to the immunohistochemical staining by using the antibodies against HSP90 (ab13495 Abcam), SSTR2 and SSTR5 (EPITOMICS) and p-mTOR (Cell Signaling). HSP90 staining was interpreted as positive if the cytoplasm of the tumor cells was stained. Staining intensity(0,1+,2+,3+) and proportion of positive cells(%) were estimated. SSTR2 and SSTR5 were interpreted score(S)0, S1, S2, S3 according to Volante et al. (2007), S2 and S3 being positive. P-mTOR was positive when cytoplasm was stained.
Results: HSP90 was positive in 11 of 12 cases. All 11 cases revealed high intensity(3+), and many positive tumor cells (100%: 8cases, 90%: 1 case, 80%: 1 cases, 70%: 1 case). SSTR2 was positive in 8 of 12 cases[S3(7/12), S2(1/12)], SSTR5 positive in 5 cases[S3(2/12), S2(3/12)], S1(1/12), S0(3/12) and p-mTOR was positive(8/12). One case of metastatic rectal NET G1 was negative for HSP90 and p-mTOR but was positive for SSTR2 and SSTR5.
Conclusions: In many(11/12) cases of the metastatic NET/NEC cases, HSP90 was positive very strongly and diffusely in the cytoplasm of the tumor cells. These results

suggest that the HSP90 should be a possible next generation target for the effective molecular target therapy with corresponding inhibitors, such as AUY922 and HSP990 in, the SSTR-negative or p-mTOR- negative cases, or in the SSTR-positive and p-mTOR-positive cases which are resistant to Octreotide or Everolimus therapies.

548 Immunohistochemical Detection of Therapeutic Biomarkers, SSTR2, SSTR5 and p-mTOR, in Various Human Pituitary Adenomas

Robert Osamura, Midori Matsuda, Chie Inomoto, Hiroshi Kajiwara, Akira Matsuno, Akira Teramoto. International University of Health and Welfare Mita Hospital, Tokyo, Japan; Tokai University School of Medicine, Isehara, Japan; Teikyo University School of Medicine, Tokyo, Japan; Tokyo Rosai Hospital, Tokyo, Japan.

Background: Molecular targeted therapy on pituitary adenomas now include Octreotide, Pasireotide and Everolimus which target somatostatin receptor(SSTR)2, SSTR5 and phosphorylated(p)-mTOR , respectively. This immunohistochemical(IHC) study is aimed at to elucidate the IHC expression of SSTR2, SSTR5 and p-mTOR in order to suggest the appropriate therapeutic options in various types of pituitary adenomas.

Design: From our FFPE tissue bank of pituitary adenomas, 33 representative cases have been extracted for the IHC study. The types of tumors, numbers, gender(Male/Female), ages of the cases are as follows; GHomas(5 cases, M4/F1, 25-66 y/o), PRLomas(5 cases, M4/F1, 21-59), TSHomas(5 cases, 3/2, 40-73), ACTHomas(8 cases, M5/F2, 30-72), Gonadotropin adenomas(Gn-omas)(5 cases, M4/F1, 55-83), Null cell adenomas(5 cases, M4/F1, 59-71). IHC detection was performed on FFPE tissue sections using the antibodies against SSTR2 and SSTR5(EPITOMICS) and p-mTOR(Cell Signaling). Interpretation of IHC staining was done according to scores(S) by Volante et al. (2007); negative(S0, S1), positive(S2, S3 stained on cell membrane). P-mTOR was interpreted positive(+) when cytoplasmic staining was observed.

Results: For the various types of pituitary adenomas, the results of IHC detection of SSTR2, SSTR5 and p-mTOR were as follows.

GHomas: SSTR2 S3(5/5), SSTR5 S2(4/5),S1(1/5), p-mTOR +(5/5), PRLomas: SSTR2 S0(5/5), SSTR5 S0-1(5/5), p-mTOR +(3/5), TSHomas: SSTR2 S3(5/5), SSTR5 S2-3(5/5), p-mTOR +(3/5), ACTHomas: SSTR2 S2(1/8), S0(7/8), SSTR5 S2-3(7/8), S1(1/8), p-mTOR +(8/8), Gn-omas: SSTR2 S2(5/5), SSTR5 S2(2/5), S0-1(3/5), p-mTOR +(1/5), Null cell adenomas: SSTR2 S2(2/5), S0(3/5), SSTR5 S0-1(5/5), p-mTOR +(1/5).

Conclusions: GHomas and TSHomas revealed high incidence of SSTR2, SSTR5 and p-mTOR suggesting various therapeutic options. For ACTHomas which are low in SSTR2 and high in SSTR5 and p-mTOR, Pasireotide and Evelorimus are suggested to be effective. Gn-omas with high SSTR2 expression may respond to Octreotide. Null cell adenomas and PRLomas demonstrated the least expression of these biomarkers. Thus, various types of pituitary adenomas revealed their unique expression of therapeutic biomarkers according to the hormone-specific groups which suggests the role of IHC detection of these biomarkers in order to pursue appropriate treatment.

549 Absence of BRAF V600E Supports the Indolent Nature of Non-Infiltrative, Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma

Vera Paulson, Brooke Howitt, Justine Barletta. Brigham & Women's Hospital, Boston, MA.

Background: Identifying which thyroid tumors are indolent is important to avoid overtreatment. Recent studies have described an infiltrative form of FVPTC that has significant metastatic potential and a risk of recurrence, and an encapsulated or partially-encapsulated/well-circumscribed form (i.e. non-infiltrative) that, in the absence of capsular penetration or lymphovascular invasion (i.e. non-invasive), has virtually no metastatic potential or risk of recurrence. While some studies have demonstrated a lack of *BRAF* V600E mutation in small cohorts of these tumors, other studies have reported conflicting results. Immunohistochemistry (IHC) for BRAF V600E has been shown to be strongly correlated with *BRAF* mutation status. The aim of this study was to evaluate *BRAF* mutation status using IHC as a correlate in a relatively large cohort of non-infiltrative, non-invasive FVPTCs.

Design: We identified 79 consecutively resected non-infiltrative, non-invasive FVPTCs. All tumor slides were reviewed to confirm that the tumors were encapsulated, partially-encapsulated, or well-circumscribed and lacked infiltrative growth, capsular invasion, and lymphovascular invasion. IHC for BRAF V600E (VE1) was performed. A melanoma sample with a known *BRAF* V600E mutation was used as a positive control. Homogeneous, strong cytoplasmic staining was scored as positive (BRAF-Pos) and no staining (or a focal weak cytoplasmic or nuclear blush) was scored as negative (BRAF-Neg). Clinicopathologic parameters recorded included: gender, age at diagnosis, and tumor size.

Results: Our cohort included 79 non-infiltrative, non-invasive FVPTCs from 62 (78.4%) women and 17 (21.6%) men, with a mean age of 53 years at diagnosis (range 27-87 years). Tumors ranged in size from 1.0 to 6.5 cm, with a mean size of 2.5 cm. All of the tumors were BRAF-Neg by IHC. While some cases showed non-specific staining of colloid and/or a weak nuclear blush, this staining was clearly distinguishable from the diffuse cytoplasmic staining seen in the positive control.

Conclusions: All 79 non-infiltrative, non-invasive FVPTCs in our cohort were negative for BRAF V600E by IHC. This finding provides additional evidence that these are indolent tumors that should be distinguished from infiltrative FVPTC and classical type PTC.

550 Molecular Analysis of Oncocytic Thyroid Carcinomas

Vera Paulson, Yonghui Jia, Elizabeth Garcia, Neal Lindeman, Justine Barletta. Brigham & Women's Hospital, Boston, MA.

Background: While oncocytic thyroid carcinomas (OTCs) account for a small subset of thyroid carcinomas overall, understanding the molecular alterations of these tumors would be clinically valuable since metastatic OTCs are often refractory to radioactive iodine treatment. The aim of our study was to investigate the molecular alterations of a cohort of OTCs to further understand the pathobiology of these tumors and to evaluate for potential targetable mutations.

Design: Slides from 16 OTCs were reviewed to confirm the diagnosis, and DNA was extracted from formalin-fixed paraffin-embedded tumor blocks. Targeted next generation sequencing (Illumina HiSeq 2500) was performed on libraries prepared with custom designed hybrid capture (Agilent Sure Select) baits of ~5000 exons from 275 genes to identify mutations, insertion/deletions, and copy number variations (CNVs) plus 91 introns of 30 genes to identify cancer-associated rearrangements. Mutations identified were evaluated for frequency of alteration, sequence coverage, and presence in the Catologue of Somatic Mutations in Cancer (COSMIC) database. Novel mutations were assessed for potential functional impact using MutationAssessor, which predicts the likelihood that an amino acid change will affect protein function using evolutionary conservation patterns.

Results: Our cohort included OTCs from 10 women and 6 men with a mean age of 56 years at diagnosis (range 27-75 years) and a mean size of 3.5 cm (range 1.3-6.5 cm). Approximately two-thirds of cases demonstrated aneuploidy. The most frequent CNVs included the loss of chromosomes 2 (7 cases, 44%), 9p/9 (7 cases, 44%), and 22 (5 cases, 31%) and gains of chromosome 17 (5 cases, 31%), and 7, 12, 16, and 20 (4 cases each, 25%). The most frequent focal segmental amplification involved *NKX2-1* which was observed in 6 cases (38%). Mutations and focal deletions were observed in a subset of cases and included a known activating mutation of *NRAS* (Q61R), a nonsense frameshift mutation in *CDKN1A*, a deletion of *CDKN1B*, a known pathogenic mutation in *p53*, and novel mutations of uncertain significance predicted to affect protein function in *APC*, *MYCN*, *RET*, *VEGFR2*, *VEGFR3*, *KEAP1*, and *MSH6*.

Conclusions: The vast majority of OTCs in our cohort lacked known molecular alterations associated with well differentiated thyroid carcinomas indicating that OTCs represent a distinct group at the genetic level.

551 Overlapping Morphologic and Immunohistochemical Features of Hashimoto Thyroiditis and IgG4-Related Sclerosing Disease in the Thyroid

Philipp Raess, Arlette Habashi, Edward El-Rassi, Mira Milas, Megan Troxell. Oregon Health & Science University, Portland, OR; University of Kansas, Kansas City, KS.

Background: Immunoglobulin G4-related sclerosing disease (IgG4-RSD) is an emerging clinicopathological entity characterized by both IgG4+ plasma cell infiltration and fibrosis in one or more organs, prototypically pancreas or salivary/lacrimal glands. IgG4-RSD in the thyroid is an area of active study, and the relationship between IgG4-RSD and Hashimoto thyroiditis is not fully delineated due to their overlapping histologic features. Increased levels of IgG4+ plasma cells have also been identified in conditions not related to IgG4-RSD.

Design: Of 606 patients undergoing thyroidectomy at OHSU from 2010-2013, 38 cases (6%) of chronic or lymphocytic thyroiditis were selected by searching the pathology database and excluding thyroidectomies performed for malignancy. Clinical information was abstracted from the electronic medical record. IgG4 and IgG immunostaining was performed on a representative block of each case. IgG4+ and IgG+ plasma cells were counted in the 3 high power fields (hpf) with the highest density of cells. Histologic features associated with IgG4-RSD were semi-quantitatively assessed.

Results: Patients were predominantly female (35/38) with a mean age of 50 years (range 17-84 years). Approximately half (22/38) of patients had a clinical diagnosis of Hashimoto thyroiditis (HT). 9/38 patients had increased absolute and relative numbers of IgG4+ plasma cells. Patients with a clinical diagnosis of HT had increased lymphoplasmacytic inflammation, but the relative proportion of IgG4+ plasma cells was not increased compared to patients without HT. There was no positive correlation between IgG4 levels and the amount of fibrosis in patients with or without HT. Patients identified as having the fibrosing variant of HT were not more likely to have increased levels of IgG4+ plasma cells than those without (1 IgG4+ high; 5 IgG4+ low).

Conclusions: No correlation between amount of IgG4+ plasma cells (number and proportion) and histologic features of IgG4-RSD was identified in an analysis of consecutive thyroidectomy specimens collected over a four year period at a single institution. A wide range of IgG4 plasma cell levels was identified, but amount of IgG4+ plasma cells did not correlate with increased fibrosis. Given the morphologic and immunohistochemical overlap between HT and IgG4-RSD, future studies to identify specific characteristics of IgG4-RSD involving the thyroid are necessary to define this entity.

552 INSM1 Expression Correlates With Metastasis and Functional Hormone Production in Neuroendocrine Neoplasms

Jason Rosenbaum, Richard Yang, Rebecca Baus, William Rehrauer, Wei Huang, Ricardo Lloyd. University of Wisconsin Hospital and Clinics, Madison, WI.

Background: INSM1 is a transcription factor associated with terminal differentiation of neuroendocrine (NE) tissue. We previously demonstrated diagnostic and prognostic utility in evaluating expression of INSM1 in neuroendocrine neoplasms (NENs). Others have shown the role of INSM1 varies in different subtypes of endocrine tissue (e.g. pancreatic α cells versus β cells). As the functional status of NENs often carries clinical and prognostic significance, we examine the correllation between INSM1 expression and hormone production in functional NENs.

Design: Immunohistochemistry (IHC) for INSM1 and pancreatic peptide hormones was performed on 21 pancreatic neuroendocrine neoplasms (Pan-NENs) from a variety

of sub-types (6 non-functional, 7 β-cell, 1 α-cell, 2 G-cell, and 2 Δ-cell in pancreas). In 9 pituitary neoplasms, INSM1 IHC was correlated with IHC for prolactin (PRL), growth hormone (GH), and adrenocorticotropic hormone (ACTH). We used a tissue microarray and Vectra imaging software to semi-quantitatively assess IHC expression of INSM1 in 39 adrenal pheochromocytomas, 29 extra-adrenal pheochromocytomas (paragangliomas), and 8 normal adrenal medullas as controls. We used qRT-PCR to quantitatively assess INSM1 expression in 34 GI-NENs. We correlate our expression data with clinical features and laboratory values.

Results: In Pan-NEN, INSM1 is highly expressed in β-cell, α-cell, and G-cell neoplasms, but is diminished or lost in 2/2 Δ-cell and 2/6 non-functional neoplasms. All 4 cases have proven metastases. In pituitary NEN, INSM1 is lost in 2/9 neoplasms, both of which have lost or diminished PRL staining. INSM1 is detected by IHC in 31/39 pheochromocytomas and 26/29 paragangliomas, and staining is diminished in neoplasms compared to benign adrenal medulla ($p = 0.01$). INSM1 expression is strongest in neoplasms that produce epinephrine, intermediate in mixed and non-functional neoplasms, and weakest in neoplasms that produce norepinephrine or dopamine. Diminished INSM1 expression corresponds with increased tendency for metastasis. In GI-NENs, increased expression of INSM1 mRNA corresponds with elevated serum CGA levels.

Conclusions: In hormone producing neoplasms, loss or diminished expression of INSM1 often correlates with loss or diminished production of functional hormone. Loss of hormone production by neuroendocrine neoplasms also often correlates with dedifferentiation and worsened clinical outcomes. Evaluation of INSM1 may add valuable prognostic information in the assessment of NENs.

553 Impact of Grossing Technique on Occult Papillary Thyroid Microcarcinomas

Roberto Ruiz-Cordero, Francesca Polit, Christina Kovacs, Carmen Gomez-Fernandez. University of Miami Hospital, Jackson Memorial Hospital, Miami, FL; Universidad de Especialidades Espíritu Santo, Guayaquil, Ecuador.

Background: Papillary thyroid microcarcinoma (PTMC) is defined as an incidental focus of papillary carcinoma (PTC) measuring less than 1 cm. Recent reports indicate an increasing incidence of PTC mostly attributable to PTMC, probably due to increased detection, environmental factors, and systematic study of surgical specimens. "Occult" PTMC generally refers to PTMC detected at final histologic exam after surgery for benign disease. We analyzed whether differences in grossing techniques had an effect on the incidence of occult PTMC in our institution.

Design: A retrospective review of the surgical pathology reports of thyroid resections between 2005 and 2014, under a University's Institutional Review Board was performed. The study period was divided into period 1 (P1 = January 2005 to February 2010) and period 2 (P2 = March 2010 to January 2014) based on a change of policy with regards to thyroid specimen grossing. Thyroidectomies were predominantly sampled with representative sections of grossly visible lesional areas in P1. In P2, the specimens were submitted entirely for microscopic evaluation. We included only patients who underwent a thyroid resection for non-neoplastic pathology (e.g. chronic thyroiditis, multinodular hyperplasia, etc.) and were found to have an "occult" PTMC in the statistical analysis. Size and number of slides per case were stratified per time interval. The difference between variables in each time interval was assessed using Fisher's exact test.

Results: A total of 731 consecutive histopathology reports from 607 patients who underwent a surgical procedure for thyroid pathology were retrieved. Of the total 731 cases, 429 cases were diagnosed as PTC. Of these, 146 (34%) were PTMC, ranging from 0.1 to 1 cm. (0.6, ±0.3). Eighty (54%) PTMC were diagnosed during P1 versus 66 (45%) in P2. Overall, 60 PTMC were "occult", i.e. detected after surgery for benign disease. During P1, 28 (35%) occult PTMC were detected in contrast to 32 (48%) in P2 ($p=0.006$, 99%CI=1-8, OR=2.9). The average number of slides per case increased from P1 (11 ±5) to P2 (16 ±8) by a factor of 5.

Conclusions: An "occult" PTMC is 2.9 times more likely to be found when thyroid resection specimens are entirely submitted for microscopic evaluation. The implications of entire submission and increased number of slides per case will be the subject of further study.

554 miRNA Sequencing of Thyroid Tumors Revealed Distinct Molecular Profiles in Hyalinizing Trabecular Tumor and Medullary Carcinoma

Theresa Scognamiglio, Nicole Panarelli, Thomas Tuschl, Zhengming Chen, Neil Renwick, Yao-Tseng Chen. Weill Cornell Medical College, New York, NY; Rockefeller University, New York, NY.

Background: microRNAs (miRNAs) are small noncoding RNAs that bind to messenger RNAs and target them for down-regulation/degradation. Deregulation of miRNAs has been shown to contribute to tumorigenesis in many organ systems. Most studies to date on thyroid tumors have focused on papillary thyroid carcinoma (PTC) and follicular carcinoma using miRNA expression arrays and qRT-PCR. In this study, we used small RNA sequencing to comprehensively examine the miRNA profiles of other thyroid tumors, including follicular adenoma (FA), oncocytic follicular adenoma (OA), hyalinizing trabecular tumor (HTT) and medullary thyroid carcinoma (MTC).

Design: Total RNA was extracted from formalin-fixed paraffin-embedded tissues from 10 FA, 10 OA, 10 MTC and 8 HTT, and was subjected to small RNA sequencing. For comparison to MTC, neuroendocrine tumors from other organs, including pancreas, GI tract, lung and adrenal (N=84) were also sequenced. Unsupervised clustering was performed using normalized data and mean miRNA expression levels were statistically compared.

Results: Unsupervised hierarchical clustering separated MTC, HTT, and FA/OA into three distinct groups. However, OA were indistinguishable from FA. 52 miRNAs showed significantly higher expression in MTC vs. all follicular tumors, 14 of them with $p<0.001$ and >10 fold differences. Many of these, including miR-375, miR-129, miR-137, miR-153, miR-7, were likely neuroendocrine lineage-specific miRNAs, as they were also highly expressed in some or most of the non-thyroid neuroendocrine tumors profiled. 72 miRNAs showed significant differences ($p<0.05$) between HTT and FA. These included 12 miRNAs that were highly expressed in HTT (vs. FA, $p<0.01$ and >10 fold differences for all comparisons), the most notably being miR-483-3p (216 fold) and miR-888/890/891a/891b/892b cluster (29-94 fold). miRNAs known to be over expressed in PTC (miR146-b, miR-181b, miR-222, and miR-221) showed low expression in HTT.

Conclusions: microRNA sequencing revealed distinct miRNA profiles for HTT, MTC, and FA/OA. High expression of miR-888/890/891a/891b/892b cluster appears to be unique to HTT. HTT showed low expression of markers known to be overexpressed in PTC and provides support for HTT as an entity distinct from PTC. MTC differs from follicular tumors mostly by their neuroendocrine signatures, reflecting their neural crest origin.

555 Enhancer of Zeste Homolog 2 (EZH2) and Global H3K27 Trimethylation Expression During Progression of Thyroid Cancer

Kaitlin Sundling, Celina Montemayor-Garcia, Heather Hardin, Darya Buehler, Sofia Asioli, Alberto Righi, Ricardo Lloyd. University of Wisconsin, Madison, WI; University of Bologna, Bologna, Italy; Rizzoli Institute, Bologna, Italy.

Background: EZH2 is a histone lysine methyltransferase that is a member of the polycomb group protein family. EZH2 plays an important role in the epigenetic maintenance of H3K27 trimethylation (H3K27me3). Abnormal EZH2 expression has been associated with various cancers. We examined the expression of EZH2 and H3K27me3 to determine their roles in thyroid cancer progression.

Design: Anaplastic thyroid carcinomas (ATC, n=35), poorly differentiated thyroid carcinomas (PDTC, n=21), papillary thyroid carcinomas (PTC, n=28), follicular variant of papillary thyroid carcinomas (FVPTC, n=29), follicular thyroid carcinomas (FTC, n=28), follicular adenomas (FA, n=31) and normal thyroids (NT, n=10) on a tissue microarray (TMA) were analyzed by immunohistochemistry (IHC) using formalin-fixed paraffin-embedded tissues. Antibodies to EZH2 (Novus, 1:100) and H3K27me3 (Cell Signaling, 1:200) were used. IHC was scored based on the intensity and percentage of nuclear staining. Survival analysis was performed using the statistical computing software R.

Results: Both EZH2 and H3K27me3 showed strong nuclear staining. The highest level of EZH2 expression was observed in ATC (97%) followed by PDTC (90%). Levels were lower in FA (71%), FTC (64%), PTC (79%) and FVPTC (79%). EZH2 expression was significantly higher in ATC than in well-differentiated carcinomas ($P<0.04$). H3K27me3 expression was high in both benign and malignant thyroid tumors as well as in NT. H3K27me3 expression was not significantly different in higher-grade tumors compared to well-differentiated carcinomas and FA. The carcinoma types expressing the highest levels of EZH2 (ATC and PDTC) had worse prognoses than the carcinoma types with lower expression ($P<0.001$).

Conclusions: EZH2 is expressed in thyroid malignancies, and its expression correlates with higher-grade tumors. EZH2 effects may also include H3K27me3-independent mechanisms. Our findings suggest that EZH2 may play an important role in thyroid tumor progression.

556 94 Cases of Encapsulated Type Follicular Variant of Thyroid Papillary Carcinoma

Lester Thompson. Southern California Permanente Medical Group, Woodland Hills, CA.

Background: The encapsulated type, follicular variant of thyroid papillary carcinoma (EFV-TPC) is a difficult tumor type to reproducibly classify and consequently manage. There is no large, comprehensive, community hospital based longitudinal evaluation of this tumor type with a specific evaluation of possible over-treatment.

Design: Ninety-four cases of EFV-TPC were identified in a review of all thyroid gland surgeries (721 surgeries; 11,130 patients with thyroid disease; 3,064,661 patients) performed in 2002 within the 11 hospitals of the Southern California Permanente Medical Group. All slides were reviewed and patient follow-up obtained.

Results: The tumors affected 75 women and 19 men, aged 20 to 80 years (mean, 45.6 years). The majority of patients presented with a mass (n=91). The tumors were single (n=61), multiple (same lobe; n=20), or bilateral (n=13). The dominant tumor ranged from 0.7—9.5 cm in diameter (mean 3.3 cm). Capsular or lymph-vascular invasion (LVI) was present in 13 cases; 81 cases showed no evidence of capsular or LVI (adequately sampled: mean 12 sections; weight 58 grs). No cases showed extrathyroidal extension or lymph node metastases. Histologically, all cases demonstrated an easily identified, usually thick, well formed, completely encapsulated tumor. The tumors showed a predominantly follicular architecture (without significant papillary structures), hypereosinophilic colloid, intratumoral acellular fibrosis and classical papillary carcinoma nuclear features. The nuclear features of TPC were multifocal and widespread, but not seen uniformly throughout the tumor (best at the tumor capsule). Lobectomy alone (n=41), thyroidectomy alone (n=34), or completion thyroidectomy (n=19) was the initial treatment. Radioablative iodine was used in 25 patients, while 69 patients had no additional therapy. All patients were alive (n=92) or had died (n=2) without evidence of recurrence or metastasis with mean follow-up of 11.2 years.

Conclusions: The EFV-TPC shows a benign clinical behavior irrespective of age, gender, tumor size, presence of limited capsular or lymph-vascular invasion and stage. Patients managed by lobectomy alone or thyroidectomy alone (without radioablative iodine, suppression therapy or thyroglobulin level monitoring) had an identical outcome to patients managed with these methods. Therefore, lobectomy alone without additional therapy can be employed in the management of this tumor type. Towards this end, adoption of a new name highlighting the benign nature of this tumor is recommended.

557 Minimal Extrathyroidal Extension (ETE) and Extranodal Extension (ENE) in Papillary Thyroid Carcinoma: A Consensus Evaluation

Andrew Turk, Sylvia Asa, Zubair Baloch, Eugenie Du, William Faquin, Ronald Ghossein, Thomas Giordano, Virginia LiVolsi, Ricardo Lloyd, Ozgur Mete, Henry Su, Saul Suster, Lester Thompson, Bruce Wenig. Mount Sinai Beth Israel, New York, NY; Columbia University, New York, NY; University of Pennsylvania, Philadelphia, PA; Toronto General Hospital, Toronto, ON, Canada; University of Michigan, Ann Arbor, MI; Massachusetts General Hospital, Boston, MA; Memorial Sloan Kettering Cancer Center, New York, NY; University of Wisconsin, Madison, WI; Medical College of Wisconsin, Milwaukee, WI; Southern California Permanente Medical Group, Woodland Hills, CA.

Background: ETE and ENE are important pathologic parameters in the evaluation of thyroid carcinomas, in terms of staging and/or potentially predicting biologic behavior. Histopathologic criteria to determine ETE and ENE are not well defined, and their determination can be subjective. The goal of this study was to determine evaluation-concordance of ETE and ENE among thyroid pathologists, and to establish histologic criteria for diagnosis of ETE and ENE.

Design: The case cohort included 130 slides: 69 for ETE and 61 for ENE. These were scanned at 40x using the Aperio ScanScope AT Turbo for review by 10 expert thyroid pathologists, specifically evaluating for perithyroid / perinodal involvement of fat (F), skeletal muscle (SkM), nerves (N) and thick-walled vessels (V). Data were collected and analyzed.

Results: Overall, intraclass correlation coefficient (ICC) was 20% for ETE and 39% for ENE. ICC for specific parameters included: SkM-90% ETE, 96% ENE; F-79% ETE, 80% ENE; N-62% ETE, 85% ENE; V-29% ETE, 36% ENE.

Conclusions: Overall, ICC in diagnosing ETE and ENE was poor. Minimal criteria for ETE and ENE correlated best for tumor in SkM, F and N. Presence or absence of tumor around V factored least in determination of ETE and ENE. Establishing specific criteria to confirm the type of extension may help to improve ICC and achieve consensus in reporting these prognostically significant findings.

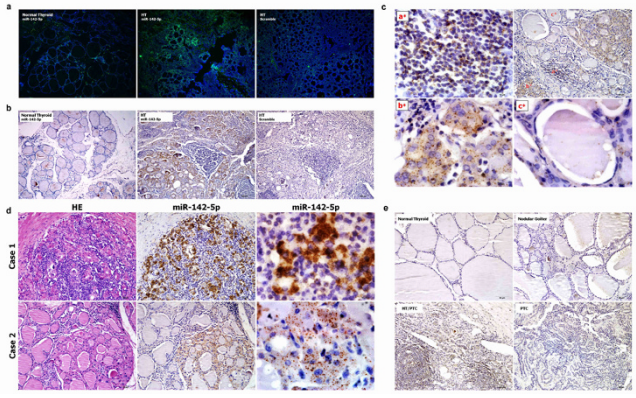
558 MicroRNA-142-5p Contributes to Hashimoto's Thyroiditis By Targeting CLDN1

Zhe Wang, Jin Zhu, Qingguo Yan, Gaosheng Huang. Fourth Military Medical University, Xi'an, Shanxi, China.

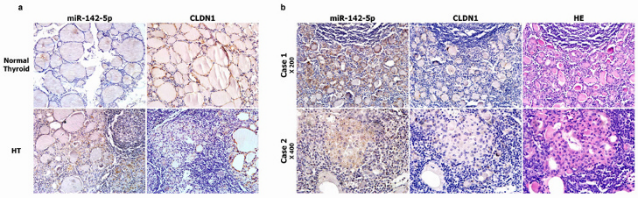
Background: Since little is known about the role of microRNAs related to Hashimoto's throiditis (HT), we, for the first time, systematically investigated the microRNA expression profile of HT.

Design: Firstly, we examined the microRNAs expression profile in HT by using microRNA microarray analysis, and verified the highly expressed specific microRNAs in HT by using quantitative RT-PCR. Then, we detected the specific microRNA expression in the patient's serum. We also detected the specific microRNAs expression in the HT tissues by using locked nucleic acid-*in situ* hybridizition. We identified the taget gene of the specific microRNA, and investigated the expression and function of the taget gene in HT and thyroid epithelial.

Results: Using microRNA microarray analysis and quantitative RT-PCR, we found that miR-142-5p, miR-142-3p, and miR-146a had desregulated expression in HT thyroid, but not in the other thyroid diseases. Clinical data indicated that miR-142-5p was detected in HT patient serum and positively correlated with thyroglobulin antibody. Locked necleic acid - in situ hybridization results showed that the expression of miR-142-5p was found mainly in injured follicular epithelial cells, suggesting a crucial role in the pathogenesis of HT.



Further molecular studies demonstrated that *CLDN1* is the direct target gene of miR-142-5p and its downregulation by miR-142-5p may impaired the barrier function of fillicular epithelial cells, contributing to the pathogenesis of HT.



Conclusions: Our finding's indicated that miR-142-5p may act as a useful bimarker and could serve as a new potential therapeutic for patients of HT.

559 Differentiating Follicular Neoplasms Through Gene Methylation

Michelle Williams, Li Zhang, Gilbert Cote, Marcos Estecio. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Over 60% of adults will show thyroid nodules by imaging, with approximately 20% remaining indeterminate for classification by fine needle aspiration (FNA) biopsy. Surgery is then required for diagnostic purposes, however ultimately over 70% of these nodules will be classified as benign. Thus there is a need for biomarkers to improve classification of thyroid nodules prior to surgery. Additionally, prognostic markers for follicular carcinoma are lacking. DNA methylation may allow for a tool to augment classification of follicular neoplasms including behavior prediction.

Design: Utilizing a well-characterized cohort of follicular neoplasms (5 normal, 3 adenomas (FA), 7 indolent follicular carcinomas (FCi) and 8 aggressive/metastatic follicular carcinomas (FCa)) global methylation patterns were analyzed utilizing the illumina 450K platform (illumnia inc, San Diego, CA). Methylation status at each loci was evaluated utilizing the average beta value of each sample. Comparisons of methylation status between normal versus neoplasms, as well as, indolent versus aggressive carcinomas were performed.

Results: Utilizing a 30% difference in methylation as a threshold, comparing normal thyroid tissue and neoplasms resulted in 6116 hypomethylated and 1648 hypermethylated loci/genes differentially methylated. Of which 725 hypomethylated and 1079 hypermethylated loci were unique to the aggressive FC group. Similarly, when comparing FCs based on biologic behavior (indolent vs. aggressive) 95 loci were differentially methylated.

Conclusions: While FNA features overlap in thyroid follicular neoplasms, genomic level changes in DNA methylation do differ by biologic behavior and may provide future biomarkers for augmenting evaluation of thyroid nodules. Further studies are on-going evaluating individual genes and smaller panels which may have diagnostic and prognostic significance.

560 Parafibromin, APC, and MIB-1 Are Useful Markers for Distinguishing Parathyroid Carcinomas From Parathyroid Adenomas

Brian Willis, Snehal Patel, Joe Sharma, Colin Weber, Cynthia Cohen. Emory University, Atlanta, GA.

Background: The diagnosis of parathyroid carcinoma relies on histologic demonstration of invasive behavior or distant metastasis. Tumors that display atypical histopathologic features, but do not show definite invasion, present a dilemma. Parafibromin and APC expression have been shown to be absent or decreased in parathyroid carcinomas, while Galectin 3 and MIB-1 expression are reported to be increased. We used a panel of these markers to assess for aggressive behavior.

Design: We examined 21 parathyroid carcinomas, 3 atypical adenomas, and 72 adenomas for expression of parafibromin, APC, and galectin 3 by immunohistochemistry. Each case was scored for percentage of tumor cells staining (0-100) and staining intensity (0-3). A product of >100 for percentage of positive cells and intensity was considered positive. MIB-1 proliferation indices were calculated using image cytometry of five 20x fields (>5% considered elevated).

Results: Negative staining for parafibromin and APC was seen in 7/21 (33%) and 20/21 (95%) cases of carcinoma, respectively. Only one parathyroid adenoma demonstrated loss of parafibromin (1%), while 38/73 (52%) adenomas showed loss of APC. MIB-1 indices were elevated in 18/21 (86%) of parathyroid carcinomas; all adenomas were <5%. Of the 3 atypical adenomas, none showed loss of parafibromin, 2 showed loss of APC, and 1 was positive for Galectin 3. 2 atypical adenomas showed a MIB-1 index >5%. The data is summarized below (excludes atypical adenomas):

	Parafibromin (negative stain)	APC (negative stain)	Galectin 3 (positive stain)	MIB-1 (>5%)
Sensitivity	88%	35%	6%	78%
Specificity	84%	97%	29%	100%
PPV	33%	95%	19%	100%
NPV	99%	48%	10%	95%
P-value		0.002		

Conclusions: Parafibromin and APC are useful markers for differentiating malignant parathyroid neoplasms from adenomas, especially in conjunction with MIB-1. Loss of staining for parafibromin is both sensitive and specific for parathyroid carcinoma, while loss of APC is specific. A high MIB-1 index is also sensitive and highly specific for carcinoma. Galectin 3 positivity does not predict malignancy in parathyroid lesions. Borderline lesions referred to as atypical adenomas are more difficult to characterize. In cases with features suspicious, but not definitive, for malignancy a panel consisting of parafibromin, APC, and quantitative MIB-1 may be useful for identifying parathyroid carcinoma.

561 Molecular Profiling Reveals Actionable Mutations and TP53 Gain of Function Mutations in Anaplastic Thyroid Carcinoma

Thomas Wilson, Anup Tilak, Sarah Hornberg, Matthew Zubcic, Robert Robinson, Deqin Ma. University of Iowa, Iowa City, IA.

Background: Anaplastic thyroid carcinoma (ATC), accounting for only 2% of thyroid cancers, is the most aggressive form of thyroid malignancy. It is chemoradiation resistant and universally lethal. There is no targeted therapy available due to a lack of understanding of its genomic alternations. We employed next generation sequencing (NGS) to search for mutations underlying oncogenesis, tumor progression and actionable targets in ATC.

Design: Twelve cases of ATC were identified from our archives. NGS was performed using the Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies, Carlsbad, CA) on Ion Torrent PGM with 5% low limit of detection. The data was analyzed using the Ion Torrent Suite Software followed by a laboratory developed pipeline. In 3 cases with coexisting non-ATC variants of thyroid carcinoma, all variants were tested in parallel.
Results: Pathogenic mutation was identified in 92% (11/12) of ATCs. *BRAF* V600E was the most frequent mutation (8/12, 67%), followed by mutations in *TP53* (5/12, 58%) and *PIK3CA* (2/12, 17%). 58% (7/12) of tumors had multiple mutations. Three of the *BRAF* wildtype tumors had mutations in *TP53* and/or other tumor suppressors (*PTEN, CDKN2A*). Of note, gain of function (GOF) mutations in *TP53* were seen in 3/11 of cases (#6, 9, 10). When co-existing variants of thyroid carcinoma (papillary thyroid carcinoma, Warthin-like and tall cell variants) were tested, the same findings were seen in both areas.

Case	Gene	Variants Detected * targeted therapy available
1	BRAFPIK3CA	c.1799T>A (p.V600E)*c.1624G>A (p.E542K)*
2	BRAFPIK3CA	c.1799T>A (p.V600E)*c.1624G>A (p.E542K)*
3	BRAFSTK11	c.1799T>A (p.V600E)*c.1801delC (p.Y60X)
4	BRAFTP53KIT	c.1799T>A (p.V600E)*c.454C>T (p.P152S), c.380C>T (p.S127F)c.1158G>A (p.V530I)*
5	BRAFTP53	c.1799T>A (p.V600E)*c.405C>A (p.C135X)
6	BRAFTP53	c.1799T>A (p.V600E)*c.818G>A (p.R273H)
7	BRAF	c.1799T>A (p.V600E)*
8	BRAF	c.1799T>A (p.V600E)*
9	TP53PTEN	c.733G>A (p.G245S)c.892C>T (p.Q298X)*
10	TP53	c.527G>T (p.C176F)
11	CDKN2A	c.230C>T (p.T77I)
12	-	-

Conclusions: *BRAF* (V600E) mutation is frequent in ATC and most likely acts as the driver mutation in tumorigenesis. Additional mutations in other oncogenes or tumor suppressors may promote tumor progression and contribute to the aggressiveness of ATC. The high incidence (83%) of oncogenic mutations in ATC, and especially the newly identified GOF mutations in *TP53,* opens the door for targeted therapy against this deadly tumor.

562 The Association of Sodium Iodide Symporter and BRAFV600E Mutation in Classical Variant Papillary Carcinoma

Aylin Yazgan, Nilufer Yildirim, Aysegul Gozalan, Sinem Gumustas, Gulten Kiyak, Aydan Kilicarslan, Cevdet Aydin, Reyhan Ersoy, Bekir Cakir, Serdar Balci. Ankara Atatürk Education and Research Hospital, Ankara, Turkey; Yildirim Beyazit University, Ankara, Turkey.
Background: Functional expression of sodium iodide symporter (NIS), a membrane transporter of iodine is essential for postoperative radioiodine treatment in papillary thyroid cancer (PTC). It is reported that $BRAF^{V600E}$ mutation was correlated with worse clinicopathologic features due to impaired NIS expression. In this study we evaluated the relationship of the BRAF mutation and functional NIS expression in patients with classical variant (CV) PTC.
Design: BRAF mutation was analyzed by RT-PCR in 106 consecutive cases with CV-PTC and immunohistochemical staining of NIS protein was evaluated in 96 cases. Localisation (intracellular or membraneous), intensity were characterized semiquantitatively. The density of cytoplasmic staining was also noted. Clinicopathologic features such as extrathyroidal invasion, lymph node metastesis were correlated with BRAF mutation and functional NIS expression. 88 patients who completed at least 24 months follow up were used for the outcome.
Results: Mean age was 46,2±13,7. Gender F/M: 79/17. 75/96 (78.1%) cases had BRAF mutation. Functional (membranous) NIS was higher in BRAF mutation positive group (78.7%) than in BRAF negative group (57.1%) (p=0.047). Nonfunctional (cytoplasmic) NIS was lower in BRAF mutation positive group (21.3%) than in BRAF negative group (42.9%) (p=0.047). Those with moderate and intense cytoplasmic staining had 6.43 times higher BRAF mutation (90%) than those with weak staining (58.3%) (95% CI=2.20-18.80,p=0.001). There was no association between NIS expresion patterns and lymph node positivity, extrathyroidal extension ans surgical margin positivity. Extrathroidal extension was present in 54.7% of cases with BRAF mutation and 33.3% without (p=0.084). Surgical margin positivity was present in 85.7% of cases with BRAF mutation and 14.3% without (p=0.084). There was no association for lymph node involvement (p=0,625). 7/88 patients without remission had BRAF mutation; 5 had functional NIS expression.
Conclusions: Functional NIS expression is higher among CV-PTC with BRAF mutation. But the clinical features were not found to be associated with NIS expression. There may be different mechanisms determining the outcome of therapy.

563 Utility of Immunohistochemical Expression of Ki-67, Beta-Catenin, P53 and AR in Assessment of Primary Adrenal Cortical Neoplasms (CAN)

Wendong Yu, Mouhammed Amir Habra, Jane Zhou, Raghu Vikram, Ruth Katz. University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX.
Background: Diagnosis of ACN, including "incidentalomas", can be challenging by fine needle aspiration or core needle biopsy, where the use of the Weiss scoring (WS) system on those limited materials is suboptimal. To identify aggressive ACN that will require surgical excision, we investigated the utility of a panel of biomarkers known to be involved in the pathogenesis of adrenocortical carcinoma (ACC) on a tissue microarray (TMA).
Design: A TMA composed of normal adrenal cortex (NAC) (12), adenomas (15 with a WS of 0, and 11 "atypical" with a WS of 1 or 2) and ACCs (48) were studied for the expression of Ki-67, p53, beta-catenin (CTNNB1), and androgen receptor (AR) by immunohistochemistry (IHC). IHC of TMAs were scored according to intensity of staining and percentage of cells stained, as well as location of staining.
Results: Using Ki-67 ≥ 5% as a cutoff, 32/48 cases of ACC were positive, but no benign lesions (NAC or adenomas) were positive (p<0.001; sensitivity (Se) 66.7%, specificity (Sp) 100%). CTNNB1 was positive in 20/48 cases of ACC, and in 4/38 cases of benign lesions (p=0.002, Se 41.7%, Sp 89.5%). Besides ACCs, one adenoma and three atypical adenomas were positive for CTNNB1. P53 was positive in 12/48 cases of ACC but absent in 37/38 cases of benign lesions (p=0.005, Se 25%, Sp 97.4%). Besides ACCs, one atypical adenoma was positive for p53. AR was positive in 12/48 cases of ACC but negative in all benign lesions (p=0.001, Se 25%, Sp 100%). 84% of ACC could be identified if they were positive for one or more biomarkers. Additionally, CTNNB1 positivity was associated with decreased overall survival (p=0.009) and disease-free survival (DFS) (p=0.074). Ki-67 ≥ 5% was also associated with shortened DFS (p=0.012).
Conclusions: Based on our findings, utilization of a panel of biomarkers comprising Ki67, p53, AR, and CTNNB1 could be very useful in predicting the biological behavior of an indeterminate adrenal neoplasm thus guiding clinical management. Our findings supported the complexity of the pathogenesis of ACC with multiple pathways involved. In particular, Wnt/β-catenin activation, demonstrated by the positive CTNNB1 staining, appears to be an important prognostic factor in ACC patients.

564 NKX2.2 Expression in Well Differentiated Neuroendocrine Tumors

Chaohui Zhao, Shamlal Mangray, Kara Lombardo, Leila Noble, Shaolei Lu, Murray Resnick, Evgeny Yakirevich. Alpert Medical School of Brown University, Providence, RI.
Background: NKX2.2 is a transcription factor that plays a critical role in the development and differentiation of gastrointestinal/pancreatic neuroendocrine cells. NKX2.2 is expressed in normal human adult pancreatic islets; however, little is known about its expression in digestive tract or pumonary well-differentiated neuroendocrine tumors (WDNETs). We investigated whether NKX2.2 is differentially expressed in WDNETs and compared its expression with Islet 1, PAX8, CDX2, and TTF-1.
Design: One hundred and fifty seven cases of WDNETs (grade 1-2) including 39 pulmonary, 11 gastric, 8 duodenal, 17 pancreatic, 55 small intestinal (SI), 15 appendiceal, and 12 rectal tumors were studied. Tissue microarrays were analyzed for IHC expression of NKX2.2, Islet-1, PAX8, CDX2 and TTF-1.
Results: Normal pancreatic islets and scattered gastric and intestinal neuroendocrine cells demonstrated strong nuclear NKX2.2 staining. NKX2.2 was frequently positive in rectal (95%), duodenal (88%), appendiceal (79%), small intestinal (60%), and pancreatic (59%) WDNETs. In pancreatic and rectal WDNETs staining for NKX2.2 was strongly associated with Islet 1 and PAX8 (P<0.0001). Immunoreactivity for all three markers was concordant in the vast majority of pancreatic WDNETs (82.4%), including 8 cases positive, and 4 cases negative for all three markers. Lower NKX2.2 positivity (36%) was seen in gastric carcinoids, while all pulmonary carcinoids were negative. In contrast to NKX2.2, all small intestinal WDNETs were negative for Islet 1 and only 5% were positive for PAX8. CDX2 was expressed predominantly in small intestinal (84%) and appendiceal (87%) WDNETs, while TTF-1 immunoreactivity was restricted to pulmonary carcinoids.

	Number (%) positive				
Anatomic site	NKX2.2	Islet 1	PAX8	CDX2	TTF-1
Lung (n=39)	0	0	2 (5)	0	30 (77)
Stomach (n=11)	4 (36)	2 (18)	5 (45)	1 (10)	0
Duodenum (n= 8)	7 (88)	4 (50)	5 (63)	3 (38)	0
Pancreas (n=17)	10 (59)	10 (59)	11 (65)	2 (14)	0
Small bowel distal to ampulla (n=55)	34 (60)	0	3 (5)	46 (84)	0
Appendix (n=15)	11 (79)	2 (13)	2 (13)	13 (87)	0
Rectum (n=12)	11 (95)	10 (83)	10 (83)	1 (8)	0

Conclusions: This study is the first to comprehensively examine NKX2.2 expression in a wide spectrum of WDNETs. NKX2.2 is variably expressed in digestive tract WDNETs and is negative in pulmonary carcinoids. NKX2.2 is as sensitive as Islet 1 and PAX8 for pancreatic WDNETs; however, its ability to distinguish pancreatic from gastrointestinal WDNETs is limited in contrast to Islet 1 and PAX8.