565 Four Years of Virtual Pathology Teaching Using "SlideAtlas", a Web-Based Open-Source Digital Pathology Platform Supporting Multi-Touch Interaction

Kristen Smith, Kuda Maloney, Brian Helba, Charles Law, Beverly E Faulkner-Jones. BIDMC, Bosotn, MA; MD Anderson Cancer Center, Houston, TX; Kitware Inc, Clifton Park, NY.

Background: There are significant advantages to using whole slide images (WSIs) for resident education. We have developed and deployed a web-based open digital pathology system for virtual pathology to teach dermatology and pathology residents for the past four years.

Design: We developed a web-based open-source digital pathology platform SlideAtlas (https://slide-atlas.org) that supports automated upload of scan files from multiple scanners. The high performance interactive image viewing experience on both desktop (Mac and PC) and mobile devices approximates the use of glass slides. Access control, search features and annotation tools are built into the system and readily accessible. A new embedded PowerPoint-like presentation function displaying WSIs is now used for board review-type sessions.

Results: WSIs have been used for more than four years to teach dermatopathology to residents and fellows. The database currently contains approximately 8, 000 dermatopathology-related images. For dermatology residents ~10 - 40 dermatopathology images are selected and posted for weekly resident preview prior to an attending-led interactive session. Images are archived weekly for later reference (~ 600 images for the last academic year). WSIs have been used for over a year to teach surgical pathology to residents and fellows. ~ 10 – 15 surgical pathology images are posted twice a week for attending-led "unknown" conference. Images are archived post session for later resident self-review (~ 1000 images in 15 months). In addition, subspecialty digital slides sets for resident self-teaching have been recently introduced. Resident satisfaction with the digital interface, responsiveness of image navigation over standard (hospital and wireless) networks and the image quality remains high. The annotation features, boards-style reviews and multi-touch interaction are all popular.

Conclusions: There is continued support and enthusiasm from dermatology and pathology residents for using WSIs and SlideAtlas for their pathology education. The development of a vendor neutral and platform independent open source digital pathology system helps provide a relatively low cost and flexible option for resident education.

566 Big Data: Bioinformatics Education during Residency Demonstrates Immediate Value

Amelia Sybenga, Riyam T Zreik, Amin Mohammad, Arundhati Rao. Scott & White Memorial Hospital, Temple, TX.

Background: Bioinformatics education is becoming increasingly recognized as an important component of physician training. Although bioinformatics as a field encompasses many different subsets, the ability to effectively analyze big data sets to uncover trends, efficiency correlations and create new pathways is an essential component of the value based healthcare system. Education of residents in this field is difficult with few active learning resources available. We have begun implementation of formalized bioinformatics education for residents through quality projects that serves to actively introduce concepts of big data analysis, and simultaneously provide quality data to improve efficiency, utilization and treatments. We hypothesize that this active learning will give residents familiarity with vocabulary, data analysis tools and databases.

Design: A preliminary survey is administered to junior residents to assess familiarity with big data topics. Junior level residents are assigned simple projects that allow them to learn spreadsheet formulae and data base development that eliminate the tedious and potentially error ridden process of manual data analysis. Senior level projects are graduated to complex concepts such as multifactorial laboratory cost analyses for the integration of potential new technologies. Competency of senior residents' project results are evaluated by staff during a multidisciplinary conference.

Results: Of the 11 residents in the program only 1 has familiarity with bioinformatics and big data analysis. After introductory education in big data analysis concepts, residents are able to rapidly analyze large sets of data to answer simple questions. Senior residents are able to engage in complex problem solving requiring management and application of multiple seemingly unrelated resources, and successfully present those results. Additionally, several utilization projects to demonstrate overutilization of repeat chemistry tests have resulted in ordering practice changes in the electronic medical record (EMR).

Conclusions: Through step-wise education beginning with basic data analysis concepts, residents become increasingly secure in managing big data and progressively more competent through the milestones progression. The ability to understand, analyze and manage big data is of growing importance in demonstrating the value of pathology in a value based healthcare system, and formalized resident education in bioinformatics can better prepare residents for this future while providing immediate and valuable quality services to their institution.

567 Multidisciplinary Tumor Board: A Ready-Made Tool for Competencies, Professionalism and Capturing Medical Student Interest in Pathology

Rebecca Wilcox. University of Vermont Medical Center, Burlington, VT.

Background: Medical educators are expected to build action plans to address new core competencies, including interprofessional education and core entrustable activities for entering residency, in an already full medical school curriculum. We hypothesized that early medical student (MS) exposure to a pathology run multidisciplinary tumor board (MDTB) could provide a practical means of addressing core objectives without adding to the current curriculum. We also proposed the additional benefit of capturing MS interest in pathology. This is an important aim given the dire combination of the aging of our specialty and the trend towards decreased applicants to pathology residencies.

Design: As part of their 8-week integrated course entitled Nutrition, Metabolism and the Gastrointestinal System (NMGI), first year MS were given the opportunity to attend one of four patient care MDTB sessions that occurred during the course. Participation was voluntary and limited to 15 students per MDTB session. All four of the offered MDTBs were related to gastrointestinal oncology/disease and therefore innately covered topics taught in the NMGI course. A single pathologist who served as both an instructor in the NMGI course as well as an active clinical member of the MDTB team served as the liaison for these sessions. This faculty met with the students for 15 minutes prior to the MDTB to discuss the process and team players involved in a MDTB as well as issues of professional conduct. The faculty member then accompanied the students to the MDTB, immediately followed by a 15 minute debrief session where questions could be addressed in a separate small group setting. All other members of the patient care MDTB team were made aware of both visiting dates and learning objective of the MS visitors from the NMGI course.

Results: Each of the four volunteer sessions filled to capacity. MDTB was cited as one of the strengths of the course by student evaluations and the student education group course review report. Several student evaluations of this course also noted the field of pathology when addressing the MDTB sessions: "it made me consider a field of medicine that I previously thought would be a bad fit for me."

Conclusions: The MDTB is a premade medical education tool that brings clinical relevance to classroom material while providing opportunities to address competencies such as professionalism and interprofessional team-based patient care. An additional benefit of engaging medical students in MDTBs at this stage is early exposure to pathologists as physicians.

Endocrine Pathology

568 Prospective Experience with Routine SSTR2A Immunohistochemistry in Neuroendocrine Epithelial Neoplasms

Deema Alkapalan, Jessica E Maxwell, Thomas O'Dorisio, James R Howe, Andrew M Bellizzi, Iowa NET SPORE. University of Iowa, Iowa City, IA.

Background: Neuroendocrine epithelial neoplasms (NENs) express high levels of somatostatin receptors, the basis of octreotide therapy and somatostatin receptor imaging (SRI). In the fall of 2014 we began routine immunohistochemistry (IHC) testing of neuroendocrine tumors (NETs) for SSTR2A, using the monoclonal antibody UMB-1. Cases are scored based on criteria proposed by Korner and Reubi (*AJSP* 2012). We also test neuroendocrine carcinomas (and, rarely, other tumors) upon request. Herein, we report our first year's prospective experience with the UMB-1 monoclonal antibody.

Design: We searched the pathology database for all SSTR2A IHC orders. Most stains (>90%) had been interpreted by a single pathologist in the context of routine care. The following clinicopathologic data was obtained: SSTR2A result (postive, indeterminate, probably negative, negative), SSTR2A H-score (extent*intensity), age, gender, anatomic site, differentiation (well, poor), WHO 2010 grade (G1-3), SRI results, prior somatostatin analogue treatment.

Results: We performed 214 IHC in 203 patients (M:F, 1:1; mean/median age 57/59). The H-score in positive and indeterminate cases was 209 (mean) and 279 (median). In 11 tested matched primary-metastatic pairs, there was 100% concordance (all positive). Detailed results are presented in the Table.

	Total # of cases	*SSTR2A Positive*
Anatomic Origin:		
Lung	24	(8) 33%
Pancreas	44	(40) 90%
Stomach	6	(5) 83%
Duodenum	6	(5) 83%
Small bowel	86	(84) 98%
Colon	3	(3) 100%
Rectum	11	(10) 90%
Appendix	14	(13) 93%
Unknown	14	(8) 63%
Differentiation:		
WD	193	(175) 91%
PD	18	(4) 22%
Grade:		
I	85	(77) 91%
II	97	(87) 90%
III	29	(14) 48%
SRI:		
Positive OctreoScan	64	(68) 94%
Negative OctreoScan	25	(16) 64%
Positive DOTA scan	19	(17) 90%
Negative DOTA scan	6	(2) 33%
Prior Somatostatin analogue treatment:		
Yes	135	(124) 92%
No	58	(37) 64%
Gender:		
M	99	(85) 86%
F	104	(83) 80%

Conclusions: Routine SSTR2A in NENs is clinically feasible. As expected, SSTR2A is highly expressed in NETs. Of interest, expression appears less common in lung. There is no significant difference in SSTR2A-positivity between G1 and G2 tumors. A surprising number of poorly differentiated and G3 tumors are positive, with follow up imaging and treatment implications. Patients with negative SRI are also often positive, opening up somatostatin-based therapy in these patients.

569 The Presence of Combined BRAF, TP53 and PIK3CA Mutations Have Prognostic Impact in the Hobnail Variant of Papillary Thyroid Carcinoma

Sofia Asioli, Luca Morandi, Francesca Maletta, Alberto Righi, Marco Gallo, Anna Sapino, Ricardo V Lloyd. University of Bologna, Bologna, Italy; University of Turin, Turin, Italy; Rizzoli Institute, Bologna, Italy; University of Wisconsin School of Medicine and Public Health, Madison, WI.

Background: Hobnail variant of papillary carcinoma (HPTC) represents a recently described aggressive and rare group of moderately differentiated thyroid tumors with poorly understood pathogenesis. Molecular data in this group of tumors are heterogeneous, possibly reflecting the rarity of this entity, and there is the need for a more detailed molecular characterization of these tumors. The objective of the study was to define a comprehensive molecular typing of HPTC.

Design: Twelve cases of HPTC, including eight primary tumors and four lymph node metastases, from eight patients selected through the files of University of Turin, Italy, have been screened for *N-K-H-RAS, BRAF, TP53, PIK3CA* and *NOTCH1* gene mutations generating locus-specific amplicon libraries with tagged primers for the above genes. Sequencing was conducted on the Roche/454 GS junior system and quality-filtered reads were analyzed with the GS Amplicon Variant Analyzer (AVA 2.7, 454 Life Sciences). Molecular data were compared with clinical-pathologic data and follow up.

Results: The patients included 5 women and 3 men. Ages ranged from 31 to 87 years. All twelve cases of HPTC showed more than 30% hobnail features. BRAF and HRAS mutations were by far the most common genetic alterations in HPTC (41.7% and 33.3% respectively). V600E BRAF mutation was detected both in primary and metastasis in two patients and in one primary tumor in one patient. TP53 deleterious mutations were found in two primary HPTC and in one metastasis. PIK3CA gene mutations was found in 3 cases and KRAS in one case. No mutation was detected in the NRAS and NOTCH1 genes. Interestingly, only the three patients (37.5%) who died of disease after a mean of 30.6 months, showed the same complex molecular characterization constituted by the presence of combined mutations.

Conclusions: The present study confirms that HPTC are genetically heterogeneous and complex. The presence of combined mutations in *BRAF, TP53* and *PIK3CA* genes appear to have a negative prognostic factor. The detection of these multiple mutations by molecular profiling should be clinically relevant for the prognostic stratification and treatment of these patients.

570 Clinical-Pathological Features of 55 Papillary Thyroid Carcinomas Carrying BRAF Mutations Other Than V600E

Fulvio Basolo, Elisa Sensi, Mirella Giordano, Liborio Torregrossa. DIPINT-University of Pisa, Pisa, Italy; AOUP, Pisa, Italy.

Background: *BRAF* V600E mutation is widely observed in papillary thyroid carcinomas (PTCs), with a prevalence of about 45%. Many researchers have shown a significant association between *BRAF* V600E mutation and high-risk clinical-pathological features of patients with PTCs. *BRAF* mutations other than V600E are more rarely observed in PTC. Although some authors have not found any association between tumor aggressiveness and these types of *BRAF* mutations in PTC, their clinical significance remains still unclear.

Design: 2,961 consecutive cases of PTCs were enrolled from 2006 to 2013. DNA samples were screened for *BRAF* mutations in exon 15 using high-resolution melting analysis (HRMA) followed by Sanger sequencing.

Results: *BRAF* alterations were found in a total of 1,186 out of 2,961 PTC cases (40.0%). In particular, we found *BRAF* V600E mutation in 1,131 cases and *BRAF* mutations other than V600E in 55 cases. According to the histological variant of PTC, *BRAF* V600E mutation was present in 41.4% of microPTCs (500 out of 1,207), in 10.1% of follicular variants (83 out of 820), in 62.8% of classic variants (426 out of 678), in 80.6% of tall cell variants (116 out of 144), and in 10.5% of PTCs with prevalent trabecular and/or solid pattern of growth (6 out of 57). *BRAF* mutations other than V600E were found in 18 microPTCs, 33 follicular variants, 3 classic variants, and 1 solid variant of PTC. In detail, we found *BRAF* K601E mutation in 35 cases (63.6%), *BRAF* V600_K601delinsE mutation in 7 cases (12.7%) and *BRAF* T599I-V600_R603del in 2 cases (3.6%). Other 11 rare *BRAF* alterations (T599R+K601N, T599del, A598_T599insV, V600K, T599delinsIYI, K601_S605delinsN, V600_K601delinsQ, I592_A598dup, I582_A598dup, D594_T599dup, and V600_K601insNTV) were found in single cases. Some of these last *BRAF* mutations (6 out of 11) have never been described in the literature. At histological evaluation, the large majority of these tumors resulted completely encapsulated (46 out of 55, 83.6%) and only one case presented lymph node metastasis (1.8%). Clinically, the majority of these patients were classified as AJCC stage I or II (51 out 55, 92.7%).

Conclusions: *BRAF* alterations other than V600E are very rare in PTC and in general seem to be more prevalent in PTCs with low-risk clinical-pathological features (follicular variant, presence of tumor capsule, absence of lymph node metastasis).

571 No Detection of Circulating BRAF Mutant DNA in Thyroid Cancer Patients

Fulvio Basolo, Vincenzo Condello, Elisabetta Macerola, Clara Ugolini, Luigi De Napoli. University of Pisa, Pisa, Italy.

Background: Circulating cell-free DNA (cfDNA) in plasma is considered a promising noninvasive instrument for cancer monitoring. BRAF mutations in cfDNA may represent an appropriate indicator of the progression and aggressiveness of thyroid cancer. The aim of our study is to determine the presence of free circulating BRAF mutant DNA in thyroid cancer patients and to assess its clinical significance as a diagnostic and predictive marker.

Design: We preoperatively collected plasma samples from 70 patients with thyroid nodules cytologically diagnosed as TIR3, TIR4, TIR5, and for each patient we collected also FFPE tumor tissues. We evaluated BRAF mutational status both on plasma and tumor tissues of those patients histologically diagnosed for malignant nodules. The mutational analysis was performed by high sensitivity techniques as real- time PCR and digital PCR.

Results: At histological examination, 39 out of 70 samples resulted papillary thyroid carcinomas (PTCs), and were classified as follows: 22 classic variant (CVPTC, 56.4%), 15 follicular variant (FVPTC, 38.5%), 2 tall cell variant (TCVPTC, 5%). Of these, 8 (20.5%) showed perithyroidal soft tissues invasion and 7 cases (18%) had lymphnode metastases. On the 39 tissue samples, BRAF analysis showed 11 mutated cases (28.2%), including 9 CVPTC (40.9%) and 2 TCVPTC (100%). However, using both techniques, neither real-time PCR nor digital PCR were able to detect BRAF mutant alleles among the 39 plasma samples.

	Tumor Type	BRAF mut Tissue	BRAF mut Plasma	Perithyroidal invasion	LN metastases
	22 CVPTC (56.4%)	9 (40.9%)	0	7 (31.9%)	6 (27.3%)
	2 TCVPTC (38.5%)	2 (100%)	0	1 (50%)	1 (50%)
	15 FVPTC (5%)	0	0	0	0
Total PTCs	**39**	**11 (28.2%)**	**0**	**8 (20.5%)**	**7 (18%)**

Conclusions: We used high sensitivity techniques to determine the presence of BRAF mutant alleles on cfDNA of thyroid cancer patients. In our series, the 28.2% of malignant tumors showed BRAF mutations. In addition, many cases had aggressive pathological features, such as perithyroidal soft tissues invasion and lymphnode metastases. Even though, BRAF mutant DNA in the respective plasma samples was never detected, in contrast to literature data. In conclusion, contrary to other human cancer models, our preliminary results indicate that the BRAF molecular analysis on cfDNA in thyroid cancer patients might be not as useful as diagnostic clinical tool. The clinical significance of cfDNA analysis for other molecular markers involved in thyroid tumorigenesis is still under investigation.

572 Old Versus New: Individual and Combined Performance of CK19, HBME-1, CD117, and TROP-2 in Distinguishing a Wide Morphologic Range of Normal, Benign, and Malignant Thyroid Tissue Cores

Peter N Bonneau, Christopher Hartley, Bryan Hunt. Medical College of Wisconsin, Milwaukee, WI.

Background: TROP-2 and CD117 are new markers used in the diagnosis of papillary thyroid cancer (PTC). TROP-2 cytoplasmic and membranous staining along with loss of CD117 staining are touted as differentiating PTC from benign thyroid lesions. Historically, membranous and cytoplasmic staining with CK19 and HBME-1 have been used to assist in the differential between PTC and benign lesions; however, these more remotely described antibodies in the thyroid are thought to lack both sensitivity and specificity. The aim of the current study was to evaluate TROP-2, CD117, CK119, and HBME-1 expression in PTC against benign and normal thyroid, using H&E morphology as the gold standard.

Design: A tissue microarray was constructed with a total of 816 cores representing 23 cases of nodular hyperplasia, 21 lymphocytic thyroiditis (LT) cases, 18 Hurthle cell adenomas, 73 PTC cases (including multiple variants), 1 hyalinizing trabecular adenoma, and 1 insular carcinoma. Morphology confirmed on H&E stained cores was considered the gold standard. Immunohistochemical stains for CK19, HBME-1, TROP-2, and CD117 were performed. CK19, HBME-1, and CD117 IHC were considered positive when ≥10% cytoplasmic and/or membranous staining was present. TROP-2 IHC was considered positive when ≥25% membranous staining was present.

Results: On univariate analysis all evaluated markers showed significant abberant staining in tumor versus benign thyroid. Multivariate logistic regression analysis demonstrated the combinatorial ability of the markers to predict thyroid malignancy versus benign lesions. While statistically significant on univariate analysis, CK19 staining was not significant in the model including the other 3 markers. HBME-1 showed the best overall sensitivity and specificity.

Table 1. Sensitivity, Specificity, and Statistical Distinction of Tumor versus Benign Thyroid					
	% Sensitivity	% Specificity	Univariate p-value	Multivariate p-value	# of cores
CK19(+)	94	51	<0.01	0.33	654
HBME-1(+)	94	96	<0.01	<0.01	648
TROP-2(+)	97	63	<0.01	0.01	649
CD117(-)	94	78	<0.01	<0.01	643

Conclusions: Our study highlights the individual and combined ability of CK19, HBME-1, CD117, and TROP-2 to differentiate benign and malignant thyroid epithelium. Surprisingly, HBME-1 out-performed the newer antibodies, and should perhaps be reconsidered as a specific and sensitive marker of thyroid malignancy.

573 Adrenal Cortical Neoplasms: Are Immunostains Useful to Distinguish Benign from Malignant?

Shikha Bose, Shefali Chopra, Ann E Walts. Cedars-Sinai Medical Center, Los Angeles, CA; University of Southern California, Los Angeles, CA.

Background: Distinguishing benign from malignant adrenal cortical neoplasms and/or predicting the metastatic potential of carcinomas (CAs) can be difficult based on morphology alone. The role of p53, Ki67, androgen receptor (AR), and beta-catenin (BCAT) as diagnostic and/or prognostic adjuncts in adrenal cortical neoplasms is controversial.

Design: Formalin fixed paraffin embedded sections from 29 CAs (21 with and 8 without metastases), 5 adenomas and 5 normal adrenal glands were stained using antibodies against p53 (clone D07 Ventana), Ki67 (clone 30-9 Ventana), AR (clone F39.4.1 Biogenex) and BCAT (clone 14 Cell Marque). Nuclear staining in ≥ 5% of viable lesional cells was scored as positive for p53, Ki67, and AR. For BCAT, nuclear staining in > 10% or membranous staining in ≥ 50% of viable lesional cells was scored as positive. Follow-up for the CAs (range 2-241 mos.; median 20 mos.) was obtained from institutional cancer registries. Results were compared by diagnosis and by metastatic status. P-values < 0.05 were considered significant.

Results: The immunoprofiles of the CAs and normal/adenomas are compared in the table below. P53, Ki67, and nuclear BCAT expression were significantly different in benign and malignant cases. Positivity in any 2 of these 3 immunostains identified 15 (52%) CAs and none of the benign cases. No significant difference in expression was observed between CAs with and without metastases. Although 8 of the 21 CAs with metastases showed Ki67 > 30%, this did not reach statistical significance (p = 0.06). None of the 8 CAs without metastases showed Ki67 > 30%.

Immunoprofile of Normal Adrenal Cortex, Cortical Adenomas, and Cortical Carcinomas

Biomarker expression	Normal (n=5)	Adenoma (n=5)	Carcinoma (n=29)	Benign vs. CA p-value
p53 = or > 5%	0	0	14 (48.3%)	0.007
Ki67 = or > 5%	0	0	21 (72.4%	< 0.001
Androgen receptor = or > 5%	0	1	6 (20.7%)	0.65
Beta-catenin (nuclear) > 10%	0	0	10 (34.5%)	0.04
Beta-catenin (membranous) = or > 50%	100	100	23 (79.3%)	0.31

Conclusions: Our findings suggest that p53, Ki67, and/or nuclear BCAT positivity may be helpful in distinguishing benign from malignant adrenal cortical neoplasms.

574 Incidental Metastatic Papillary Thyroid Carcinoma: A Clinicopathologic Study

Kathleen Byrnes, Rebecca Chernock. Washington University in St. Louis, St. Louis, MO.

Background: Incidentally discovered metastatic papillary thyroid carcinoma (iPTC) in lymph nodes (LNs) removed for another reason is uncommon. A few small published cases series suggest that they behave in an indolent manner and thyroidectomy may not be indicated in all cases. Here, we characterize the clinicopathologic features of a larger series of iPTCs in LNs.

Design: All pathology reports of metastatic PTC from 1997 to May of 2015 were reviewed to identify iPTCs in LNs. Slides of iPTCs were reviewed to record pathologic features [size, histologic type, mitoses, necrosis, extracapsular extension]. Thyroid imaging, treatment, and patient follow-up were obtained by chart review.

Results: Of 25 iPTCs identified, most were in patients undergoing surgery for head and neck squamous cell carcinoma (SCC, 60%) or hyperparathyroidism (32%). The mean age was 49 years and 76% were male. Of 21 cases with slides available for review, the iPTCs ranged from <0.1 mm to 8 mm (mean of 2.5 mm) and involved up to 7 LNs. Tall cell features were identified in 29% of the cases (n=6). Extracapsular extension, mitoses or necrosis was not seen. BRAF mutation analysis was negative in 3 cases tested. Imaging (CT scan or ultrasound) reports were available in 20 cases, of which 6 had indeterminate or suspicious findings in the thyroid gland. Fifteen patients underwent total thyroidectomy (including 4 of 6 with abnormal imaging) and 2 underwent a lobectomy (including 1 with abnormal imaging). The 1 patient with thyroid nodules who did not have surgery has had multiple benign or non-diagnostic biopsies. All primary tumors measured ≤1.1cm and 7 were stage pT3. Eleven patients received radioactive iodine (RAI) post-operatively. RAI treatment was unknown in 3 cases. The length of follow-up ranged from 2 to 242 months (mean 50 months). One patient recurred (positive LN on RAI whole body scan 1 year after initial RAI). This patient had had focal (<5%) tall cell features in the 1 iPTC LN, a suspicious initial thyroid ultrasound, and a 7 mm T3 primary tumor. Another had an indeterminate RAI whole body scan at 20 months and a 3rd had uptake in regional LNs on initial RAI whole body scan but was lost to follow-up. While 5 died of metastatic SCC, none died of PTC or developed RAI resistant disease.

Conclusions: iPTC in LNs is indolent but recurrence after RAI treatment is possible. Thyroidectomy may not be necessary in all patients, especially if no primary tumor is found on thyroid ultrasound and/or the prognosis from a concurrent SCC is poor.

575 Does Reclassification of Follicular Variant of Papillary Thyroid Carcinoma Affect Rates of Malignancy by Bethesda Category?

Allison B Cavallo, Daniel N Johnson, Saaduddin Saddiqui, Raymon Grogan, Peter Angelos, Edwin L Kaplan, Tatjana Antic, Nicole A Cipriani. The University of Chicago, Chicago, IL.

Background: Potential reclassification of encapsulated or well-circumscribed follicular variants of papillary thyroid carcinoma (PTC-FV) to benign, non-invasive tumors with atypical nuclei decreases the rates of malignancy by Bethesda category. However, interobserver and interinstitutional variability in the diagnosis of thyroid malignancy is well-recognized. We hypothesize that decreased rates of malignancy by Bethesda category after reclassification are institution-dependent.

Design: Search of the archives of a large tertiary care center for thyroid resection specimens during 2011-2013 identified 617 nodules subject to fine needle aspiration biopsy (FNA). Bethesda category and final diagnosis for each nodule were recorded and malignancy rates by Bethesda category were calculated. Twenty nodules with prior FNA were called PTC-FV at histologic exam. Cases of PTC-FV were reviewed and reclassified as classic papillary thyroid carcinoma or non-invasive tumor. Malignancy rates by Bethesda category were then recalculated.

Results: 15 cases were reclassified as non-invasive tumor (2 in Bethesda II, 6 in III, 3 in IV, 3 in V, 1 in VI).

Rates of malignancy by Bethesda category						
	Before reclassification		After reclassification		% Decrease	
	Benign n (%)	Malignant n (%)	Benign n (%)	Malignant n (%)	Absolute	Relative
I	23 (85.2)	4 (14.8)	23 (85.2)	4 (14.8)	0	0
II	174 (96.1)	7 (3.9)	176 (97.2)	5 (2.8)	-1.1	-28.2
III	127 (81.9)	28 (18.1)	133 (85.8)	22 (14.2)	-3.9	-21.5
IV	42 (63.6)	24 (36.4)	45 (68.2)	21 (31.8)	-4.6	-12.6
V	12 (23.1)	40 (76.9)	15 (28.8)	37 (71.2)	-5.7	-7.4
VI	4 (2.9)	132 (97.1)	5 (3.7)	131 (96.3)	-0.8	-0.8
Total	382 (62)	235 (38)	397 (64)	220 (36)	-2	-5.3

Conclusions: Our findings show an absolute and relative decrease in malignancy rates for all Bethesda categories except I (non-diagnostic). The relative decrease in malignancy rate is greatest for nodules called benign on FNA (category II). The rate of change decreases with increasing category. The relative decrease in the rate of malignancy is less than reported by other institutions (up to 59% relative decrease). The expected change in malignancy rates following reclassification may vary significantly by institution and reported rates may not reflect institutional practices.

576 Molecular and Histopathologic Characteristics of Radioiodine-Refractory Papillary Thyroid Cancer

Sung-eun Choi, Ju Yeon Pyo, Ji-seop Kim, Eunah Shin, Soon Won Hong. Gangnam Severance Hospital, Seoul, Republic of Korea; Hanyang University Guri Hospital, Guri-Si, Kyeonggi-Do, Republic of Korea; CHA Gangnam Hospital, Seoul, Republic of Korea.

Background: Radioiodine (RI) ablation after surgical approach with suppression of thyroid stimulating hormone is an effective therapeutic agent for papillary thyroid cancer (PTC) and leads to excellent prognosis. Meanwhile, RI refractory tumors have aggressive behavior and poor outcome. Recently, along with the genetic abnormalities associated signaling pathways, mutation of telomerase reverse transcriptase (TERT) activity proved to be accused of the poor outcome in PTC. We comparatively analyzed the proportion of BRAF V600E or *TERT* promoter mutations, and the clinicopathologic differences between RI refractory and RI responsive PTCs.

Design: Among 82 patients that developed recurrence or distant metastasis following the administration of an accumulated dose of radioactive iodine of >600mCi, we determined 26 cases of RI-refractory group obtainable of formalin-fixed, paraffin-embedded tissue from initial thyroidectomy. 89 cases of PTCs were sampled as matched RI responsive group without distant metastasis during 5 following years after surgery. We performed PNA-mediated clamping PCR for BRAF V600E mutation and pyrosequencing for *TERT* promoter C228T and C250T mutation.

Results: *TERT* promoter mutation (P=0.000) was found in 14/26 (53.8%, 13 in TERT C228T, 1 in TERT C250T) cases of RI-refractory PTCs while only 1/82 (1.2%, in TERT C228T) case in RI-responsive PTCs. The BRAF V600E mutation was found in more than 80% in both groups (21/26, 80.8% versus 67/82, 81.7%). Coexistence of *TERT* promoter and *BRAF* mutations was found in 13/108 (12%) in the total cases of PTCs. In the histopathologic comparison of the two groups, RI-refractory PTCs showed significant increase in small tumor clusters that are composed of micropapillae without fibrovascular cores (≥20% cut off, $P<0.001$, especially in tumor center ($P=0.019$)), in hobnail features (≥ 5% cut off, $P=0.002$, especially in tumor center ($P=0.037$)) and in Height/Width of tumor cell (maximum≥3, $P<0.001$). The nuclei of RI-refractory PTCs had tendency to be located within the middle to base area of the tumor cells (Linear-by-Linear Association, $P<0.001$).

Conclusions: Our results suggest that RI refractory PTCs may be highly associated with *TERT* mutations as well as more aggressive histopathologic features, being prominent in the tumor center, which include small clusters and hobnail components.

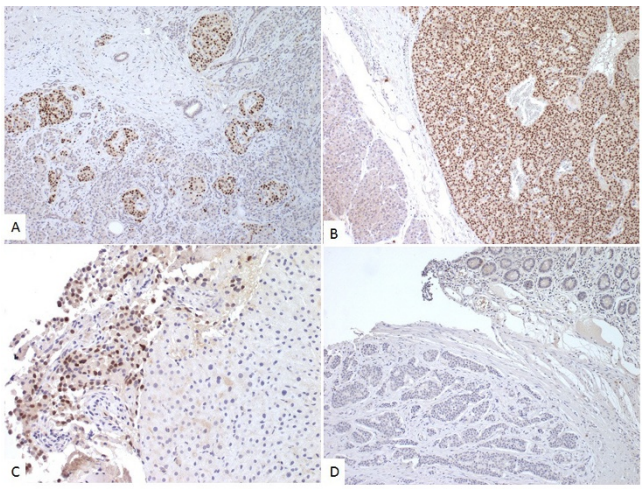
577 Progesterone Receptor Expression Is a Sensitive and Relatively Specific Biomarker for Well-Differentiated Pancreatic Neuroendocrine Tumors

Todd DeJulio, Xiaoming You, Haonan Li, Jie Liao, Sambasiva Rao, Xiaoqi Lin, Guang-Yu Yang. Northwestern University, Chicago, IL.

Background: Well-differentiated pancreatic neuroendocrine tumors (WD-PNET) are commonly encountered in pancreatic resections and occasionally seen in the metastatic setting. While these tumors consistently express neuroendocrine markers, there are not great markers for identifying metastatic WD-PNET as being of pancreatic origin. Although progesterone receptor (PR) is expressed consistently in pancreatic islet cells, whether or not PR expression could be a potentially useful tool in identifying primary and metastatic WD-PNET is not well studied. Thus, progesterone receptor expression is extensively evaluated immunohistochemically in primary WD-PNET and metastatic WD-PNET as well as primary and metastatic intestinal carcinoid tumors.

Design: A total 48 neuroendocrine tumors, including 22 primary WD-PNET, 11 metastatic WD-PNET and 15 primary and metastatic intestinal carcinoid tumors were assessed. Formalin-fixed paraffin-embedded sections were immunohistochemically labeled with a monoclonal antibody to PR with appropriate positive and negative controls. Cases were scored as positive if dense nuclear staining was observed. Pancreatic islet cells served as a positive control (Fig. 1a).

Results: 17 of 22 (77%) primary WD-PNET were strongly and diffusely positive for PR expression (Fig. 1b), while three of nine (33%) metastatic WD-PNET showed strong and diffuse positivity (Fig.1c). All fifteen of the primary and metastatic intestinal carcinoid tumors were negative for PR expression (Fig. 1d), but positive for CDX2. For the three WD-PNET in which the primary and metastatic tumors were both available for analysis, only one of the three primary tumors showed focal PR expression, and the metastatic tumors were all negative for PR.



Conclusions: Our results demonstrate that PR is a sensitive marker for WD-PNET. Compared to intestinal neuroendocrine tumors, PR could serve as a relatively specific biomarker to distinguish metastatic WD-PNET. The higher degree of PR negativity in metastatic tumors also suggests that lack of PR expression in WD-NET is a poor prognostic factor.

578 Oncocytic Adrenocortical Adenomas: An Important Cause of False Positive Malignant Results in Preoperative Urine Steroid Profiling

Salvador Diaz-Cano, Cathy Zhang, Norman Taylor. King's College Hospital/King's College London, London, United Kingdom.

Background: The diagnosis of malignancy in adrenocortical neoplasms can be challenging, especially for lesions of heterogeneous appearance and large size that suggest a malignant behavior. The urinary steroid profile (USP) has been used as a successful preoperative tool to distinguish adenomas (ACA) from carcinomas (ACC). The presence of oncocytic changes in adenomas (ACA-Onc) can contribute to confusion with ACC and its USP remains unknown.

Objective: To evaluate the features of ACA-Onc in comparison with other adrenocortical neoplasms.

Design: We selected consecutive ACA (15), ACA-Onc (7) and ACC (18) cases for which USP analysis was performed before surgery and tissue were available for histological evaluation (King's College Hospital, 2005-2014). Cases were classified according to WHO and Armed Forces Institute of Pathology criteria. USPs were obtained by gas chromatography/mass spectrometry. Total excretion of individual steroids and Indices (sums and ratios chosen to reflect steroid metabolic activity) were compared between ACA-Onc, ACA, and ACC. Steroids that have proved to be useful markers of ACC were also compared empirically between groups, including tetrahydro-11-deoxycortisol, pregnene3,16,20-triols, 16a- and 21-hydroxypregnenolone and tetrahydro-11-deoxycorticosterone.

Results: In comparison with ACA, tumors in ACA-Onc were significantly larger (10.3±2.1 vs. 3.5±1.0, P=0.002), presented in older patients and showed relatively higher incidence in males. Mitotic figure counts were significantly lower (0.32±0.04 vs. 0.95±0.10 in ACA, p=0.001) and revealed higher frequency of apoptotic cells (97% vs. 9% in ACA, p= 0.001). The USP of ACA-Onc showed diagnostic features of ACC, including 3α/3β ratios of pregnanetriol metabolites.

Conclusions: Oncocytic ACA reveals distinctive histological features, and USP markers suggestive of malignancy. It is important to recognize ACA-Onc because its size and heterogeneous appearance raise the possibility of ACC; in this context, USP alone cannot be used as a reliable tool for a correct preoperative diagnosis predicting the behavior of oncocytic adrenocortical neoplasms.

579 Metastatic Follicular Thyroid Carcinoma: Retrospective Institutional Experience and Insight into Primary Follicular Thyroid Neoplasia

Krzysztof Glomski, Peter M Sadow. Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Background: Solitary thyroid nodules biopsied as suspicious for a follicular or Hürthle cell neoplasm are generally surgically excised via thyroid lobectomy. It is accepted by endocrine pathology experts that proper grossing involves submitting the entire lesional capsule. As genetic testing becomes more commonplace and as assessment for invasion, both vascular and capsular, is subjective, especially in minimally invasive disease, we revisit our institutional experience with follicular thyroid carcinoma over the past twenty-five years.

Design: With IRB approval, 650 MGH cases from 1990-2015 bearing a diagnosis of follicular thyroid carcinoma were reviewed. Several parameters, including primary diagnosis among metastatic tumors, progression of primary minimally/widely-invasive (MI, WI), and poorly differentiated (PD) follicular carcinomas, completeness of capsule submission, tumor size, and available follow-up data were analyzed. Primary carcinoma cases with missing gross descriptions, <6 months or unknown follow up, and mixed lesions were excluded, with 293 cases included.

Results: Of 87 metastatic cases, 26 presented with metastasis and 5 were diagnosed as benign. Of 37 primary carcinomas that progressed to metastasis, capsule submission was complete in 10 and incomplete in 11 (16 unknown). Completely submitted nodules in this cohort were smaller (3.2cm) than incompletely submitted nodules (5.0 cm), p<0.05. Non-metastasic primary cases with incomplete capsule submission (72) showed metastatic progression in 12 cases; only 1 MI. In cases with complete capsule submission (145), metastatic progression was seen in 12 ; 1 FA and 1 MI. Metastatic lesions were associated with larger primary tumors (median, 4.5 cm) than non-metastatic cases (median, 3.4 cm), p<0.001. Average size in completely submitted specimens was 3 cm and 4.5 cm in incompletely submitted tumors (p<0.001).

Conclusions: Our cohort of 293 cases show wide invasion and poor differentiation in primary carcinoma correlated strongly with metastasis, regardless of extent of capsule submission; of minimally invasive FTC, incomplete submission did not increase likelihood of metastatis. As this study begins to address "appropriate" sampling of follicular thyroid lesions, additional parameters, including more quantitative analysis of capsule submission as well as investigating molecular alterations and their relationship to metastatic progression will be critical for accurate, efficient diagnosis and prognosis.

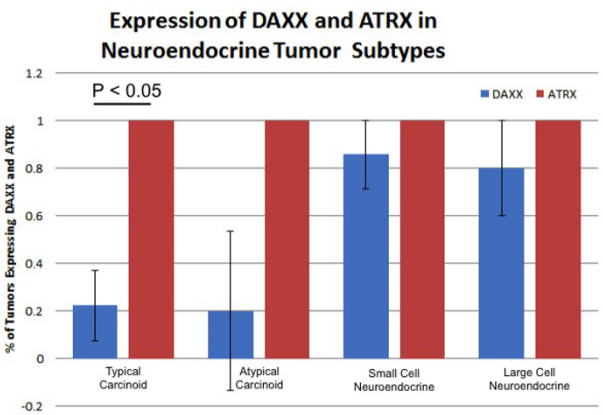
580 DAXX Gene: A Higher Rate of Mutation than ATRX Gene in Neuroendocrine Tumors

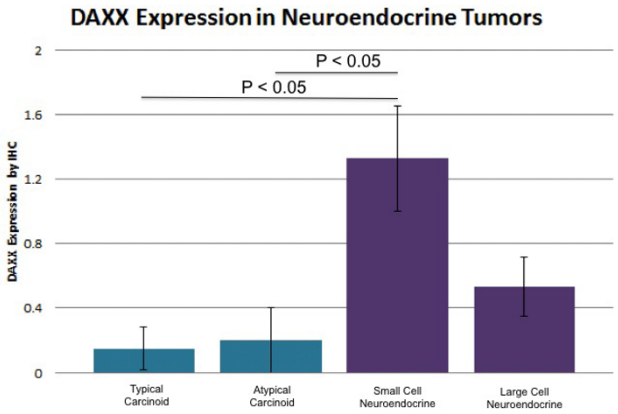
Abigail L Goodman, Vaidehi Avadhani, Cynthia Cohen, Momin T Siddiqui. Emory University Hospital, Atlanta, GA.

Background: Death domain-associated protein 6 (DAXX) and alpha thalassemia/mental retardation syndrome X-linked (ATRX) are tumor suppressors, with mutations resulting in loss of expression. Loss of DAXX and ATRX is associated with alternative lengthening of telomeres (ALT). In pancreatic NT, loss of DAXX or ATRX is associated with a worse prognosis. We investigated the expression of DAXX and ATRX in NT arising from other sites.

Design: Immunostaining for DAXX and ATRX was performed on 24 NT. These included; lung (n=17) & GI (n=7). The tumor sub-types included carcinoid tumor (CT, n=9), atypical carcinoid tumors (AC, n=3), small cell neuroendocrine carcinoma (SCNC, n=7), and large cell neuroendocrine carcinoma (LCNC, n=5). Staining was considered positive if staining intensity was 2+ or greater.

Results:





ATRX was positive in all NT (Fig 1). There is a significant decrease in the percent of tumors with DAXX immunostaining in CT and AC compared to SCNC and LCNC ($p < 0.01$). There is a significant difference in the DAXX combined score between CT and SCNC, and AC and SCNC (Fig. 2). There was a trend toward a decreased combined score in the LCNC, however the staining pattern was positive in most of these tumors (Fig. 1 and 2). A large proportion of the tumors were metastatic at the time of diagnosis (14 of 24); no difference in DAXX or ATRX expression was observed in metastatic versus non-metastatic tumors.

Conclusions: We conclude that NT have a much higher rate of DAXX mutation than ATRX. DAXX expression is altered between NT subtypes suggesting they are using different mechanisms of telomere lengthening. Loss of DAXX is suggestive of the use of ALT as the mechanism of telomere lengthening in CT and AC. This alteration in telomere lengthening may account for some of the prognostic differences between these subtypes as DAXX expression is known to correlate with prognosis in multiple tumor types and may respond differently to treatment, particularly radiotherapy.

581 Succinate Dehydrogenase Deficiency in Pheochromocytoma and Paraganglioma

Evan Holleran, Brandon Boldt, Devin McKissic, Jason L Hornick, Andrew M Bellizzi. University of Iowa, Iowa City, IA; Brigham and Women's, Boston, MA.

Background: Succinate dehydrogenase (SDH), a heterotetramer that resides in the inner mitochondrial membrane, participates in the Krebs cycle and electron transport chain. SDH-subunit mutations have been implicated in the autosomal dominant hereditary paraganglioma-pheochromocytoma (PARA-PHEO) syndrome and have been described in a subset of gastrointestinal stromal tumors and rare renal cell carcinomas. There is clinical interest in identifying SDH-deficient PHEOs and PARAs as an indicator of a potential hereditary cancer syndrome. Any SDH-subunit mutation destabilizes the SDH complex, resulting specifically in markedly reduced to absent SDHB expression, detectable by routine immunohistochemistry (IHC). *SDHA* subunit mutations result in additional loss of SDHA expression.

Design: We searched our pathology database for all PHEOs and PARAs diagnosed since 1991. Slides were reviewed and the diagnoses confirmed. Tissue microarrays were constructed (tumors arrayed in triplicate). IHC was performed with mouse monoclonal antibodies to SDHB (21A11AE7, 1:100) and SDHA (2E3, 1:750). Expression was scored as normal (intense, granular cytoplasmic staining) or abnormal (markedly reduced or completely absent staining). The following clinical data were obtained: tumor diagnosis, age, gender, anatomic location, primary/metastatic status. Two-sided Fisher's exact tests were used to analyze categorical data.

Results: We arrayed 111 PHEOs from 101 patients (ages 11-88) and 149 PARAs from 131 patients (ages 14-85). Abnormal and completely absent SDHB IHC were more common in PARAs than PHEOs ($p<0.0001$, $p=0.0126$). Among PARAs, SDHB absence was more common in thoracoabdominal (30%) than head and neck (13%) primaries ($p=0.0331$). SDH-deficiency due to SDHA was distinctly uncommon (up to 2%; 6/260). Detailed data are presented in the Table.

	M:F	Median Age	% SDHB Abnormal	% SDHB Absent	% SDHA Abnormal/Absent
PHEO (Total) (n=111)	46:55	46	13	6	1/1
PHEO (Primary) (n=103)	40:53	45	12	6	0/0
PHEO (Metastatic) (n=8)	6:2	51	25	13	13/13
PARA (Total) (n=149)	53:78	47	50	17	3/0
PARA (Primary) (n=111)	40:71	50	47	14	3/0
PARA (Metastatic) (n=6)	4:2	33	83	17	3/0

Conclusions: SDH-deficiency is common in PHEO and especially in PARA. We advocate screening all PHEOs and PARAs for SDH-deficiency using SDHB IHC, as the identification of SDH-deficiency should direct genetic counseling. SDH-deficiency due to *SDHA* mutation appears rare, and performing SDHA IHC routinely in SDH-deficient tumors does not appear warranted.

582 How Reliable Are TTF-1 and PAX8 in Confirming Thyroidal Origin in Undifferentiated (Anaplastic) Thyroid Carcinomas?

Daniel N Johnson, Tatjana Antic, Nicole A Cipriani. The University of Chicago, Chicago, IL.

Background: Undifferentiated (anaplastic) thyroid carcinoma (UTC) is an aggressive malignancy that usually presents as a rapidly-growing neck mass, possibly with metastases. It may not demonstrate evidence of thyroid origin on H&E, especially in the absence of a well-differentiated component. The morphologic appearance varies from large pleomorphic epithelioid cells to spindled sarcomatoid cells. Immunomarkers of thyroid origin (thyroglobulin, TTF-1, and PAX8) are variably expressed in UTC, with thyroglobulin considered least reliable. This study aims to determine rates of positivity of nuclear markers of thyroid origin (TTF-1 and PAX8) in UTCs.

Design: Full histologic sections from 11 UTCs were stained with PAX8 and 13 were stained with TTF-1.

Results: 73% (8 of 11) undifferentiated carcinomas expressed PAX8, while 15% (2 of 13) expressed TTF-1.

Conclusions: As thyroid carcinoma becomes less differentiated, it retains PAX8 in greater frequency than TTF-1. A majority of UTCs retain expression of PAX8. In the setting of an undifferentiated pleomorphic spindle cell or epithelioid neoplasm in the neck or cervical lymph node, PAX8 is a reliable marker of thyroidal origin.

583 Neuroendocrine Neoplasms of the Pancreas and the Lungs: Potential Differences in Morphological Characteristics and Cell Cycle Regulation

Atsuko Kasajima, Samaneh Yazdani, Tomoyoshi Tachibana, Hirotaka Ishida, Kazuma Kobayashi, Keigo Murakami, Hironobu Sasano. Tohoku University Hospital, Sendai, Miyagi, Japan; Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan.

Background: Neuroendocrine neoplasms (NENs) in the pancreas and the lung exhibit similar morphological features but classified by different systems, the former by WHO2010 and the latter WHO2015. However, differences of morphological and biological features of NEN arising in these two organs have remained relatively unexplored in details.

Design: 95 cases of pancreatic NEN (G1 47, G2 42, G3 6) and 22 cases of pulmonary NEN (typical carcinoid 9, atypical carcinoid 4, small cell carcinoma 2, large cell carcinoma 6) were retrieved. Morphological characteristic (fibrous capsule, necrosis, cellular atypia, stromal pattern, degrees of inflammation) were assessed and cell cycle regulators including p53, BCL2, p16 were evaluated using immunohistochemistry.

Results: When the WHO2010 classification was employed to pulmonary NEN, the cases were assigned as G1 6, G2 6 and G3 10 respectively. Ki67 index (%) were evenly distributed in both organs (pancreas 0-89.9, lung 0.07-78) but its deviation of mitotic index (/10HPF) in pancreatic NEN was smaller than that in the lung (pancreas 0-8, lung 0-98). Fibrous capsules were rarely identified in the lung (pancreas 75%, lung 5%). Foci of necrosis were rare in the pancreas (pancreas 1%, lung 50%). Degrees of cellular atypia and WHO2010 were correlated in NEN of both organs (pancreas $p=0.02$, lung $p<0.01$). Immunoreactivity of cell cycle regulators p53, BCL2 and p16 were significantly higher in G3 in the lung (p53 $p=0.005$, BCL2 $p=0.05$, p16 $p=0.02$) but they were not detected in the high grade pancreatic NEN ($p>0.05$ for p53, BCL2 and p16).

Conclusions: The stromal environments were different in the NEN arising in the pancreas and the lung and the higher mitotic indexes as well as increased immunoreactivity of G1-phase cell cycle regulators (p53, BCL2 and p16) in the lung NEN compared to the pancreatic one indicated the different mechanisms of cell cycle regulation between these two NENs.

584 Node-Negative Versus Node-Positive Papillary Thyroid Carcinoma: A Comparison of Clinicopathologic and Molecular Findings

Justin A Kotliar, Peter Kragel. The Brody School of Medicine at East Carolina University, Greenville, NC.

Background: Papillary carcinoma of the thyroid (PCT) is a common malignancy with a number of well-documented molecular genetic abnormalities. Evidence of how these molecular abnormalities correlate with nodal status and effect prognosis or treatment stratification is not well-demonstrated.

Design: The surgical pathology database was electronically searched and 37 cases of PCT with lymph node metastases and 36 cases of PCT without lymph node metastases were identified. Hematoxylin-and-eosin stained sections were reviewed, and representative areas of tumor nodules in the thyroid tissue marked. Tissue microarrays were created, and immunostains using standard methods were performed for BRAF, MET, PDL1, MUC1, P16, SPP1, and CLIP2. Stains were examined and graded. Patient age at diagnosis, sex, tumor size, focality, and extrathyroidal extension were also noted. Statistical analysis using chi-square with Yates correction was performed.

Results: The average age for all patients was 45 years, with a range of 9 years to 79 years. There was no significant difference in patient age when node-negative and node-positive groups were compared. 32 of 36 patients were female in the node-negative group while 23 of 37 patients were female in the node-positive group. This difference was statistically significant ($p=.0174$). Average size of the PCT tumor nodule was 2.0 cm for node-negative patients and 1.7 cm for node-positive patients. 2 node-negative and 5 node-positive cancers had extrathyroidal extension, and 18 node-negative and 21 node-positive cancers were multifocal. Differences in tumor size, multifocality, and extrathyroidal extension were not statistically signifcant. BRAF showed expression in 10 of 36 patients with negative nodes and in 22 of 37 patients with positive nodes ($p=.0127$). p16 showed expression in 4 of 36 node-negative and 13 of 37 node-positive patients ($p=.0153$). There was no statistically significant difference in expression of the other markers studied.

Conclusions: In our series, PCT with positive nodes was more commonly seen in males than females. Patient age at diagnosis, tumor size, focality, and extrathyroidal extension were comparable in both node-negative and node-positive groups. BRAF and p16 expression were associated with the presence of lymph node metastasis. When BRAF and p16 are expressed, especially in male patients, careful evaluation for possible lymph node metastases should be considered.

585 Calcitonin-Producing Pancreatic Neuroendocrine Neoplasms: Clinico-Pathologic Study of 25 Cases

Stefano La Rosa, Roberta Maragliano, Annika Blank, Silvia Uccella, Aurel Perren, Fausto Sessa. Ospedale di Circolo, Varese, Italy; University of Insubria, Varese, Italy; University of Bern, Bern, Switzerland.

Background: Calcitonin-producing pancreatic neuroendocrine neoplasms (C-PanNENs) are rare tumors and less than 50 cases have been reported in the English literature to date, mainly as case reports.

Design: Two specific anti-calcitonin antibodies (Signet, Dedham, MA, USA; Leica Biosystem-Novacastra, Nussloch, Germany) were used to detect calcitonin expression in a series of 262 PanNENs. The clinico-pathologic features of the immunohistochemically identified C-PanNENs were evaluated

Results: Twenty five (9.5%) C-PanNENs were identified. 15 patients were females and 10 males (mean age 53 years; range 30-84 years). All cases were sporadic. 17 neoplasms were clinically nonfunctioning while 8 patients presented the insulinoma syndrome. The mean tumor size was 4.4 cm (range: 0.8-20 cm). The most common site was the head of pancreas (10/25 cases). 12 cases were classified as NETs G1, 9 cases as NETs G2 and 3 cases as NECs, according to the WHO criteria. In one tumor, the Ki67 index was not evaluable. Focal necrosis was observed in six cases. 17/25 tumors showed vascular invasion. Amyloid deposits were present in only one tumor. Calcitonin-immunoreactivity (IR) was observed in all C-PanNETs (mean percentage of positive cells: 30%; range: 5-100%). 6/17 cases were CD117-IR and 17/23 were CK19-IR. Scattered cells with IR for glucagon, PP, and gastrin were also observed in a few cases. Insulinomas were strongly insulin-IR. Eight patients were at stage I, 6 patients at stage II, 8 patients at stage III and 3 patients at stage IV, according to the ENETS criteria. Four patients died of disease after a mean follow-up time of 5 months, 15 patients were alive (three of them with clinically evident disease) after a mean follow-up time of 90.6 months, while two patients died of other causes. Four patients were lost at follow-up.

Conclusions: Calcitonin is expressed in a fraction of PanNENs, both nonfunctioning and insulinomas. C-PanNENs encompass the spectrum of neuroendocrine neoplasms ranging from NET G1 to NEC and the outcome depends on the tumor type rather than on calcitonin expression.

586 GATA3 Expression in Pituitary Adenomas

Mirna Lechpammer, Jessica G Rogers, Kristin A Olson, Kiarash Shahlaie, Alexander Borowsky, John W Bishop. University of California Davis, Sacramento, CA.

Background: GATA3, zinc-finger transcription is sensitive for urothelial and breast carcinomas, but has low specificity. The aim of this study is to investigate the expression of GATA3 in typical and atypical histopathological subtypes of pituitary adenomas and its correlation with hormonal expression, proliferative activity, and p53 status.

Design: 29 patients who underwent transsphenoidal surgery for newly diagnosed pituitary adenomas were retrospectively reviewed for GATA3 expression by immunohistochemistry (IHC). Staining scores were determined by multiplying the percentage of lesional cells staining with an intensity score of 1+, 2+, or 3+ (H-score; range, 0 to 300). Functional status was determined by review of the laboratory findings and IHC.

Results: GATA3 was expressed in thirteen typical adenomas (13/29; 45%) and in none of the adenomas that met the criteria for atypical lesions; 11 (85%) of the 13 were macroadenomas. GATA3-expressing tumors occurred in 7 female (54%) and 6 male (46%) patients. Patient age ranged from 33 to 82 years (mean 58 years). Seven patients (54%) had hormonally active tumors, and 6 had nonfunctional lesions. IHC analysis demonstrated GATA3 expression in the following tumor subtypes: FSH/LH-producing adenomas (5/5 patients [100%]); null-cell adenoma (6/9 patients [67%]); GH-secreting adenoma, including those with plurihormonal staining (1/6 patients [17%]); prolactinoma (1/6 patients [17%]) and ACTH-staining tumors with Cushing disease (0/3 patients). The MIB-1 (Ki67) labeling index ranged from 0 to 5% (mean 2.7%). p53 was positive in 38% of GATA3-expressing adenomas. GATA3 expressing tumors showed a mean score of 201.5, and these results were consistent irrespective of the subtype examined.

Conclusions: GATA3 was expressed in approximately half of newly diagnosed pituitary adenomas. Expression was most commonly seen in FSH/LH and null-cell adenomas, and there was no correlation with histologically atypical features. Nevertheless, GATA3 must be interpreted with caution when evaluating pituitary lesions for a metastatic process.

587 Mixed Gangliocytoma-Pituitary Adenoma: Insights on the Pathogenesis of a Rare Sellar Tumos

M Beatriz Lopes, Emily A Sloan, Julie G Polder. University of Virginia, Charlottesville, VA.

Background: Gangliocytomas originating in the sellar region are rare; the majority are tumors composed of gangliocytic and pituitary adenomatous elements, forming the so-called "mixed gangliocytoma–pituitary adenoma" (GC-PA). Most GC-PAs are associated with endocrinopathies, mainly acromegaly and
less often Cushing's disease and hyperprolactinemia. The histogenesis of these tumors is debatable. Three theories have been proposed: 1. a primary gangliocytoma inducing an adenoma by hormonal stimulation; 2. transdifferentiation of adenomatous cells into gangliocytic cells; 3. a common progenitor/stem cell capable of transformation in the two cellular components. With the identification of specific transcription factors implicated in pituitary cytogenesis and differentiation, we have investigated if GH-secreting GC-PAs show similar pattern of differentiation and, therefore, possible common histogenesis.

Design: Nine cases of patients with acromegaly and tumors consistent with GC-GH adenoma were retrieved from the pathology files under the guidelines of the institutional IRB. The tumors were characterized by immunohistochemistry (IHC) for pituitary hormones, CAM 5.2, Pit-1, TTF-1, and the neuronal markers NeuN, neurofilaments (SMI33), and MAP2. Double-labeling IHC (DL-IHC) was performed for GH/Pit-1, SMI33/Pit-1, MAP2/Pit-1 and GH/NeuN.

Results: All 9 cases were diagnosed as sparsely granulated GH adenomas and had strong/diffuse Pit-1 nuclear expression on the adenomatous cells. In all cases, the gangliocytic cells expressed NeuN, SMI33 and MAP2. On single and double IHC, adenomatous cells did not express SMI33; however, focal NeuN and MAP2 expression was seen in adenomatous cells in 4/9 and 9/9 tumors, respectively. DL-IHC for GH/Pit-1 was positive in the majority of adenomatous cells. In 5/9 tumors, DL-IHC of Pit-1 and neuronal markers (SMI33 and/or MAP2) showed focal co-expression in gangliocytic cells. In 4/9 tumors, focal co-expression of NeuN and GH was present in gangliocytic cells. TTF-1, a transcription factor expressed by pituicytes, was negative in all cases in both cellular elements.

Conclusions: Although mixed GC-GH adenomas show histologically distinct cellular populations, there is at least a small population of cells that co-express the acidophilic-linked Pit-1 transcription factor and neuronal-associated cytoskeletal proteins. Therefore, it seems that the two cellular elements of GC-PAs may be histogenetically linked by either a common progenital/stem cell or due to transdifferentiation.

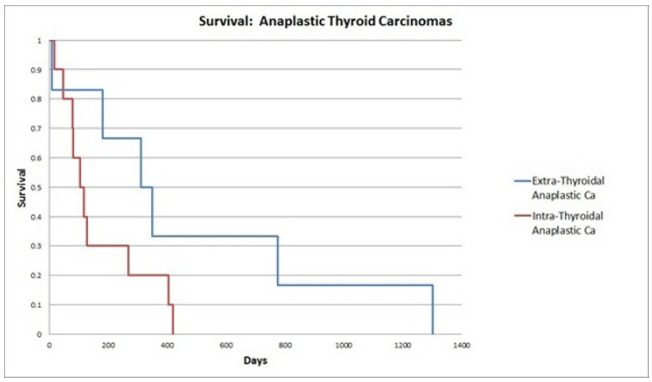
588 Anaplastic Thyroid Carcinoma Arising Outside the Thyroid Gland: A Comparison of Clinical Outcomes

Jonathon Mahlow, Deborah J Chute. Cleveland Clinic, Cleveland, OH.

Background: Anaplastic thyroid carcinoma is a rare and aggressive form of thyroid cancer, often with a prognosis of < 6 months survival. However, we have identified a subset of cases in which the transformation to anaplastic thyroid carcinoma has occurred outside the thyroid gland, typically arising from metastasis of a well-differentiated thyroid carcinoma. The clinical outcome of patients with extra-thyroidal anaplastic transformation has yet to be described in the literature.

Design: A retrospective records search from 1995-2015 identified six patients with a pathologic interpretation of anaplastic thyroid carcinoma arising outside the thyroid gland. In all cases, the thyroid gland was totally or near totally-submitted for histologic evaluation to rule-out intra-thyroidal anaplastic transformation. A Kaplan-Meier survival curve was created using the six identified cases and compared to ten randomly selected cases of typical anaplastic thyroid carcinoma arising within the thyroid.

Results: Patients with extra-thyroidal transformation (ET-ATC) (average age: 65.5 years; M:F ratio = 1:1) survived an average of 487 days after their initial diagnosis of ATC. All patients were diagnosed with papillary thyroid carcinoma prior to development of ET-ATC. Patients with intra-thyroidal transformation (C-ATC) (average age: 66.4 years; M:F ratio = 6:4) survived an average of 166 days after their initial diagnosis. ET-ATC occurred in cervical lymph nodes in 4 cases and peripheral soft tissues of the neck in 2 cases. All six cases of ET-ATC were treated by total thyroidectomy with neck dissection. All patients in both groups died of disease.



Conclusions: Our findings demonstrate that, while all patients eventually died of disease, ET-ATC has a more favorable prognosis than C-ATC. However, we recognize aggressive surgical resection in the cases of ET-ATC likely lowered the tumor burden relative to those cases of C-ATC in which invasion precluded complete surgical debulking. ET-ATC results from the delayed acquisition of molecular mutations in well differentiated thyroid carcinomas; in our series all arose from papillary thyroid carcinoma. Although rare, surgical pathologists should recognize that anaplastic transformation can occur outside the thyroid gland, with important prognostic implications.

589 Low PASS Is an Effective Tool for Predicting Non-Metastatic Pheochromocytomas

Julia R Maisel, Lauren Fishbein, Katherine L Nathanson, Virginia A LiVolsi, Debbie L Cohen, Kathleen Montone. Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Background: The Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) is a histologic measure to predict metastatic potential of pheochromocytomas. The score has significant intra and inter-observer variability, and therefore, many institutions do

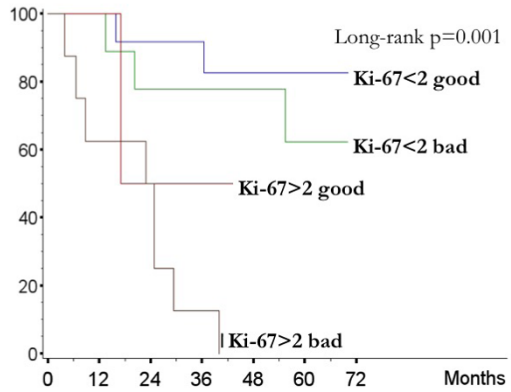
not use it. Despite this variability, our experience suggests there may be utility in the PASS. We hypothesize that the PASS can be an effective prognostic tool for determining which adrenal pheochromocytomas will not become metastatic.
Design: We examined all primary pheochromocytomas resected at The Hospital of the University of Pennsylvania before December 2008 to allow for adequate follow-up or that were resected between 2008 and 2013 with known distant metastases. The PASS was assessed by pathologists blinded to the clinical outcomes.
Results: Seventy-eight primary pheochromocytomas were available for review. Of those, 59% were female and the mean age at the time of surgery was 48.18±14.60 years. The median follow up was 5.36 years (range 0-18). The average PASS for the seven cases which developed metastatic disease was 7.71±3.04 (range 3-12), while the 71 non-metastatic cases had an average PASS of 4.24±2.30 (range 2-16). Only one metastatic case had a primary tumor PASS less than six. This patient had a separate primary head-and-neck paraganglioma which could be the source of metastatic disease. Removing this case, the six remaining metastatic cases had an average primary tumor PASS of 8.5 ± 2.43 (range 6-12).
Conclusions: Our study confirms that a PASS less than four, perhaps even less than six, can identify patients at very low risk of developing metastatic pheochromocytoma. PASS values higher than six have no utility in prediction of metastatic disease. A low PASS value (<4) has clinical utility in that patients with no susceptibility gene mutation and a low tumor PASS may not need long term follow up.

590 Gene Expression Profiling by Nanostring nCounter miRGE Assay May Identify Malignant Thyroid Nodules

Danielle Meunier, Iyare Izevbaye. University of Alberta, Edmonton, AB, Canada.
Background: Fine needle aspirate biopsy (FNAB) is a key screening test in evaluating thyroid nodules for cancer. Although widely used, 24% of FNAB results are indeterminate for malignancy and surgery is required to establish a diagnosis. Several studies have proposed molecular tests to supplement FNAB cytology, however they lack negative predictive value and fail to prevent diagnostic surgery in patients with benign nodules.
Design: Here we used a new technology, the Nanostring nCounter miRGE assay, which simultaneously detects messenger RNA (mRNA) and micro RNA (miRNA) species. We characterized gene expression levels of 109 genes from 40 tumor and 8 normal formalin fixed paraffin embedded thyroid samples; this panel included 97 mRNA and 12 miRNA targets chosen from the literature that are known to exhibit abnormal expression in thyroid malignancies. Fold change was used to identify differentially expressed genes. Hierarchical clustering by Euclidean distance was used to identify targets that could discriminate between the different tumor types.
Results: Our results show loose clustering of normal thyroid and papillary thyroid cancer samples when considering the entire 109-gene panel. Furthermore, papillary thyroid carcinoma, follicular thyroid carcinoma, poorly differentiated thyroid carcinoma and follicular adenoma can all be individually separated from normal thyroid tissue with a set of only 14 genes arranged in different combinations. Follicular adenoma and follicular thyroid carcinoma are loosely separated from each other by genes with greater than 2.8-fold difference in expression.
Conclusions: These results demonstrate the ability of this assay to detect potential patterns of variable gene expression between distinct classes of thyroid neoplasms. In the future, this assay may be used to discriminate malignant from benign thyroid nodules in clinical settings. However, more work is needed to determine the optimal combination of genes that will best separate each group and to translate the results to FNAB samples. Further analysis with clinicopathologic correlation will identify prognostic and predictive biomarkers for thyroid malignancies.

591 Pancreatic NENs <2 cm Could Represent a New Entity Worth of Further Investigation

Massimo Milione, Patrick Maisonneuve, Alessio Pellegrinelli, Giovanni Centonze, Mauro Scotti, Joregelina Coppa, Vincenzo Mazzaferro. Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; European Institute of Oncology, Milano, Italy.
Background: According to current guidelines, surgical approach for the treatment of well differentiated pancreatic neuroendocrine neoplasms (pNENs) is based on tumor size and Ki67. In particular, surgical removal is not recommended for tumors <2cm, due to their indolent nature. Ki67≤2% and size <2cm are considered, according to ENETS guidelines, indicative of benign tumors. However, size and Ki67 may not be sufficient to correctly predict the prognosis of patients with pNENs <2cm. We propose here a multiparametric approach to the classification of pNENs.
Design: In total, 75 patients (33≤2 cm, 42>2cm; 35 G1, 36 G2, 4 G3) were evaluated. All patients with <2cm had received surgical treatment. We investigated the following morphological parameters: mitotic count, parenchymal infiltration, vascular invasion, perineural infiltration, tumor necrosis. Progression-free survival (PFS) was evaluated for all patients.
Results: No difference in PFS were observed between NENs ≤2cm and those >2cm (p=0.920), whereas Ki67<2% was predictive of longer PFS irrespective of tumor size (p=0.001). Parenchymal infiltration (p=0.040), perineural infiltration (p=0.005), tumor necrosis (p=0.034) were predictive of shorter PFS.
We then defined two distinct morphological categories for all NENs: 'good', i.e. absence of parenchymal infiltration, perineural infiltration and tumor necrosis, and 'bad', i.e. presence of any of these parameters. 40/42 (95.2%) of NENs>2cm were classified as 'bad'; 16 NENs (48.4%) <2cm were classified as 'good' and 17 (51.6%) as 'bad'. 'Bad' NENs were associated with a significantly shorter PFS compared with 'good' tumors (p=0.024). The combination of Ki67 and the morphological category ('good' and 'bad') allowed a further characterization of prognosis.



Conclusions: A multiparametric approach based on morphological parameters may allow improved prognosis characterization also in patients with pNET <2cm. According to this multiparametric approach, differences in PFS emerged also among pNENs <2cm, until now considered an unique clinical entity. We believe that further investigation is required to better characterize this subgroup of pNENs in terms of prognostic features.

592 T-cell Infiltrate in GEP-NENs: identification and Evaluation of Its Distribution among the WHO G1, G2 and G3 NENs

Massimo Milione, Alessio Pellegrinelli, Giovanni Centonze, Francesca Dominoni, Joregelina Coppa, Sara Pusceddu, Roberto Buzzoni, Luca Giacomelli, Vincenzo Mazzaferro, Filippo De Braud, Andrea Anichini. Anatomic Pathology 1 Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; University of Genoa, Genoa, Italy.
Background: Given the results reported by immunotherapy in several advanced solid tumors, a key question is whether this therapeutic option may be potentially effective even in some gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs). The identification of T-cell infiltrate and the expression of inhibitory molecules in tumor microenvironment would support this therapeutic approach.
Design: This study investigates the presence of T-cell infiltrate in primary and metastatic lesions from 52 patients with GEP-NENs at all gastroenteropancreatic sites.106 specimens (52 primitive tumors (P), 33 lymph node metastases (N) and 21 liver metastases (L); 39 Stage IV and 13 Stage IIIb; 54 G1, 20 G2, 32 G3) were analyzed by immunochemistry directed against CD3, CD4, CD8, HLA1, HLADR and PDL1, and were compared with internal controls. The expression (E) of each antibody was defined as follows: 1+, immunoreactivity in <25% of neoplastic cells; 2+, 26–50%; 3+, 51–75%; 4+, 76–100%. Intensity (I) was ranked as low (1+), normal (2+), or strong (3+), compared with controls. E and I were combined into a single score (S; E×I). IHC positivity was given by score more than 1.
Results: CD3 positivity was detected in 21% of P, 21% of N and 9% of L; CD4 positivity was detected in 8% of P, 6% of N and 5% of L and CD8 positivity was detected in 13% of P, 12% of N and 10% of L. Specimens of stage IV L presented a higher staining score of HLA1 than lower-stage specimens (39/52, 75% vs 13/52, 25%; p=0.0001). HLA1 was frequently retained in P and N, but in L its staining score was lower in association with G3. PDL1 positivity was revealed only in the subset of N specimens with Ki67>20%.
Conclusions: A correlation between Ki67>20% and CD3, CD4, CD8, HLA and PDL1 was disclosed. HLADR was absent in all specimens. T cell infiltrate is present in a subset of GEP-NENs. Moreover, a subset of patients with Ki67>20% and loss of HLA1 expression was identified. These patients also appear to lack infiltrating T cells and may have a poorer prognosis than those with Ki67>20%, retention of HLA1 expression and presence of T cell infiltrate. Expression of PDL1 in stage IV patients with Ki67>20% may prompt studies on checkpoint inhibitors in this population.

593 Clusterin as Ancillary Marker of Medullary Thyroid Carcinoma

Najat Mourra, Malika Bennis, Beatrix Cochand-Priollet, Marine Lefevre, Charles Lepine, Jerzy Klijanienko, Fabrice Menegaux, Frederique Tissier. St-Antoine Hospital, Paris, France.
Background: Clusterin (CLU) is a sulfated glycoprotein, widely distributed in various tissues, and implicated in many physiological and pathological processes, including tumorigenesis. CLU overexpression occurs in various human neoplasms and represents a potential target therapy being tested in a phase III clinical trial in prostatic cancer.
However, there is no extensive data on its role in thyroid; here we investigate CLU expression in thyroid tumors and the potential correlation between this expression and clinicopathologic parameters.
Design: Immunohistochemistry with Anti-Clu (α/β rabbit H330, 1:300 dilution) was performed on paraffin sections from:16 adenomas, 12 papillary carcinomas, 6 follicular carcinomas, and 5 medullary carcinomas (MTC). Only MTC were positive.
To confirm these results, 130 more cases (including 4 C-Cell hyperplasias) collected from 5 centers, their matched lymph node metastases (46 cases) and lymph node recurrences (10 cases) were analysed.
Cytoplasmic positivity was scored qualitatively (weak, moderate, strong) and quantitatively on a 4-tired scale: 0, 1+ (less than 10% of cells positive), 2+ (less than 25%), 3+ (less than 75%), and 4+ (more than 75%).
Results: There were 59 men and 76 women, with a mean age of 55 years (13-90). The tumor was multiple in 34 cases (up to 7) with a mean size of 2 cm (0.1-7.5).

MTCs were classified according to WHO into variants: papillary (n=5), follicular (n=7), paraganglioma-like (n=12), oncocytic (n=52), small (n=28), spindle (n=21), and clear cells (n=1); 5 cases remained unclassified.
Scattered cells in the normal thyroid corresponding to C-cells were strongly stained, and so was C-cell hyperplasia.
All MTCs were positive; only 12% had a score of 1 and 8% of weak positivity. CLU expression seemed to be related to the cellular type, as only 9% of oncocytic type had a score 1 positivity, comparing to 32% of the small cell type. There was no correlation with other clinicopathologic parameters.
CLU was also positive in the metastases and recurrences.
Conclusions: In this first study of its kind, CLU was highly expressed in normal C-cells, C-cell hyperplasia, all MTCs, and their matched metastases and recurrences. In addition to calcitonin, CLU could be an ancillary marker in the diagnosis of MTC, and may represent a potential target therapy in metastatic and recurrent tumors.

594 BRAF and RAS Mutation Analysis to Distinguish Benign Ectopic Thyroid Tissue and Metatastatic Thyroid Carcinoma

Diana Murro, Sara Javidiparsijani, Alexandra Vardouniotis, Lela Buckingham, Paolo Gattuso. Rush University Medical Center, Chicago, IL.

Background: Ectopic thyroid tissue is a rare and controversial finding. Some experts consider it to always be metastatic thyroid carcinoma, while others consider it benign as long as it is restricted to few follicles without cytoarchitectural features of papillary thyroid carcinoma (PTC). To our knowledge, molecular studies have not yet been performed to further characterize this entity. We evaluate ectopic thyroid tissue for BRAFV600E and RAS mutations, which are commonly found in PTC and follicular thyroid carcinoma.
Design: We retrospectively searched our pathology files for all ectopic thyroid inclusions diagnosed in the past 23 years (1992-2015) and reviewed clinicopathological characteristics and concurrent thyroid pathological findings. Formalin-fixed, paraffin-embedded blocks were obtained from 9/11 cases for immunohistochemistry (IHC) and macrodissection. 7 cases had sufficient material for molecular studies, and 5 had available thyroidectomy blocks. BRAF V600E (VE1) IHC, BRAFV600E allele-specific polymerase chain reaction (PCR) and NRAS/KRAS pyrosequencing were performed. BRAF positive controls were 15 primary and metastatic PTC and NRAS/KRAS positive controls were 2 lung adenocarcinomas.
Results: The 11 patients (8 F, 3 M; mean age 52 yrs) were diagnosed incidentally at surgery (10 cases) or autopsy (1). Surgeries included thyroidectomy (5/10 cases, 50%), parathyroidectomy(3, 30%), neck dissection (1, 10%) and thyroglossal duct cyst excision (1, 10%). Locations were neck lymph nodes (7/11, 64%), neck soft tissue (3, 27%), and thymus (1, 9%). In 10 cases, diagnosis was based on lack of PTC features. 1 case had intranuclear inclusions and grooves suggestive of metastatic PTC; however, subsequent thyroidectomy revealed no malignancy. 8 patients had concurrent or subsequent thyroidectomies; the other 3 had benign thyroid follow-up imaging. All the ectopic cases were BRAF V600E (IHC, PCR) and NRAS/KRAS mutation negative (specificity = 100%) and correlated with thyroidectomy results. Compared with PCR, BRAF IHC had 75% sensitivity and 100% specificity.
Conclusions: Ectopic thyroid tissue most frequently occurs in neck lymph nodes. Lack of common carcinoma-associated mutations supports benign nature of this entity. BRAF IHC is accurate and helpful when not enough tissue is available for molecular studies. BRAFV600E and RAS mutation analysis is more specific than morphology alone in identifying benign thyroid rests. This ancillary method may help prevent unnecessary surgery.

595 Cytology/Histology Correlation of Follicular Variant of Papillary Thyroid Carcinoma: The UCSF Experience

Dianna Ng, Zheng Ma, Britt-Marie Ljung, Elham Khanafshar. UCSF Medical Center, San Francisco, CA.

Background: Follicular variant of papillary thyroid carcinoma (FVPTC) is a subtype of of papillary thyroid carcinoma. By definition these tumors are almost exclusively composed of follicles and tubules. There are two distinctive groups of FVPTC: an encapsulated subvariant (EFVPTC) and an infiltrative subvariant (IFVPTC). EFVPTC is more common. Recent studies have suggested that EFVPTC is a controversial entity that has indolent behavior with extremely low rate of recurrence if any. As a result, there has been a recent impetus to revise the nomenclature for EFVPTC to reduce overdiagnosis and overtreatment. One foreseen complication of this revision would be for the cytopathology community since the diagnosis of papillary thyroid carcinoma (PTC) is based solely on nuclear features.
Design: We searched the University of California, San Francisco (UCSF) Pathology database for thyroid nodule aspirates performed from 2005-2014 with diagnoses of PTC, follicular neoplasm /lesion (FN/L), suspicious for PTC (SUS), indeterminate, non-specific pattern, and atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS). Cases with subsequent surgical resection were identified and reviewed by three of the authors including an endocrine pathologist.
Results: 217 cytology cases were identified with 216 correlating surgical specimens. 191 surgical specimens were reviewed. Eighteen cases were identified as FVPTC comprising a total of 19 nodules with size range 0.4-4.5 cm (average 1.95 cm). Patients (4 men, 15 women) had an age range 29-70 (median age 48). Eight cases were classified as EFVPTC; on cytology 4 were diagnosed as FN/L, 3 as AUS/FLUS and 1 as PTC. Ten cases were classified as IFVPTC; on cytology 6 were diagnosed as PTC, 3 as SUS, and 2 as FN/L.

Table 1: Cytology/Histology correlation of FVPTC

	EFVPTC	IFVPTC*
PTC	1	6
SUS	0	3
FN/L	4	2
AUS/FLUS	3	0

*1 case had 2 FNAs performed with diagnosis of SUS and PTC, respectively
Conclusions: On cytology, EFVPTC and IFVPTC have divergent morphologies. At UCSF, most cases of EFVPTC had a cytologic diagnosis of FN/L or AUS/FLUS; only one case was categorized as PTC. Most cases of IFVPTC were called SUS or PTC. In most FNAs, cases of EFVPTC demonstrated microfollicular architecture but nuclear features of PTC were not as well-developed. Cases of IFVPTC showed more typical nuclear features of PTC on FNA. The ability to differentiate these two subvariants will be important if the nomenclature for EFVPTC is revised, and will have therapeutic and prognostic implications.

596 Immunohistochemical Expression of OCT3/4 in Adrenal Gland Pheochromocytomas and Prevertebral Paragangliomas

Marlo Nicolas, Patricia Dahia. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Background: OCT3/4 is a known marker for germ cell tumors, specifically, embryonal carcinoma and seminoma. Recently, we have observed that the prevertebral/paravertebral paraganglia in specimens from retroperitoneal dissections for metastatic germ cell tumors of testicular origin were diffusely staining for OCT3/4. In this study, we stained tumors originating from paraganglia, adrenal gland pheochromocytomas (ADP) and prevertebral paragangliomas (PVP) to evaluate the expression of these neoplasms for OCT3/4.
Design: OCT3/4 immunohistochemistry was performed in 19 cases of ADP and 2 cases of PVP. The immunoreactive score (0 negative; 2-4, weakly +; 6-8, moderately +; 9-12, strongly +) was calculated by multiplying the extent of staining (0%, 0; 1-10%, 1; 11-50%, 2; 51-80%, 3; 81-100, 4) with the staining intensity (negative, 0; weak, 1+; moderate, 2+; and strong, 3+).
Results: All 21 cases showed strong cytoplasmic staining for OCT3/4. Only 1 case of ADP had 80% of the tumor cells staining (immunoreactive score of 9) for OCT3/4. The rest of the ADP (18 cases) and the 2 cases of PVP had 90-100% of the tumor cells immunoreactive for OCT3/4 (immunoreactive score of 12). The adrenal medulla included in some of the stained slides were also positive for OCT3/4 while the adrenal cortex and mesenchymal tissues were negative.
Conclusions: OCT3/4 is strongly expressed in the cytoplasm of component cells of ADPs and PVPs in contrast to the nuclear staining of embryonal carcinoma and seminoma. In conjunction with the histomorphology and other established immunohistochemical markers, expression for OCT3/4 in ADPs and PVPs may facilitate the identification of these tumors of paraganglia. Awareness of this unusual cytoplasmic immunoreactivity in ADPs and PVPs may help prevent misdiagnosis when evaluating retroperitoneal dissections in patients with germ cell tumors.

597 Immunohistochemical Detection of NRASQ61R Protein in Follicular-Patterned Thyroid Tumors

Naoki Oishi, Tetsuo Kondo, Huy Vuong, Tadao Nakazawa, Kunio Mochizuki, Kazunari Kasai, Tomohiro Inoue, Ippei Tahara, Mitsuyoshi Hirokawa, Akira Miyauchi, Ryohei Katoh. University of Yamanashi, Chuo, Yamanashi, Japan; Kuma Hospital, Kobe, Hyogo, Japan.

Background: The *NRAS*^{A182G} mutation, which results in the NRAS^{Q61R} protein, is a major driver mutation in follicular-patterned thyroid neoplasms. Although new immunohistochemistry (IHC) for NRAS^{Q61R} is now available, its sensitivity, specificity and diagnostic utility for thyroid tumors are not yet established.
Design: We performed IHC for NRAS^{Q61R} and direct sequencing for *NRAS* codon 61 in four thyroid cancer-derived cell lines and 73 follicular-patterned thyroid tumors that included 22 follicular adenomas (FTAs), 35 follicular carcinomas (FTCs) and 16 cases of nodular hyperplasia (NH).
Results: Two cell lines with *NRAS*^{A182G} showed selective immunoreactivity for NRAS^{Q61R}. In tumor tissues, NRAS^{Q61R} IHC was positive in 18% (4/22), 29% (10/35) and 0% (0/16) of FTAs, FTCs and NH samples, respectively. All tumors with NRAS^{Q61R} expression exhibited cytoplasmic positivity with or without accumulation in their cell membranes.

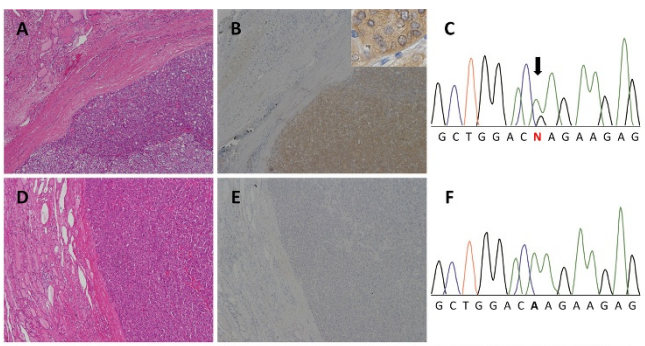


Figure 1 HE staining (**A** and **D**), NRAS^{Q61R} IHC (**B** and **E**) and Sanger sequencing (**C** and **F**)of FTCs with *NRAS*^{A182G} and *NRAS*^{WT}, respectively.

Of the 14 FTA/FTCs with NRAS^{Q61R} expression, 12 cases showed *NRAS*^{A182G} in direct sequencing, while all of the FTA/FTCs without NRAS^{Q61R} expression were negative for the mutation. Sensitivity and specificity of the IHC was 100% and 97%, respectively.

Table 1. Summary of *NRAS* mutation status and NRAS^{Q61R} IHC

				NRAS^{Q61R} IHC	
Histology	***NRAS* genotype**	**n**	**(%)**	**Negative**	**Positive**
FTA (*N*=22)	*NRAS*^{A182G}	4	(18)	0	4
	NRAS^{WT}	18	(82)	18	0
FTC (N=35)	*NRAS*^{A182G}	8	(23)	0	8
	NRAS^{G179A}	1	(3)	1	0
	NRAS^{WT}	26	(74)	24	2
HN (*N*=16)	*NRAS*^{A182G}	0		0	0
	NRAS^{WT}	16	(100)	16	0
Overall (*N*=73)	*NRAS*^{A182G}	12	(16)	0	12
	NRAS^{G179A}	1	(1)	1	0
	NRAS^{WT}	60	(82)	58	2

WT, wild type; FTA, follicular thyroid adenoma; FTC, follicular thyroid carcinoma; NH, nodular hyperplasia.

Conclusions: The IHC for NRAS^{Q61R} has excellent sensitivity and specificity in follicular-patterned thyroid tumors and expected to be useful in routine pathological diagnosis.

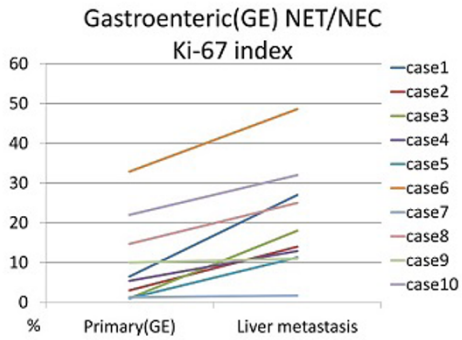
598 Ki67 Indices and Status of SSTR2 and SSTR5 in the Primary Tumors and in Their Liver Metastases of Gastro-Enteric(GE) and Pancreatic Neuroendocrine Neoplasms(NEN): Do They Change When They Metastasize?

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

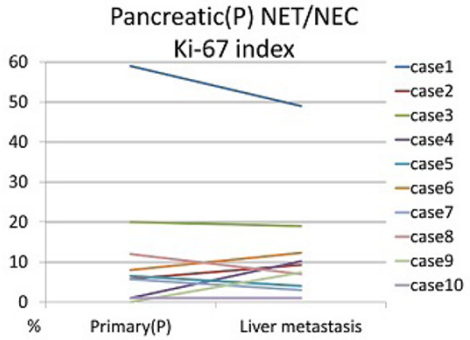
Background: Gastro-enteric(GE) and pancreatic(P) neuroendocrine neoplasms(NEN) has been graded into NET Grade(G) 1 and G2 and NEC(G3) according to the mitotic counts or proliferative Ki67 indices. This study is aimed at to compare Ki67 indices and SSTR2,5 expressions between the primary GE and P NENs and their liver metastases in order to pursue the better patient care.

Design: Total twenty cases(10 cases of GE NENs and 10 cases of P NENs) with both primary and liver metastases available were subjected to the following immunohistochemical analysis which was performed on FFPE sections using the antibodies, anti-Ki67(MIB1 DAKO) and anti-SSTR2 and anti-SSTR5(Epitomics Inc). The interpretation was done according to the criteria by Rindi G(for Ki67 2007) and by Volante M(SSTR2 Score 2, 3 positive 2007).

Results: For the GE NENs(NET and NEC), Ki67 indices in the liver metastases(LM) are higher than those of primary tumors(PT), average9.78% in the PT and average 20.5% in the LM.



Among ten cases, two cases converted from G1(PT) to G2 (LM) and wo cases from G2(PT) to NEC(G3)(LM). For SSTR2, one case from Score 0(PT) to 2(LM), another case from Score 2(PT) to 3(LM). For SSTR5, one case from Score 2(PT) to 3(LM). For the PNENs(PNET, NEC), the average Ki67 indices were 11.9 %(PT) and 12.2 %(LM).



From PT to LM, Ki67 indices were lowered in five cases, elevated in four cases. Two cases converted from G1(PT) to G2(LM). SSTR2 in one case converted from Score 1(PT) to 2(LM). SSTR5 increased from PT to LM in two cases, Score 1 to 2 and Score 1 to 3.

Conclusions: Our studies suggest that Ki67 and SSTR may change from PT to LM and that the metastatic NENs, especially GE NENs, should be examined for the better understanding and therapeutic application.

599 Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma Accounts for over Half of Carcinomas Harboring RAS Mutations

Vera Paulson, Priyanka Shivdasani, Brooke E Howitt, Matthew Ducar, Trevor Angell, Erik K Alexander, Edmund Cibas, Jeffrey F Krane, Neal Lindeman, Justine A Barletta. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.

Background: Molecular testing of thyroid nodules is increasingly being utilized to guide clinical management decisions. *RAS* mutations are the most frequent mutations detected in the context of an indeterminate fine needle aspiration (FNA) diagnosis. At the 2015 USCAP meeting, the Endocrine Pathology Society announced a proposal to change the terminology for non-invasive follicular variant of papillary thyroid carcinoma (NFVPTC) to promote conservative treatment (i.e. lobectomy only). This change was based on the indolent clinical behavior of these tumors and their molecular profile, which includes frequent *RAS* mutations. The aim of this study was to determine the percentage of *RAS*-mutant carcinomas that are NFVPTCs.

Design: From a database of 199 thyroid carcinomas from patients presenting for molecular characterization of their tumors as part of Profile:Oncopanel, we searched for cases with known activating *RAS* mutations. The preceding FNA diagnoses were recorded, and cases with a preceding indeterminate FNA diagnosis (defined as a diagnosis of atypia/follicular lesion of undetermined significance, suspicious for follicular neoplasm, or suspicious for malignancy) were identified. Tumor slides from resection specimens were reviewed to identify tumors that would be categorized as NFVPTCs.

Results: There were 27 cases in our cohort. Fifteen (56%) cases had an *NRAS* mutation, 9 (33%) had an *HRAS* mutation, and 3 (11%) had a *KRAS* mutation. Twenty-four (89%) cases had a preceding FNA, 19 (79%) of which had an indeterminate FNA diagnosis. The surgical resection specimen demonstrated FVPTC in 20 (74%) cases, classical type PTC in 2 (7%), solid variant of PTC in 1 (4%), and follicular carcinoma in 4 (15%). Of the 20 FVPTCs, 16 (80%) were NFVPTCs. NFVPTCs accounted for 59% of *RAS*-mutant carcinomas overall and 63% of *RAS*-mutant carcinomas with a prior indeterminate FNA diagnosis.

Conclusions: NFVPTCs accounted for over half of *RAS*-mutant carcinomas in our cohort. In cases where clinical and sonographic data support a low risk phenotype, these results suggest that a diagnostic lobectomy should be considered over total thyroidectomy as the initial surgical approach for a nodule with an indeterminate FNA diagnosis and a *RAS* mutation.

600 GATA3 Immunohistochemistry Differentiates Malignant Pheochromocytoma from Adrenal Cortical Carcinoma

Carmen M Perrino, Alex Ho, Debra L Zynger. The Ohio State University Medical Center, Columbus, OH.

Background: GATA3 is a relatively new immunohistochemical (IHC) marker which shows consistent nuclear expression in a variety of tumors, including urothelial carcinoma, breast carcinoma and paraganglioma. The staining pattern of GATA3 in adrenal lesions is poorly studied. We aim to describe the expression of GATA3 in adrenal tumors and to determine if there is differential staining of pheochromocytoma and adrenal cortical carcinoma (ACC).

Design: A retrospective search of the pathology database at our institution identified the following adrenal tumors: pheochromocytoma (n=13), pheochromocytoma with features concerning for malignancy (n=15), malignant pheochromocytoma (n=9), composite pheochromocytoma with ganglioneuroma (n=3) and ACC (n=10). Pheochromocytomas were defined as concerning for malignancy if they had ≥3 of the following features: periadrenal adipose tissue invasion, capsular invasion, vascular invasion, >1 mitoses per 30 high powered fields, atypical mitoses, necrosis, spindling or marked pleomorphism. ACC and malignant pheochromocytoma had histologically confirmed metastases. GATA3 IHC staining with a monoclonal antibody was performed on a representative slide (clone L50-823, 1:400, Biocare Medical). Nuclear reactivity was semi-quantitatively evaluated for percent of cells stained (0, <5%; 1+, 5-10%; 2+, 11-50%; 3+, >50%) and intensity (0-3).

Results: GATA3 was negative in 90% of ACC, with weak nuclear staining in a single case (0, 90.0%; 2+, 10.0%; mean intensity 1.0). In contrast, pheochromocytomas were frequently positive. The majority of malignant pheochromocytomas (88.9%, 8/9) had strong positivity for GATA3 (0, 11.1%; 3+, 88.9%; mean intensity 3.0). Most benign pheochromocytomas (69.2%, 9/13; 0, 30.8%; 2+, 30.8%; 3+, 38.5%) and pheochromocytomas with histologically concerning features (53.3%, 8/15; 0, 46.7%; 2+, 13.3%; 3+, 40.0%) were also positive with differential staining not identified (mean intensity score 2.3 for both). All cases of composite pheochromocytoma with ganglioneuroma were strongly positive (100.0%, 3/3; 3+, 100.0%; mean intensity 3.0).
Conclusions: GATA3 is frequently expressed in pheochromocytomas, with strong nuclear staining in malignant pheochromocytomas. GATA3 IHC may aid in the distinction between malignant pheochromocytoma (diffuse, strong positivity) and ACC (rare, weak positivity) in the clinical setting.

601 Genomic Profiling of Neuroendocrine and Neuroepithelial Neoplasms by Targeted Next Generation Sequencing

Jason N Rosenbaum, Chad Storer, Catherine E Cottrell, Eric J Duncavage. Washington University Medical School, Department of Pathology and Immunology, St. Louis, MO.
Background: The role of genetic variants in neuroendocrine neoplasms (NENs) is poorly understood, outside of inherited syndromes. One impediment to discovery may be that NENs are often understood as separate entities based on site of origin. NENs from diverse sites (as well as many neuroepithelial neoplasms - e.g. medulloblastoma) share common histologic appearances, protein expression, developmental origins, and regulatory pathways. Biologic insight may be gained into NENs and neuroepithelial neoplasms by examining them collectively, rather than treating them as entirely separate entities.
Design: From 1269 cases in which targeted NGS was performed, we identified 73 NENs or neuroepithelial neoplasms, including gastroenteropancreatic NENs, bronchial NENs, medullary thyroid carcinoma, paraganglioma, Merkel cell carcinoma, and medulloblastoma. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue, captured using probes targeting 151 cancer-associated genes, and sequenced to high depth of coverage. Probable somatic mutations were determined from single nucleotide variant (SNV) and insertion/deletion calls by removing variants present in various public databases with mean population allele frequencies >1%.
Results: Non-synonymous variants were identified in all cases, with a mean of 6.4 per case (range: 1-23), predominantly SNVs (98%). Variants were most frequently identified in *TP53* (23 variants in 22 cases) and *RB1* (11 variants in 11 cases). The variants in each of these genes were observed across a variety of tumor types. No individual category of neoplasm showed a significant association with any single variant, nor with variants in any particular gene.
Conclusions: Our findings are consistent with published literature suggesting that specific driver mutations are rare in non-syndromic NENs and neuroepithelial neoplasms. The frequency of SNVs in *RB1* is notable; alterations to *RB1* have been reported in some NENs and neuroepithelial neoplasms, but often as deletions or alterations to expression of the protein, rather than SNVs. By treating NE and neuroepithelial neoplasms as a broader class, we identify recurrently mutated genes that might otherwise remain obscure.

602 Paradoxical Decrease of TSPO Expression in Adrenocortical Carcinomas Confers a Worse Prognosis

Roberto Ruiz-Cordero, Mouhammed A Habra, Alia Gupta, Merce Jorda, Kanishka Sircar. MD Anderson Cancer Center, Houston, TX; University of Miami Jackson Memorial Hospital, Miami, FL.
Background: The 18-kDa translocator protein (TSPO) is a mitochondrial protein found in almost every tissue in the body. Its main function represents the rate-limiting step in steroidogenesis by translocating cholesterol into the mitochondrion for its conversion into pregnenolone. TSPO expression is up regulated in steroidogenic organs like the adrenal gland. Increased TSPO expression has been reported in certain tumors and in some cases, it correlated with disease progression. TSPO expression in adrenocortical neoplasms has not been previously reported
Design: Two tissue microarrays were prepared by randomly sampling 1 to 5 different areas of tissue per case. TSPO expression was assessed by immunohistochemistry and the staining intensity was graded from 0 (no staining) to 5+ (increased staining). In one additional case, the expression of TSPO was assessed (in tumor and normal adrenal tissue) by immunofluorescence and quantified by western blot. Correlation between TSPO expression intensity and different variables and the difference between expression and diagnoses were assessed using Pearson and ANOVA statistics. Kaplan-Meier survival curves were also performed. Significance was set at p-values< 0.05
Results: TSPO expression was studied in a total of 102 cases: 23 controls, 31 adenomas (20 with Weiss score (WS) of 0 and 11 "atypical" with WS of 1 or 2), 1 borderline adrenal oncocytic neoplasm (AON), and 48 adrenocortical carcinomas (ACC). TSPO expression was similar in all controls (3+) and variable among all tumors (0 to 5+). TSPO expression in ACC was inversely correlated to stage, metastasis, and a longer survival (p=0.008). No correlation between different diagnoses and TSPO expression was observed. Vital status and TSPO expression did not correlate with age or gender. Decreased expression was more frequent in functional ACC. A 70% paradoxical loss of expression was observed by western blot in the borderline AON. TSPO expression did not correlate with WS or Ki-67 proliferative index
Conclusions: We report for the first time the variable expression of TSPO in adrenocortical neoplasms. Conversely to what has been described in the literature, decreased expression of TSPO indicates a worse prognosis in ACC. TSPO expression seems to be an independent prognostic marker involved in the physiopathology of ACA

603 Frequency of TERT Promoter Mutations in Thyroid Carcinoma Metastases

Karan Saluja, Ying C Henderson, Maria Cabanillas, Elizabeth G Grubbs, Stephen Y Lai, Michelle D Williams. UT MD Anderson Cancer Center, Houston, TX.
Background: Identifying biomarkers of aggressive disease are needed for thyroid cancer. Recent observations of mutations in the promoter region of the telomerase reverse transcriptase (TERT) gene suggest a possible association with metastatic tumor potential. The incidence of these mutations in the spectrum of thyroid cancer types and any association with specific distant metastatic sites (DM) of disease involvement are unknown. The aim of this study was to investigate the frequency and clinicopathologic associations of *TERT* promoter mutations at various metastatic sites across thyroid carcinoma phenotypes.
Design: 26 patients, who had undergone surgery for DM (10 lung, 10 brain, 6 bone/soft tissue) were identified. The primary thyroid diagnoses included 15 papillary thyroid carcinoma (PTC), 5 follicular carcinoma (FC), 2 Hurthle cell carcinoma (HCC), 3 medullary thyroid carcinoma (MTC), and 1 anaplastic thyroid carcinoma (ATC). DNA was extracted from formalin fixed paraffin embedded tissue and amplified by PCR for the TERT promoter region. Nested PCR was performed and the PCR product was sequenced by Sanger sequencing. The clinicopathologic features were compared between *TERT* mutated (-124 loci) versus TERT wild-type using Fisher exact probability test and unpaired t-test.
Results: The *TERT* promoter mutation was identified in 7 of 26 (26.9%) metastases from 6 of 15 men and 1 of 11 women with advance thyroid carcinoma. *TERT* mutations occurred in PTC (4 of 15, 26.7%) and FC (3 of 5, 60%) with a median age of 59 years at the time of 1st DM (range, 46-86). No mutations were identified in MTC (n=3), HCC (n=2) or ATC (n=1). Amidst various metastatic sites, *TERT* mutations were highest in lung metastasis (40%) followed by bone/soft tissue (33.3%) and brain (10%). Three of the 4 *TERT* mutated PTCs were identified in lung metastases that developed a median of 242 months after the primary thyroid diagnosis (which were at <45 years of age). The clinicopathologic features were similar between *TERT* mutated and wild-type groups including the rate of progression following DM presentation (36 vs 56 median survival in months respectively) (P>0.05).
Conclusions: *TERT* promoter mutations occur in a subset of follicular derived thyroid carcinoma metastases involving various sub-sites, however did not predict a more rapid disease progression. Thus TERT's role as a biomarker in advanced thyroid disease is unclear. The association between *TERT* mutation and late metastases in young patients with PTC requires future investigation.

604 Clinical and Morphologic Features of ETV6-NTRK3 Translocated Papillary Thyroid Carcinoma

Raja R Seethala, Simion I Chiosea, Cheng Z Liu, Marina Nikiforova, Yuri E Nikiforov. University of Pittsburgh, Pittsburgh, PA; NYU Medical Center, New York, NY.
Background: While the morphologic and clinical correlates for common molecular alterations in thyroid carcinomas are established, newly described alterations such as the *ETV6-NTRK3* translocation are less characterized. We report our experience with a series of papillary thyroid carcinomas harboring this translocation.
Design: Clinicopathologic features were reviewed on 9 cases (2004-2015) confirmed to harbor the *ETV6-NTRK3* translocation using next-generation sequencing (DNA/RNA ThyroSeq v2 on Ion Torrent Personal Genome Machine) for mutational testing of > 300 hotspots in 14 genes as well as 42 gene fusions. TTF-1 immunostaining was performed on 5 cases (clone:8G7G3/1; 1:50 dilution; Dako, Carpinteria, CA).
Results: Clinical features are in Table 1.

Case	Age (years)	Sex	Size (cm)	Extrathyroidal Extension	pN	pM
1	21	F	2.2		0	
2	47	F	5.0		1b	
3	19	F	1.8	Y	1b	
4	22	F	2.1		0	
5	55	F	4.3		0	
6	38	M	5.9		---	
7	35	F	5.0	Y	1b	1(brain)
8	42	F	3.0		0	
9	60	F	2.6	Y	---	

Mean age was 38 years (range: 19-60 years) with a female predilection (8:1). Extrathyroidal extension was present in 3/9 cases. 3/7 cases had positive lymph nodes (pN1b), while one case showed a brain metastasis. In 2 cases, the absence of a radiation history was noted.
All cases showed foci with clear, vacuolated cytoplasm; 5/9 cases were partly oncocytic. Growth patterns included: encapsulated (n=2), encapsulated with capsular invasion (n=3) to infiltrative (n=4). All cases with lymph node or distant metastases were infiltrative. The encapsulated tumors were follicular patterned (<1% papillary growth). The remainder showed follicular predominance with variable papillary growth. Nuclear features were prominent (pseudoinclusions in 5/9 cases). The brain metastasis in case 7 was deceptively bland with a prominent follicular pattern and abundant colloid. All tested cases were TTF-1 positive.
Conclusions: *ETV6-NTRK3* translocated papillary thyroid carcinomas are predominantly follicular patterned, and range from encapsulated to infiltrative tumors with papillary growth and regional and distant spread. Nuclear features are overt, though metastases may show discordantly bland nuclei. Distinctive features include vacuolated, clear to oncocytic cytoplasm reminiscent of the secretory phenotype noted in *ETV6-NTRK3* translocated carcinomas of other sites.

605 Sclerosing Mucoepidermoid Carcinoma with Eosinophilia: Clinicopathologic & Molecular Analysis of a Distinct Entity

Akeesha Shah, Kristin La Fortune, Stacey E Mills, Zubair Baloch, Virginia LiVolsi, Sanja Dacic, Alyssa M Luvison, Marina Nikiforova, Yuri E Nikiforov, Raja R Seethala. University of Pittsburgh, Pittsburgh, PA; Indiana University, Indianapolis, IN; University of Virginia, Charlottesville, VA; University of Pennsylvania, Philadelphia, PA.

Background: Sclerosing mucoepidermoid carcinoma with eosinophilia (SMECE) is a rare thyroid neoplasm of uncertain pathogenesis that resembles salivary gland mucoepidermoid carcinoma. We characterize the clinicopathologic and molecular features in a multi-institutional series.

Design: 10 SMECE from 3 institutions were retrieved. Molecular testing included breakapart fluorescence in-situ hybridization (FISH) for *MAML2* and next-generation sequencing (DNA/RNA ThyroSeq v2 on Ion Torrent Personal Genome Machine) for mutational testing of >1000 hotspots in the following 14 genes: *AKT1*, *BRAF*, *NRAS*, *HRAS*, *KRAS*, *PTEN*, *TP53*, *TSHR*, *GNAS*, *CTNNB1*, *RET*, *PIK3CA*, *TERT*, and *EIF1AX* as well as 42 gene fusions involving *RET*, *BRAF*, *NTRK1*, *NTRK3*, *ALK*, *PPARG* and *THADA*.

Results: Clinicopathologic features of SMECE are reported with respect to age in years (yrs), location (R=right, L=left, I=isthmus), size (cm), nodal status (N=negative, P=positive) and follow-up (LR=local recurrence, NED=no evidence of disease, DOD=dead of disease) in table 1.

Case	Sex	Age	Location	Size	Nodal Status	Follow-Up
1	F	74	L	5.0	---	LR 4 yrs
2	M	70	R	3.0	N	---
3	F	65	R	6.0	P	DOD 1 yr
4	F	48	R	0.5	N	NED 9 yrs
5	M	41	I	0.5	N	NED 7 yrs
6	M	30	R	0.5	---	---
7	M	62	L	6.0	---	---
8	F	67	R	4.0	N	---
9	F	38	R	6.0	P	NED 5 yrs
10	F	77	R	6.0	N	NED 11 yrs

Mean age was 57 yrs (range: 30 to 77 yrs) with a slight female predilection (3:2). All cases had chronic lymphocytic thyroiditis and two had papillary thyroid carcinomas. Nodal metastases were noted in 2/7 cases, LR in 1/6 cases, and DOD in 1/6 cases. Mean follow-up on patients without disease was 8 yrs. FISH for *MAML2* was negative (n=10). The 6 cases which were tested for common thyroid tumor mutations and translocations were negative. However, increased *NTRK1* and *MET* expression was seen in 6 and 3 cases, respectively.

Conclusions: SMECE is a biologically distinct neoplasm that is molecularly unrelated to salivary mucoepidermoid carcinoma as well as papillary or follicular thyroid carcinoma. They may show local recurrence, nodal disease, and rarely, mortality. The basis for *NTRK1* and *MET* overexpression requires further investigation.

606 Can Medullary Carcinoma Arise in Thyroglossal Duct Cysts? A Search for Parafollicular C-cells in 38 Resected Cases

Tracy A Stein, Paari Murugan, Faqian Li, Mohamed El Hag. University of Minnesota Medical Center, Minneapolis, MN.

Background: Thyroglossal duct cysts are present in approximately 7% of adults and develop from persistence of the midline migratory tract between the foramen cecum and anatomic location of the thyroid gland. Thyroid tissue can be identified in roughly 2/3 of thyroglossal duct cysts upon thorough examination. Additionally, up to 1% of thyroglossal duct cysts develop an associated malignancy, 90% of which are papillary thyroid carcinoma. Cases of follicular and anaplastic carcinoma have also been documented, but there are no case reports of medullary carcinoma arising in a thyroglossal duct cyst, presumably due to the distinct embryologic origin of parafollicular C-cells from the ultimobranchial body. In normal thyroid tissue, parafollicular C-cells aggregate in the upper and middle portions of the lobes, but are not found in the isthmus or other midline structures. The goal of this study is to determine whether parafollicular C-cells, a source where medullary carcinoma could potentially develop from, are present in thyroglossal duct cysts and associated thyroid tissue.

Design: 38 cases of thyroglossal duct cyst were retrieved from the laboratory medical records. H&E sections were thoroughly examined for the presence of thyroid tissue, thyroglossal duct remnants, ultimobranchial remnants, and parafollicular C-cells. The presence and absence of thyroid tissue on H&E was recorded. Immunohistochemistry was performed on selected sections for TTF-1 (thyroid transcription factor) and calcitonin. The presence and distribution of TTF-1 nuclear positivity and calcitonin positivity was recorded.

Results: On H&E examination, 82% (31/38) of resected thyroglossal duct cysts contained thyroid tissue. TTF-1 staining was positive in these 31 cases and did not reveal any additional cases with thyroid tissue remnants. In contrast, no ultimobranchial bodies or parafollicular C-cells were identified on H&E. Calcitonin did not highlight any ultimobranchial body remnants or parafollicular C-cells in all 38 cases examined, confirming the findings on H&E.

Conclusions: These findings illustrate that although thyroglossal duct cysts often contain thyroid tissue, parafollicular C-cells are absent. Therefore, unlike other thyroid neoplasms, there is no evidence to support the possibility of medullary carcinoma arising in the setting of a thyroglossal duct cyst.

607 Mathematical Modeling of Epithelial-to-Mesenchymal Transition in Thyroid Carcinoma Progression

Kaitlin Sundling, Darya Buehler, Zhenying Guo, Heather A Hardin, Rakesh Mandal, Celina Montemayor-Garcia, Ricardo V Lloyd. University of Wisconsin, Madison, WI.

Background: Low-grade thyroid carcinomas, including papillary, follicular and follicular variant of papillary thyroid carcinomas, generally carry good prognoses, although a fraction metastasize. In contrast, high-grade thyroid carcinomas, such as poorly differentiated carcinoma and anaplastic thyroid carcinoma (ATC), have high rates of metastasis and poor prognoses. We propose that complex interactions may result in a molecular "switch" governing the epithelial-to-mesenchymal transition (EMT). We created a mathematical model to investigate the control of EMT in thyroid carcinoma.

Design: Prior work quantified protein and RNA targets by immunohistochemical staining and in situ hybridization, respectively, on tissue microarrays containing normal thyroid, follicular adenomas, low-grade carcinomas and high-grade carcinomas. In the current work, we created a model of 26 ordinary differential equations based on published molecular interactions and ran simulations using Python with the SciPy stack.

Results: Experimentally, EMT proteins Slug, Twist, PRRX1 and Glut1 increased in high-grade carcinomas with concomitant loss of E-cadherin. Other known EMT markers Ezh2, PRRX1 and micro RNA (miR) 146b-5p showed no relationship to carcinoma progression. The model showed complex interconnections, with Snail and Slug integrating multiple inputs (Fig. 1), and mutually inhibitory feedback loops, with miR 200 and miR 34 inhibiting Zeb1 and Snail (Lu M, Cancer Res 2014). Simulations showed progression to ATC, with loss of E-cadherin and increasing Slug, Twist and miR 21. In ATC, however, simulated levels of Ezh2, Zeb1, SMAD7 and Glut1 varied between high and undetectable, despite experimental expression of these markers.

Conclusions: Our model predicts expression of key EMT markers in progression to ATC and shows potential for complex behavior, making a molecular "switch" possible. However, changes in molecular interaction rates led to low levels of some EMT markers with concomitant E-cadherin loss. Further work will explore which interactions lead to this behavior, and whether additional interactions are needed to simulate other tumor types. The model will be refined to predict future targets for experimental study, including potential biomarkers and therapeutic targets.

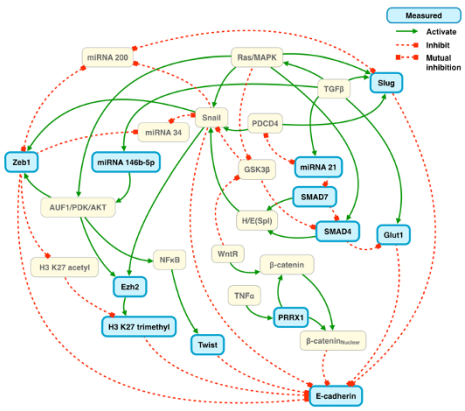


Figure 1: Diagram of molecular interactions included in mathematical model of EMT in thyroid carcinoma progression.

608 Thyroglossal Duct Cyst Carcinomas: A Clinicopathologic Series of 21 Cases

Lester DR Thompson, Hannah B Herrera, Sean K Lau. SCPMG, Woodland Hills, CA; SCPMG, Anaheim, CA.

Background: Carcinoma arising from a thyroglossal duct remnant cyst (TGDC) are rare, without well defined management and staging criteria.

Design: All TGDC (n=688) diagnosed between 2005 and 2015 were retrospectively reviewed, with 21 carcinomas identified (3% incidence).

Results: 21 patients (16 females; 5 males), aged 12-64 years (mean 40.3 years) were identified. An anterior, superior midline neck mass was the presenting symptom in all patients. A cancer diagnosis (all papillary thyroid carcinoma [PTC]) was made after the Sistrunk procedure (SP), with a Bethesda 5 or 6 classification preoperatively by FNA in 5 of 12 cases tested. A SP was performed in all patients, with total thyroidectomy concurrently (n=5) or subsequently (n=11). Neck dissection was performed in 4 patients, with metastases found in 2. Of the patients who had a thyroidectomy, concomitant PTC was identified in 5. Follow-up radioablative iodine therapy (RAI) was performed in 13 patients. Metastatic disease to local lymph nodes 57 months after presentation was seen in 1 patient, with all others alive and disease free (mean 3.4 years; range 0.3-10.5 years). This supports an origin from extra-thyroidal remnants (cyst origin) rather than metastatic tumor from a thyroid gland primary. The TGDC ranged from 0.8 to 5 cm (mean 2.3 cm), while the papillary carcinomas ranged from 0.1 to 3.8 cm (mean 1.4 cm). All of the tumors were classical papillary carcinoma, showing a sclerotic and infiltrative pattern, with a capsule present in 10. Lymphovascular invasion was detected in 11; margins were positive in 6. Using currently defined criteria, the patients were separated into Group I (n=19); II (n=1); III (n=1). But if extension into the adipose tissue (n=11), skeletal muscle (n=10) or perineural/perivascular tissues (n=10) were used to stage the patients, interpreted as "extraglandular extension" (n=13), there would be 13-Group I and 8-Group III cases. Two patients with metastatic disease would be upstaged from I to III if using extension criteria. Further 7 of 8 Group III patients were ≥45 years.

Conclusions: TGDC carcinomas are uncommon, with nearly all accounted for by papillary carcinoma. With most tumor "microscopic" (<1 cm), conservative management can be used for patients <45 years (SP only); due to the high incidence of concurrent papillary carcinoma and higher stage at presentation in older patients, completion thyroidectomy is recommended for patients ≥45 years. A good prognosis can be expected for papillary carcinoma developing in TGDC, even when there is soft tissue extension. Staging is advocated.

609 Ampullary and Duodenal Somatostatin-Producing Neuroendocrine Tumors: Two Histologically and Clinico-Pathologically Distinct Entities

Alessandro Vanoli, Stefano La Rosa, Luca Albarello, Federica Grillo, Frediano Inzani, Rachele Manca, Michele Martino, Ombretta Luinetti, Roberta Maragliano, Catherine Klersy, Fausto Sessa, Roberto Fiocca, Claudio Doglioni, Guido Rindi, Carlo R Capella, Enrico Solcia. IRCCS San Matteo Hospital and University of Pavia, Pavia, Italy; University of Insubria, Varese, Italy; Ospedale di Circolo, Varese, Italy; San Raffaele Hospital, Milan, Italy; University of Genoa, IRCCS San Martino IST, Genoa, Italy; Catholic University, Rome, Italy.

Background: Neuroendocrine tumors (NETs) arising in the duodenum and ampullary region (AR) are rare and include several tumor entities, among which somatostatin (SS)-producing tumors. These may show heterogeneous clinico-pathologic features, but data are limited.

Design: We collected 68 duodenal/AR NETs containing SS immunoreactive cells. Gangliocytic paragangliomas were excluded. Those arising in the AR or the minor papilla (Ampullary SS Tumors, ASSTs) were compared with those located in the remaining duodenum (Duodenal SS Tumors, DSSTs) with regards to histologic, immunohistochemical and clinico-pathologic patterns.

Results: 35 ASSTs and 33 DSSTs (24 in the duodenal bulbus) were identified. Compared to the DSSTs, ASSTs showed larger mean size (2.28 cm vs 0.75 cm) and higher rates of proliferative grade 2 (40% vs 3%), lymphovascular invasion (80% vs 16%), invasion beyond the submucosa (65% vs 9%) and local lymph node metastases (54% vs 12%) ($p<0.001$ for all differences). Eight ASSTs (23%, vs 3% for DSSTs) showed distant metastases (3 at diagnosis and 5 during follow up) and 3 ASST patients, but no DSST patient, died of disease. Histologically, ASSTs were characterized by the presence of tubular structures (89%), often enclosing psammomatous bodies (37%), and extensive (60% to 100% of tumor cells) reactivity for SS in 97% of cases (versus 24% of DSSTs). Moreover, ASSTs lacked membranous reactivity for SS receptor type 2A in 84% of tested cases (against 24% of DSSTs), while both retained membranous expression of type 5 SS receptor.

Conclusions: Most ASSTs harbor distinctive clinical and histologic features as well as higher invasive and metastatic potential, making their distinction from DSSTs clinically relevant.

610 C-KIT: A Useful Adjunct Marker for the Evaluation of Papillary Thyroid Carcinoma

Jaylou Velez Torres, Ernesto Martinez Duarte, Oleksandr N Kryvenko, Jennifer Chapman, Darcy A Kerr, Christina Kovacs, Carmen Gomez Fernandez. University of Miami Miller School of Medicine/ Jackson Memorial Hospital, Miami, FL.

Background: Loss of the c-KIT receptor occurs during the malignant transformation of normal thyrocytes to papillary thyroid carcinoma (PTC). Indeed, gene expression studies have identified decreased *c-KIT* expression in PTC. Immunohistochemistry (IHC) for c-KIT has been recently described as a novel ancillary diagnostic tool in a series of fine needle aspiration biopsies of histologically confirmed classic PTC. For this study, we evaluated the immunohistochemical expression of c-KIT in a series of surgical resection specimens that included classic as well as several variants of PTC, and compared the results with c-KIT expression in a series of clinically and histologically benign thyroid nodules (BTNs).

Design: We reviewed the archives of the University of Miami Department of Pathology for surgically resected cases of PTC with confirmed lymph node metastases. Fourteen cases of PTC were identified and represented several of the known morphologic variants including classical (6), classical and follicular (4), diffuse sclerosing (3), tall cell/oncocytic (1). All 14 demonstrated diffuse (>75%) expression of HBME by IHC. Fourteen cases of clinically and histologically benign thyroid nodules (BTNs) served as the control group and included follicular adenomas (1), adenomatoid (11) and hyperplastic nodules(2). All BTN were negative for HBME. The two groups were evaluated for the immunohistochemical expression of c-KIT.

Results: All of the PTC demonstrated complete lack of immunoreactivity for c-KIT. The absence of c-KIT by the tumor cells contrasted sharply with the positive expression of c-KIT by the adjacent normal follicular epithelial cells. In contrast to PTC, all of the BTNs expressed c-KIT, with variable staining intensity, predominantly localized to the cytoplasmic membrane. The positive staining of the normal follicular cells included a combination of diffuse cytoplasmic, membranous, and dense granular cytoplasmic patterns of reactivity.

Conclusions: c-KIT demonstrates a differential immunohistochemical expression pattern in clinically and histologically proven cases of PTC (diffusely negative) and BTNs (diffusely positive). The negative immunoreactivity for c-KIT in PTC contrasts sharply with the positive expression of c-KIT in the adjacent normal thyroid follicular epithelium (internal control). The use of this marker either in combination with HBME or as a sole determinant for the diagnosis of challenging or borderline cases of PTC will be the subject of further investigation.

611 Recurrent and Aggressive Pituitary Adenomas and Carcinomas: A Comprehensive Genomic Profiling Study

Olga Voronel, Kyle Fedorchak, Siraj M Ali, Julia A Elvin, Jo-Anne Vergilio, James Suh, Shakti H Ramkissoon, Vincent A Miller, Philip J Stephens, Jeffrey S Ross. Albany Medical College, Albany, NY; Foundation Medicine Inc., Cambridge, MA; Brigham and Women's Hospital, Boston, MA.

Background: Recurrent pituitary adenomas and infiltrating carcinomas (PitCA) are classically resistant to conventional treatment and pursue an aggressive clinical course. We queried whether comprehensive genomic profiling (CGP) of PitCA would uncover clinically relevant genomic alterations (CRGA) that could potentially lead to targeted therapies for patients with relapsed and metastatic disease.

Design: DNA was extracted from 40 microns of FFPE sections from 25 cases of PitCA. CGP was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 519X for up to 315 cancer-related genes. Genomic alterations (GA) included base substitutions (SUB), INDELs, copy number alterations (CNA) and fusions/rearrangements. CRGA were defined as GA linked to drugs on the market or under evaluation in mechanism driven clinical trials.

Results: The (56%) male and 11 (44%) female patients had a mean age of 45.9 years (range 18 to 76 years). All 25 (100%) of the PitCA had relapsed and progressed after primary surgical and radiation therapies. There were 9 WHO Grade 1 (36%), 3 grade 2 (12%) and 13 grade 3 (52%) tumors. 8 (32%) of the PitCA stained positively for pituitary hormone production (3 ACTH, 3 prolactin, 1 TSH and 1 GH). Ki67 labeling index (LI) averaged 16% with 91% featuring KI-67 ≥ 5%. CGP revealed an extremely low frequency of GA at 1.28 GA/sample. The most frequent non-CR GA involved *TP53* (12%). *TP53* positive PitCA had a mean Ki-67 LI of 34%. Only 5 (20%) of the PitCA cases featured at least one CRGA with a CRGA frequency of 0.4 CRGA per tumor. The 5 PitCA with CRGA included 1 GA in *BRCA2* potentially associated with use of PARP inhibitors, GA in *PTEN* and *RICTOR* potentially associated with use of MTOR inhibitors, 1 GA in *HGF* potentially associated with MET inhibitors, 1 GA in *ERRB2* potentially associated with HER2 inhibitors and 1 GA in *CDK4* potentially associated with cell cycle inhibitors.

Conclusions: Recurrent and refractory PitCA feature a high WHO grade, high Ki-67 LI, high TP53 mutation frequency and an extremely low rate of total GA when compared to other solid tumors. In this study of 25 PitCA cases, 20% of the tumors were found to have significant CRGA that have the potential to direct targeted therapies for these individual patients. Further study of genomic profiling of clinically aggressive pituitary tumors in a prospective clinical trial setting featuring the use of targeted treatments appears warranted.

612 TERT Promoter Mutation as a Prognostic Marker in Follicular Carcinomas

Michelle D Williams, Ying C Henderson, Gary L Clayman, Gilbert Cote. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The biologic potential of follicular (FC) and Hurthle cell carcinomas (HC) of the thyroid is difficult to predict by histologic parameters alone. Prognostic biomarkers to aid in identifying patients at high-risk for distant metastases are needed and may allow for development of adjuvant therapies in those patients. Recently identified telemerase reverse transcriptase (*TERT*) gene promoter region mutations have been linked to aggressive disease in several tumor types including thyroid carcinoma. As follicular neoplasms require long follow-up in order to delineate their true biologic potential, the incidence and significance of *TERT* mutations in this subgroup requires further characterization.

Design: 59 follicular thyroid neoplasms with long term follow-up (median > 140 months) representing the spectrum of biologic potential were analyzed for *TERT* promoter mutations (*TERTm*): follicular (3) and Hurthle (6) adenomas, indolent FC and HC (10 and 4 respectively without recurrence), aggressive FC and HC with metastases (18 each). DNA extracted from fresh tissue was PCR amplified for the *TERT* promoter region and Sanger sequenced beginning ~400bp upstream from the *TERT* start site. Clinicopathologic features were compared between *TERTm* @ position -124 versus wild type (WT) tumors.

Results: *TERTm* was identified primarily in aggressive FC (11 of 18, 61%) with a subset identified in aggressive HC (3 of 18, 17%), indolent FC (1 of 10, 10%) and indolent HC (2 of 2, 50%). No *TERTm* were seen in adenomas. *TERTm* occurred in 11 of 17 (65%) males with carcinoma versus 17 of 32 females (53%). Older age at FC diagnosis was seen in *TERTm* over WT tumors (median 64 vs. 41 years, t-test $p< 0.0004$). As *TERTm* in aggressive FC verses indolent FC was significant ($p=0.01$), *TERTm* as a potential biomarker has a sensitivity of 92% and specificity of 61% for identifying aggressive FC (PPV of 91%). Within the aggressive carcinomas survival curves were not significantly different regardless of *TERT* status. No correlations were identified within the Hurthle cohort.

Conclusions: *TERT* promoter mutational testing of primary thyroid follicular neoplasms (non-Hurthle) may improve risk stratification as a prognostic marker of aggressive/ metastatic potential; however, this association is less clear in Hurthle neoplasms where *TERTm* is less frequent.

613 Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma and the Afirma Gene-Expression Classifier

Kristine S Wong, Trevor Angell, Kyle C Strickland, Erik K Alexander, Edmund Cibas, Jeffrey F Krane, Justine A Barletta. Brigham and Women's Hospital, Boston, MA.

Background: At the 2015 USCAP Companion Meeting, the Endocrine Pathology Society announced a proposal to change the terminology for non-invasive follicular variant of papillary thyroid carcinoma (NFVPTC) to reflect the indolent clinical behavior of this tumor and encourage conservative management (i.e. lobectomy only). The Afirma

gene-expression classifier (GEC) helps guide clinicians in the management of thyroid nodules with indeterminate fine needle aspiration (FNA) results. Thyroid surgery is recommended for nodules with a suspicious Afirma result, whereas observation is deemed reasonable for most nodules with a benign result. Because surgery is advised for NFVPTCs, it is important that they be recognized by the Afirma GEC. The aim of this study was to evaluate if the Afirma test detects NFVPTCs and determine how many carcinomas detected by the Afirma GEC would be categorized as NFVPTC.
Design: From a database of 249 FNAs sent for Afirma testing between 1/2012 and 10/2014, we searched for cases with indeterminate cytology, a suspicious Afirma result, and a corresponding resection specimen reviewed at our hospital. The diagnoses of the prior FNAs and subsequent resection specimens were recorded. All tumor slides for cases diagnosed as FVPTC on resection were reviewed to identify tumors that would be categorized as NFVPTCs.
Results: Our cohort included 69 cases that met inclusion criteria. The preceding FNA diagnosis was atypia/follicular lesion of undetermined significance in 34 (49%) cases, suspicious for a follicular neoplasm in 30 (44%), and suspicious for malignancy in 5 (7%). The surgical resection specimen demonstrated classical type PTC in 1 (1%) case, FVPTC in 22 (32%), follicular carcinoma in 5 (7%), and a benign tumor/nodule in 41 (59%). Of the 22 FVPTCs, 20 (91%) could be characterized as NFVPTCs.
Conclusions: Our results indicate that the Afirma GEC detects NFVPTCs and that many of the carcinomas detected by Afirma are NFVPTCs. Because lobectomy only is advised for these tumors, the results suggest that a suspicious Afirma result for most nodules with indeterminate cytology should prompt lobectomy and not total thyroidectomy.

614 Clinico-Pathologic Features of Fatal Non-Anaplastic Follicular Cell-Derived Thyroid Carcinomas (Non-ANA FCDCs)

Bin Xu, Tihana Ibrahimpasic, Laura Wang, R Michael Tuttle, Ian Ganly, Ronald Ghossein. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: The vast majority of thyroid cancer, in particular the non-ANA FCDCs, are considered indolent tumors with very low mortality. Hence, it is crucial to analyze the subgroup of patients who die of disease (DOD) in order to identify those at risk of tumor progression as well as those with good outcome.
Design: All non-ANA FCDCs operated at a tertiary cancer center between 1985 and 2010 who were DOD were identified and submitted to a meticulous clinico-pathologic analysis.
Results: 55 fatal non-ANA FCDCs (1.4%) out of 3,750 thyroid carcinomas were identified. The DOD group was composed of 30 (55%) poorly differentiated carcinoma (PDTC), 14 (25%) papillary thyroid carcinomas (PTC) tall cell variant, 4 (7%) Hurthle cell carcinomas, 3 (5.5%) papillary microcarcinomas, 2 (4%) PTC classical variant and 2 (4%) PTC follicular variant. Twenty-seven patients (49%) presented with distant metastases (DM), 25 (45%) developed DM during follow ups, while the remaining 3 (5%) had locally advanced non-resectable disease. Gross extension beyond thyroid (GET) was present in 34 (62%) and extensive vascular invasion (VI) in 20 (37.7%) of cases. All microcarcinomas had PDTC in their cervical nodes at presentation. Encapsulated thyroid carcinomas were responsible for 18% of mortality and all had extensive VI and/or DM at presentation. All patients had at least one of these high risk features at diagnosis: DM at presentation, PDTC, GET, extensive VI. The average time to death was 4.7±0.3 years and was significantly shorter in patient with DM at presentation (3.4±0.4 years) compared to those without (6.1±0.7 years; p = 0.002). The majority of patients died from DM (n=49, 89%), 3 (5%) of locoregional disease, and 2 (4%) from both.
Conclusions: 1) PDTC and tall cell variant PTC are responsible for the vast majority of death in differentiated thyroid carcinomas while the few fatal classical, follicular variant PTC and microcarcinomas all harbor PDTC component, DM or GET.
2) Encapsulated differentiated thyroid carcinoma with focal capsular and/or VI without DM at presentation does not seem to cause death.
3) Lack of DM at presentation, PDTC, GET and extensive VI identify thyroid carcinomas at almost no risk of DOD.
4) The vast majority of patients die of DM rather than locoregional invasion, prompting the need for effective systemic treatment.

615 Stage Specific Embryonic Antigen-1 (SSEA-1) Expression in Thyroid Tissues

Jin Xu, Heather A Hardin, Ranran Zhang, Ricardo V Lloyd. University of Wisconsin School of Medicine and Public Health, Madison, WI.
Background: SSEA-1, also known as CD15, is a member of cluster of differentiation antigens that have been identified in various normal tissues including hematopoietic cells, as well as different types of cancers such as papillary and medullary thyroid carcinoma. Some studies suggest that SSEA-1 may be expressed in normal stem cells and cancer stem-like cells (CSCs). To evaluate the potential diagnostic and prognostic utility of SSEA-1 in thyroid tumors, we analyzed the expression of SSEA-1 in normal and neoplastic thyroid tissues by immunohistochemistry (IHC) and by flow cytometric analysis.
Design: A tissue micro array (TMA) was constructed with sections of normal thyroid (NT, n=10), nodular goiters (NG, n=10), follicular adenoma (FA, n=32), follicular carcinoma (FCA, n=28), papillary thyroid carcinoma (PTC, n=28), follicular variant of papillary thyroid carcinoma (FVPTC, n=29), and anaplastic thyroid carcinoma (ATC, n=10), and stained by immunohistochemistry (IHC) with an antibody to SSEA-1 at a 1:100 dilution (BD Biosciences). The individual sections were graded by intensity of staining from 0 (negative) to 3 (strongly positive) as well as the percentage of the tumor cells staining positive (<5% - rare, 5-25% - focal, and >25% - diffuse). Flow cytometric analyses with ATC and PTC cell lines were done to evaluate the percentage of SSEA-1 positive cells in the tumors and normal tissues.
Results: IHC stain for SSEA was negative in all benign thyroid tissues, but positive in all types of malignant thyroid neoplasms including conventional PTC, FVPTC, FCA, and ATC. Of cases that were positive for SSEA-1, the IHC staining was usually membrane associated and focal. Among different types of tumors, ATC showed the highest SSEA expression levels (80%, 8/10), which was significantly higher than FCA (32.1%, 9/28) (p = 0.023) and FVPTC (20.6%, 6/29) (p = 0.001), respectively. PTC (60.7%, 17/28) showed significantly higher expression than FVPTC (p= 0.01) as well as FCA (p=0.028). Flow cytometry analyses showed that about 5% of ATCs cells from the cell line expressed SSEA-1, which was significantly higher than in two PTCs cell lines (<2%). In univariate analyses, SSEA positivity in ATC was associated with worse survival (p=0.036). Six of six ATC cases showing SSEA expression died of cancer, while two of two ATC cases without SSEA staining were still alive on follow-up.
Conclusions: SSEA-1 is a useful marker with prognostic significance in aggressive thyroid cancers. Our study also suggests that SSEA-1 may be a marker for CSCs in thyroid tumors.

616 Encapsulated Follicular Variant of Papillary Thyroid Carcinoma with Minimal Invasion Is Biologically Indolent

Jennifer Zeng, Michael Bannan, Cheng Z Liu, Fang-Ming Deng. NYU Langone Medical Center, New York, NY.
Background: Encapsulated follicular variant papillary thyroid carcinoma (eFVPTC) is associated with indolent clinical behavior. A minority of cases of eFVPTC show invasive features, i.e. angioinvasion, capsular invasion or both. Data on the long term clinical outcome for eFVPTC with invasion are limited. In this single institution retrospective study we examine cases of eFVPTC with invasion and clinical follow-up to see whether the classification of invasiveness as minimally or widely invasive used in follicular carcinoma,predicts clinical behavior of these tumors.
Design: Between 1990-2014, 34 cases of eFVPTC with capsular and/or angioinvasion were retrieved from our pathology database. Of these, 27 cases had corresponding clinical data in medical records. Slides were reviewed by a head and neck pathologist (CL). Number of invasive foci, extension into extrathyroidal soft tissue and presence of lymph node or distant metastases were recorded. The following clinical data was recorded: age at diagnosis, gender, type of surgery and status of disease.
Results: Average age at diagnosis was 51 years (24-82 years). Female to male ratio was 2.2:1 (female, n = 20; male, n= 9). Treatment was total thyroidectomy in 22 of 27 cases (81%) and lobectomy alone in 5 of 27 cases (19%). Tumors had an average size of 2.8cm (0.5-6cm). Average follow up was 62 months (24-144 months). Capsular invasion alone was more common than angiolymphatic invasion alone, with 20 of 27 cases (63%) versus 5 of 27 cases (19%, p<0.0001). Both capsular and angiolymphatic invasion were seen in 2 of 27 cases (7%). Minimal invasion (<4 invasive foci) was seen in 24 of 27 cases; extensive angiolympatic and/or capsular invasion (>4 invasive foci) was seen in 3 of 27 cases (p<0.0001). All patients but one (26 of 27 cases, 96%) are disease free after surgical resection without metastasis or recurrence. Adverse outcome was seen in 1 of 27 cases. This patient, staged as pT1aNx at diagnosis with extensive capsular invasion (5 foci) and without angioinvasion developed metastatic disease in a regional lymph node and a distant site 47 days following initial treatment.
Conclusions: As seen in a small number of previous studies with long term follow up, the patients in this study with eFVPTC and minimal invasion had a favorable outcome. In the one case with adverse outcome, extensive capsular invasion (>4 foci) was identified. Whether semi-quantitative assessment of invasion can predict risk of adverse outcome merits further investigation.

617 Metabolic Reprograming in Adrenocortical Tumors in Children: A Promising New Pathway in the Biology of This Disease

Maria Zerbini, Celine Pinheiro, Sara Granja, Adhemar Longatto, Andre M Faria, Maria CV Fragoso, Silvana M Lovisolo, Antonop M Lerario, Madson Q Almeida, Fatima Baltazar. University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil; University of Minho, Braga, Guimaraes, Portugal; Barretos Cancer Hospital, Barretos, SP, Brazil.
Background: Adrenocortical neoplasm in children is a challenging condition for pediatric oncologists and pathologists. The incidence of childhood adrenocortical tumor in Southern Brazil is approximately 10 times greater than the worldwide incidence that ranges from only 0.3–0.38 million per year, due to the high frequency of the founder germline TP53R337H mutation found in 95% of ACTs of young Brazilian children. Despite the extensive research to better understand the biology of these tumors in childhood, it is not yet known which factors determine the aggressive behavior of a small proportion of these cases. Recently, the metabolic reprogramming of cancer cells towards aerobic glycolysis has gained renewed attention as a potential prognostic and therapeutic tool. Therefore, this study evaluated the expression of metabolism-related proteins including monocarboxylate transporter (MCT) isoforms 1, 2 and 4, MCT chaperones (CD147 and CD44), the glucose transporter GLUT1 and the pH regulator CAIX, in a series of childhood adrenocortical tumors.
Design: The immunohistochemical expression of MCT1 (AB3538, Chemicon International), MCT2 (sc-50322, Santa Cruz Biotechnology), MCT4 (sc-50329, Santa Cruz Biotechnology), CD147 (sc-71038, Santa Cruz Biotechnology), CD44 (MCA2726, AbD Serotec), GLUT1 (ab15309-500, AbCam) and CAIX (ab15086, AbCam) was evaluated in a series of 45 childhood adrenocortical neoplasias (36 clinically benign and 9 clinically malignant lesions), arranged in tissue microarrays (3 samples for each case).
Results: The immunohistochemical analysis showed a significant increase in the expression of GLUT1 in the cell membrane of malignant lesions, when compared to benign lesions (p=0.003). Although significant differences were not observed for the other proteins, MCT1 and CD147 were highly expressed in adrenocortical neoplasias (around 90%).

Conclusions: Our data point to GLUT1 as a possible key discriminator between benign and malignant adrenocortical tumors in children, and this suggests that metabolic reprogramming of cancer cells towards aerobic glycolysis can be explored for development of therapeutic strategies.

618 Long Non-Coding RNA MALAT1 Expression in Thyroid Tissues and Tumors

Ranran Zhang, Heather A Hardin, Wei Huang, Darya Buehler, Sofia Asioli, Alberto Righi, Francesca Maletta, Anna Sapino, Ricardo V Lloyd. University of Wisconsin School of Medicine and Public Health, Madison, WI; University of Bologna, Bologna, Italy; Rizzoli Institute, Bologna, Italy; University of Turin, Turin, Italy.

Background: Long non-coding RNAs (lncRNAs) participate in transcription and in epigenetic or post-transcriptional regulation of gene expression, and may contribute to carcinogenesis. MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1), a lncRNA that participates in the regulation of cell cycle and migration, is known to be deregulated in multiple cancers. Some studies suggest MALAT1 may function as both an oncogene and a tumor suppressor. We analyzed the expression of the MALAT1in thyroid tumors and compared its expression to miR-146b-5p, a microRNA known to be deregulated in papillary thyroid cancer.

Design: Tissue microarrays (TMAs) were constructed with formalin-fixed paraffin-embedded (FFPE) tissues of normal thyroid (NT, n=10), nodular goiters (NG, n=10), follicular adenoma (FA, n=32), follicular carcinoma (FCA, n=28), papillary thyroid carcinoma (PTC n=28), follicular variant of papillary thyroid carcinoma(FVPTC, n=29), poorly differentiated thyroid carcinomas (PDC, n=21) and anaplastic thyroid carcinoma (ATC, n=35). TMA sections were analyzed by in situ hybridization (ISH) using RNAscope technology with a MALAT1 probe (Advanced Cell Diagnostics). ISH for miR-146b-5p was also performed on the same set of TMAs (Exiqon). qRT-PCR was performed on a subset of the TMA cases (n=16). The results of the MALAT1 TMA ISH were analyzed with Vectra imaging technology, Nuance® and inForm ® software.

Results: MALAT1 was highly expressed in NT, NG and in benign and malignant thyroid tumors predominantly in the nucleus, but also in the cytoplasm. The highest levels of MALAT1 wwere observed in PTCs which was significantly higher than in NT ($p=0.014$) and FVPTC ($p=0.016$). In contrast NT expressed higher levels of MALAT1 than PDC ($p=0.015$) or ATC ($p<0.001$). qRT-PCR analyses supported the ISH findings. Expression of miR-146b-5p was highest in PTC (89%) followed by FVPTC (41%) and was lowest in ATC (8%).

Conclusions: MALAT1 is highly expressed in NT tissues and thyroid tumors with increased expression during progression from NT to PTCs. However both MALAT1 and miR-146b-5p are downregulated in ATC compared to PTCs, suggesting that MALAT1 may function both as an oncogene and as a tumor suppressor in different thyroid tumors and that non-coding RNAs may regulate the development of PTCs and ATCs.

Gastrointestinal Pathology

619 CD66b-Positive Tumor-Associated Neutrophils in Epstein-Barr Virus Associated Gastric Carcinoma: A Comparative Study with CD8-Positive Cytotoxic T-Lymphocytes

Hiroyuki Abe, Teppei Morikawa, Ruri Saito, Hiroharu Yamashita, Yasuyuki Seto, Masashi Fukayama. The University of Tokyo Hospital, Tokyo, Japan; Graduate School of Medicine, the University of Tokyo, Tokyo, Japan.

Background: Epstein-Barr virus-associated gastric carcinoma (EBVaGC) is one of four molecular subtypes of gastric carcinoma. EBVaGC is characterized by prominent intratumoral lymphocyte infiltration, with some infiltration by other types of inflammatory cells, such as neutrophils. In this study, the significance of tumor-associated neutrophils (TANs) was investigated in EBVaGC and compared to CD8-positive cytotoxic T lymphocytes (CTLs).

Design: After immunohistochemistry of CD66b and CD8, specific markers of TAN and CTL respectively, whole sections of EBVaGC were analyzed with digital image analysis system (Tissue Studio), and their clinicopathological significance was analyzed.

Results: There was no correlation between CD66b- and CD8-positive areas in the tumor (correlation coefficient: $R^2=0.0075$, $P=0.453$), suggesting an independent mechanism of both types of inflammatory cell infiltration. Forty-two of 77 cases of EBVaGC (55%) had some or considerable infiltration of TANs (CD66b-positive areas>0.5%, TAN^+) and 35 (45%) had no or scant infiltration (TAN^-). Thirty-two cases (42%) had more CTLs (CD8-positive area>20%, CTL-high) and 45 (58%) had fewer (CTL-low). The cases of EBVaGC TAN^+ showed correlation with intestinal type histology ($P=0.048$) and absence of lymph node metastasis ($P=0.023$), while cases of EBVaGC CTL-low with upper location ($P=0.033$) and advanced invasion depth (pT2 or more) ($P=0.045$). Neither TAN^+ nor CTL-low was associated with disease-specific survival in EBVaGC. Multivariate logistic regression analysis revealed that TAN^- was independently associated with lymph node metastasis ($P=0.036$). None of the 21 cases of EBVaGC TAN^+ with submucosal invasion showed lymph node metastasis, and such probability was estimated to be extremely low (95% confidence interval: 0-13.3%).

Conclusions: TANs in EBVaGC may suppress lymph node metastasis through anti-tumor effect. Presence of TANs in the sites of submucosal invasion indicates absence of lymph node metastasis, which may be useful for pathological diagnosis of endoscopic submucosal dissection of gastric carcinoma.

620 High Expression of the Leaky Protein Claudin-2 in Esophageal Carcinoma and Precancerous Lesions Is Significantly Associated with the Bile Salt Receptors VDR and TGR5

Sohaib Abu-Farsakh, Tongtong Wu, Amy Lalonde, Jun Sun, Zhongren Zhou. University of Rochester Medical Center, Rochester, NY; University of Illinois at Chicago, Chicago, IL.

Background: Claudins are a family of integral membrane proteins and are components of tight junctions (TJs). Many TJ proteins are known to tighten the cell structure and maintain a barrier. In contrast, Claudin-2 is a leaky protein that plays an opposing role and increases cell permeability. Recently, we found that VDR enhanced Claudin-2 expression in colon and that bile salt receptors VDR and TGR5 were highly expressed in esophageal adenocarcinoma (EAC) and precancerous lesions. Here, we examined the expression of Claudin-2 in EAC and precancerous lesions and its association with VDR and TGR5 expression.

Design: Claudin-2 expression was examined by immunohistochemistry on tissue microarrays, containing EAC, high grade dysplasia (HGD), low grade dysplasia (LGD), Barrett's esophagus (BE), columnar cell metaplasia (CM), squamous cell carcinoma (SCC), and squamous epithelium (SE) cases. Intensity (0 to 3) and percentage were scored for each case. High expression was defined as 2-3 intensity in ≥ 10% of cells.

Results: Claudin-2 was highly expressed in 77% EAC (86/111), 38% HGD (5/13), 61% LGD (17/28), 46% BE (18/39), 45% CM (29/65), 88% SCC (23/26), and 14% SE (11/76). It was significantly more highly-expressed in EAC, SCC and glandular lesions than in SE and more in EAC than in BE and CM. No significant difference in Claudin-2 expression was found between CM, BE, LGD, and HGD. A significant association was found between Claudin-2 expression and VDR and TGR5 expression. No significant association was found between expression of Claudin-2 and age, gender, grade, stage, or patients' survival time in EAC and SCC.

Conclusions: We conclude that Claudin-2 might play a novel role in the development and progression of esophageal mucosal metaplasia, dysplasia and carcinoma. Claudin-2 expression is significantly associated with VDR and TGR5 expression. The functional relationship between Claudin-2, VDR and TGR5 will be studied in future.

621 Doxycycline Induced Gastrointestinal Injury: Case Series with New Sites of Involvement

Kajsa Affolter, Kathleen Boynton, Wade S Samowitz, Erinn Downs-Kelly. University of Utah Health Sciences Center and ARUP, Salt Lake City, UT; University of Utah Health Sciences Center, Salt Lake City, UT.

Background: Doxycycline induced gastric injury is a rarely recognized side effect of this common medication. Tetracyclines, especially doxycycline, are a widely accepted cause of pill-induced esophagitis, but little attention has been given to its association with other sites of gastrointestinal injury. In the stomach, a distinctive superficial capillary degeneration with fibrinoid material is seen in a background of a reactive gastropathy. Previously, only two cases have been reported describing this gastric finding. We have encountered additional examples of this injury pattern and sought to expand the limited information by describing the associated length of drug ingestion, original and follow-up endoscopic findings, sites of involvement, gender, and age.

Design: All gastrointestinal biopsy material for cases indexed with the word "doxycycline" were retrieved and the histology reviewed and recorded. The associated medical record was used to obtain pertinent clinical findings.

Results: Four cases were identified from the indexed search with available clinical and biopsy material, including two males and two females ranging in age from 39-80 years. Doxycycline ingestion could be confirmed in three of the four cases with the length of time from start of drug to initial endoscopy ranging from 5-54 days. Gastric endoscopic findings included a non-removable white coat, a greater curvature erosion, a superficial pyloric ulcer, and linear fundic ulcers. All the patients' gastric biopsies had reactive gastropathy, variable neutrophilic infiltrates in the superficial lamina propria and marked superficial small vessel injury with fibrinoid material in a concentric fashion around the interior of the vessel. A single patient, of three in which the duodenum was biopsied, had similar vascular changes in the duodenum. One patient underwent follow-up endoscopy after drug cessation and was found to have normal gastric mucosa endoscopically and histologically.

Conclusions: Doxycycline induced gastrointestinal injury that comes to clinical attention prompting endoscopy is rare given the frequency the drug is used. Previous to this report only two cases were documented with histologic findings. Thus, our series adds to the literature by confirming the distinct vascular injury and describes the finding in the duodenum, a site not previously known to be effected. Recognition of this evolving drug-specific injury pattern is important for patient management.

622 Histologpic Spectrum of Ipilimumab Associated Colitis Is Narrow and Resembles Inflammatory Bowel Disease

Atin Agarwal, Dipen Maru. University of Texas, MD Anderson Cancer Center, Houston, TX.

Background: Ipilimumab (AntiCTLA-4), a standard of care option for metastatic melanoma, renal cell carcinoma and lung cancer, has potentially lethal side effect of enterocolitis. Histopathologic features of Ipilumimab induced colitis are not well described.

Design: In a retrospective search of the institutional pathology database (2001-2014), we identified 22 patients on ipilimumab who underwent colonoscopy for diarrhea clinically suspected due to Ipilumimab. Patients' electronic medical records were reviewed for colonoscopic findings and to exclude other etiologies of colitis. In all patients all the segments of colon and rectum were biopsied. Hematoxylin and Eosin