# Endocrine

### 555 Papillary Thyroid Carcinoma with Prominent Hobnail Features: A New Aggressive Variant of Moderately Differentiated Papillary Carcinoma – A Clinicopathological, Immunohistochemical and Molecular Study of 8 Cases

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**Background:** Papillary thyroid carcinomas (PTC) are the most common thyroid tumors and usually have a good prognosis. Recurrence, metastases and cancer death may occur in a few patients and are usually associated with more aggressive variants of PTC and with the presence of BRAF mutation. We present the clinicopathological, immunohistochemical and molecular features of a novel rare aggressive variant of PTC showing prominent hobnail features.

**Design:** We reviewed 2,534 primary PTC from patients who underwent primary surgical treatment between 1955-2004, at one institution in the USA. Six cases were identified according to following criteria: 1) non-solid type of PTC; 2) ≤ 10% of the tumor showed tall/columnar or diffuse sclerosing features; 3) loss of polarity/cohesiveness with hobnail features in ≥ 30% of the tumor cells; 4) available clinical data and follow-up. Two additional cases were included from the consultation files of the authors. We assessed epithelial membrane antigen, TTF1, thyroglobulin, HBME1, E-cadherin, β-catenin, cytokeratin 7, cytokeratin 19, p53, Ki67/MIB1 expression in all 8 cases by immunohistochemistry. Tumor samples were also analyzed for BRAF mutation using PCR amplification and DNA sequencing.

**Results:** The patients included 6 women and 2 men with a mean age of 57.6 years. Patients presented with a neck mass and cervical lymphadenopathy. Tumor size ranged from 1.0 to 4.0 cm. The tumors were usually multifocal with variably-sized complex papillary structures lined by cells with increased nuclear cytoplasmatic ratios and apically placed nuclei that produced a surface bulge leading to hobnail appearance. Thyroglobulin, TTF1, HBME1 and p53 were diffusely positive in all cases, and there was membrane staining for β-catenin and E-cadherin. The proliferative index with Ki67/MIB1 showed a mean of 10%. BRAF mutation was found in 4/7 (57.1%) cases. The average follow-up time was 77 months. Four patients died of disease after a mean of 42 months. Two patients are alive with disease after 4 and 87 months, respectively. Two patients are alive without disease after 120 and 236 months.

**Conclusions:** PTC with a prominent hobnail pattern is a moderately differentiated PTC variant with aggressive clinical behavior and significant mortality.

### 556 Molecular and Histopathological Features of Multifocal Papillary Thyroid Carcinomas

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**Background:** Papillary thyroid carcinoma (PTC) frequently presents as a multifocal tumor. The multifocality may be due to either intraglandular tumor spread or synchronous independent primary tumors (SIPT). It is not known if SIPT develop through genetically similar or different mutations and have particular histopathological characteristics.

**Design:** Sixty cases of PTC containing 2-4 discrete foci were analyzed. All tumor foci were tested for BRAF, NRAS, HRAS, KRAS and RET/PTC mutations. The following histopathological features were also analyzed: tumor location, histological variant, architectural and cytological features, encapsulation, and microscopic peritumoral dissemination (MPD) which was defined as the presence of small tumor foci in the stroma or in lymphatic channels surrounding the primary tumor.

**Results:** The molecular analysis of 60 cases revealed 4 different patterns of mutation occurrence: (i) 18 cases (30 %) with two foci containing different mutations; (ii) 19 cases (32%) with one tumor containing a mutation and another with no mutations; (iii) 15 cases (25%) with tumors containing the same mutation; and (iv) 8 (13%) with all tumors having no mutations. The 18 cases with two different mutations represent a group of tumors which are unequivocally SIPT. In this group, the most common combination of mutations was BRAF and RAS (56%), followed by different types of RAS (33%) (i.e. NRAS and HRAS or two NRAS mutations with different nucleotide change), and the least common combination was BRAF and RET/PTC (11%). Of these cases, 14 (78%) had tumors located in different lobes, 2 (11%) in different poles of the same lobe, and 2 (11%) had tumors separated by a distance of 0.6 cm. Eleven (61%) of these cases had tumors of different histological variant, and 4 of the remaining 7 cases had tumors of the same variant but displaying significantly different architectural/cytological features. Among the 18 tumor pairs, 61% were encapsulated and only 8% showed MPD.

**Conclusions:** Multiple discrete foci of PTC frequently represent SIPT tumors which develop via distinct molecular alterations. Most common combination of mutations was BRAF and RAS, followed by different types of RAS mutations. SIPT typically occur in different lobes, although they can be located as close as 0.6 cm from each other. Histopathologically, these tumors typically demonstrate distinct histological variants/microscopic features, are encapsulated, and do not show MPD.

### 557 $BRAF^{V600E}$ Mutation Analysis of Liquid Based Preparation-Processed Fine-Needle Aspiration Sample Improves the Diagnosis of Papillary Thyroid Carcinoma

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**Background:** Early detection and diagnosis of papillary carcinoma is important in the management of patients with thyroid nodule. Fine-needle aspiration (FNA) is the most useful tool in diagnosis of thyroid nodules, however 10 to 30% of papillary carcinomas are diagnosed as benign or indeterminate nodules by cytomorphologic examination alone. In recent years, liquid-based preparation (LBP, Thinprep$^{R}$) in fine needle aspiration of thyroid nodule has been widely used and replacing conventional smear, because residual samples can be used for ancillary tests. $BRAF^{V600E}$ mutation is common and specific genetic alteration of papillary carcinoma. Detection of $BRAF^{V600E}$ mutation in cytology specimen could help diagnosis of papillary carcinoma. We analyzed cytologic features and $BRAF^{V600E}$ mutation status using thyroid LBP-FNA samples.

**Design:** A total of 191 histologically confirmed thyroid LBP-FNA specimens were selected. We analyzed cytomorpholoigic features and $BRAF^{V600E}$ mutation status in both LBP-FNA samples and their corresponding formalin-fixed paraffin-embedded tissue. Melting curve analysis (MCA) with SYBR green and Seeplex$^{R}$ BRAF ACE detection kit were used to detect $BRAF^{V600E}$ mutation.

**Results:** In 191 patients, 125 were histologically confirmed papillary carcinoma and 64 were benign lesions and carcinomas of other types. In tissue samples of papillary carcinomas, $BRAF^{V600E}$ mutation was detected in 71(56.8%) using BRAF kit and in 98(78.4%) cases using MCA. Only one benign tissue sample showed $BRAF^{V600E}$ mutation in MCA. Using residual cytologic material, $BRAF^{V600E}$ mutation was detected in 95(76%) using BRAF kit and in 98(78.4%) using MCA in histologically confirmed papillary carcinoma. Using LBP cytology alone, the sensitivity for diagnosis of papillary carcinoma was 70.4%. When $BRAF^{V600E}$ mutation analysis was done in conjunction with cytologic diagnosis, the diagnostic sensitivity of papillary carcinoma was increased to 84% and 86.4%(BRAF kit and MCA, respectively). In both methods, 3 benign cytologic samples showed $BRAF^{V600E}$ mutation.

**Conclusions:** This is the first study of validating the utility of $BRAF^{V600E}$ mutation in the diagnosis of thyroid papillary carcinoma using LBP-FNA samples. Liquid-based preparation for thyroid nodule makes it possible for ancillary tests such as immunochemistry (IC) and molecular tests without any further invasive examination by using residual material. And $BRAF^{V600E}$ mutation analysis may be a useful additional diagnostic tool for diagnosis of thyroid papillary carcinoma.

### 558 Claudin Proteins, PAX8 and NIS Immunohistochemical Evaluation in Human Fetal Thyroid Development

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**Background:** thyroid gland derives from aggregates of unpolarized cells. Pax8, a family of developmental control genes that encode transcription factors, is crucial for thyroid organogenesis and differentiation. The $Na^{+}/I^{-}$ symporter (NIS) is a critical gene involved in the active $I^{-}$ accumulation into the thyroid gland, the first step in thyroid hormone synthesis. Tight junctions (TJs) are dynamic structures, that at different stages of epithelial tissue development play a role in maintaining integrity and physiological function of polarized epithelial cells. Claudins (CLDNs) form the backbone of TJs. They expression during thyroid ontogenesis is unknown.

**Design:** we analyzed the immunohistochemical expression of CLDNs-1,3,4,5,7, Pax8 and NIS in 19 human fetal thyroid glands (15-27 gestational weeks; from elective/voluntary abortions or autopsies).

**Results:** CLDN7 was constantly expressed showing strong, diffuse and linear basolateral positivity. CLDN5 and 4 staining appeared as a thin line or dot-like along the lateral membrane with discontinuous pattern. CLDN1 exhibited strong, linear or dot membranous staining on the gland periphery. CLDN3 immunoreactivity was negative. Pax8 was observed in a nuclear pattern in all samples. NIS displayed strong basolateral staining in most of the follicular cells.

**Conclusions:** CLDN7 is consistently expressed in thyroid epithelium during ontogenesis at a similar level from fetal to adult thyroid tissue, thus suggesting a pivotal role in architectural stability of follicular cells. CLDN1 was noted only at the border of the fetal gland: the exact role for this membrane protein is still not clear. Conversely, CLDN1 was weakly expressed in adult normal tissue while was up-regulated in thyroid cancer, thus emerging as an oncofetal antigen and a potential marker of thyroid cancer. CLDN4 exhibited high expression in thyroid fetal gland while was down-regulated in adult thyroid tissue, suggesting a physiological involvement in the development and functioning of thyroid follicles. Our study demonstrates, for the first time, that CLDN5 was expressed both in fetal and adult thyroid tissue showing a similar distribution and staining pattern. Finally, we confirm that Pax8 and NIS are expressed at an early stage of ontogenesis playing a key role in thyroid development and differentiation.

### 559 RAS Mutation Is an Early Event in the Development of the Follicular Variant of Papillary Carcinoma and May Predispose to Subsequent Genetic Alterations

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**Background:** The follicular variant of papillary thyroid carcinoma (FVPTC) is a common variant, which may be diagnostically challenging for pathologists. Histologically, these tumors frequently present with patchy, focally developed nucleic features of PTC intermixed with the areas of benign-appearing nuclei. The biology of these tumors is not fully defined, although about 40% of them have RAS mutations.

**Design:** We studied the distribution of RAS mutations in 8 FVPTC tumors with patchy expression of the nucleic features of PTC. In each tumor, five separate areas containing well-developed nucleic features and small, round, benign-appearing nuclei where separately microdissected and tested for RAS mutations. In addition, we used Affymetrix Genome-Wide Human SNP Array 6.0 analysis to determine the copy number change in 6 FVPTC and matched normal tissues.

**Results:** In each of the 8 cases studied, all areas within the tumor, including those with benign-appearing nuclei, were positive for RAS mutation, indicating that it represents an early event in tumor development. The SNP array analysis of 5 additional FVPTC with diffusely present nuclear features revealed on average 318 chromosomal regions of amplification and 124 regions of deletion per tumor. One of the frequently deleted region was on 21q21.1, which was previously found to be deleted in lung cancer. It contains the USP25 gene as well as several microRNA genes. One of those miRNAs is miR99a, which we found to be 4-fold downregulated in the RAS-positive FVPTC tumors.

**Conclusions:** These findings indicate that RAS mutation is an early event in the development of FVPTC and present in the tumor areas with no well developed nuclear features of PTC. The RAS-positive tumors appear to be prone to subsequent genetic alterations, including multiple amplifications and deletions that may involve carcinogenic genes and miRNAs.

### 560 Factors Contributing to Nuclear Bubbling and Clearing Mimicking the Features of Papillary Thyroid Carcinoma

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**Background:** The diagnosis of the follicular variant of papillary thyroid carcinoma (FVPTC) depends on the identification of a set of nuclear features, including ground glass chromatin, abundant grooves and pseudoinclusions (PI). Variations in tissue fixation, processing, and sectioning lead to a variety of changes that simulate these features, including nuclear bubbles and disturbed patterns of chromatin distribution leading to nuclear clearing and enlargement. These changes are responsible, in part, for the interobserver variation in the diagnosis of FVPTC. The aim of this study was to identify factors related to the development of these artifacts.

**Design:** Sections were taken from 10 thyroid adenomatous nodules, 8 formalin fixed for 24 hours and 2 formalin fixed for 1 hour (the latter 2 were essentially alcohol fixed), then routinely dehydrated. Four micron sections were mounted onto Superfrost slides in a 42C waterbath, then air-dried and baked at 65C. Variables tested included incomplete dehydration, use of freeze spray or ammonia water on the block face, varying waterbath temperature, introducing excess water between the tissue and the slide while mounting, varying the air-drying time, and altering the drying method (hot plate vs oven drying). Slides were reviewed for the presence of nuclear bubbles and clearing, as well as cytoplasmic bubbling.

**Results:** Introducing excess water while mounting the section elicited the most extensive nuclear and cytoplasmic bubbling. Short fixation time, increasing waterbath temperature to 50C, hot plate drying, or immediate oven drying created disturbed patterns of chromatin distribution mimicking nuclear clearing. Incomplete dehydration, freeze spray or ammonia water did not elicit these artifacts.

**Conclusions:** Short fixation time, excess water introduced while mounting the slide, high waterbath temperature, and incomplete air-drying prior to baking are the main factors that contribute to nuclear bubbling and clearing. In contrast to well-defined PIs, which typically appear eosinophilic with sharply defined margins, nuclear bubbles often appear clear; moreover, they are considerably more common than PIs and are present in adjacent normal thyroid. Disturbed patterns of chromatin distribution and nuclear swelling impart an "empty" appearance to nuclei. Careful attention to tissue preparative methods can circumvent many of the problems associated with the diagnosis of FVPTC.

### 561 CDX2 Expression in Columnar Cell Variant of Papillary Thyroid Carcinoma

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**Background:** Columnar cell variant of papillary thyroid cacinoma is a rare, aggressive tumor that exhibits elongated and enlarged nuclei and nuclear stratification, often without the typical nuclear characteristics seen in papillary carcinomas of the thyroid. The morphology of columnar cell variant is often reminiscent of colonic adenocarcinomas. CDX2 is known to play a critical role in the embryologic development of the GI tract and establishment of the intestinal cell phenotype. It is also highly expressed in GI adenocarcinomas, but not or rarely in tumors of other origins. CDX2 has been reported rarely in papillary carcinoma of the thyroid but has not yet been evaluated in the columnar cell variant, which shares morphologic similarity with GI tumors.

**Design:** Three cases of columnar cell variant of thyroid carcinoma were studied. All cases were located in thyroid and tested positive for TTF-1 at the time of diagnosis. Paraffin section of each case was stained with a mouse monoclonal antibody against CDX-2 (clone CDX2-88, Biogenex, San Ramon, CA) on Leica Microsystems' Bondmax autostainer after sections heated in a 70°C oven for 1 hour.

**Results:** Diffuse nuclear CDX2 reactivity was detected in all three columnar cell variants, but not in the adjacent non-neoplastic thyroid tissue.

**Conclusions:** CDX2 is a homeobox gene that encodes an intestine-specific transcription factor that is expressed in epithelial cells throughout the intestine. CDX2 has been used as a sensitive and specific marker for colonic adenocarcinomas. Previously, a single case (papillary type) of 30 thyroid cancers was found to be CDX2 positive. Our finding of all three cases of columnar cell variant staining positively for CDX2 is novel, and suggests that the genetic basis as well as the intestinal-type morphology seen in this rare thyroid tumor may be due in part to CDX2 expression. This finding may have important implications for understanding the molecular basis of columnar cell variant of papillary thyroid carcinoma. Columnar cell variants of thyroid carcinoma should be included in the differential diagnosis of carcinoma with columnar cell morphology and CDX2 reactivity.

### 562 Specific MicroRNAs Differentiate between Adrenocortical Adenomas and Carcinomas

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**Background:** Although the prediction of malignant potential is usually straightforward in adrenocortical tumors (ACTs), some present with borderline features and elude specific diagnosis. Several protocols for ACT analysis have been suggested. The most reproducible is that of Weiss, who assessed 9 microscopic features thought to be related to malignancy. The presence of 3 or more of these features in a given tumor indicates malignant potential. However, some of the features show poor correlation between pathologists. Information on the molecular pathogenesis of the tumors is continuously accumulating but it has not been utilized thus far in the diagnostic setting. MicroRNAs are a family of short non-coding RNAs that play a central role in the regulation of gene expression and are strongly associated with tissue and cancer development. Prompted by the growing data on microRNA expression in a broad spectrum of neoplastic processes, we sought to examine microRNA expression in ACTs in an attempt to differentiate adrenocortical carcinomas (ACCs) from adrenocortical adenomas (ACAs).

**Design:** Thirty-four ACTs were retrieved from our files. Both clinical and macroscopic information (weight and diameter) were available. The nine histologic criteria of Weiss were evaluated in a blinded fashion, and tumors that met 3 or more were considered malignant. High quality RNA was extracted from tumor samples using proprietary protocols and profiled with Rosetta microRNA microarrays.

**Results:** Of the 34 ACTs, 8 were considered ACCs and 26 ACAs according to Weiss's criteria. Clinical data and patient follow-up supported the diagnosis in some of the cases. Microarray analysis revealed large and highly significant differences between ACCs and ACAs. Over a dozen microRNAs were up- or down-regulated in ACCs compared to ACAs, with 4- to 50-fold differences. The results demonstrated a high level of correspondence between Weiss's protocol and microRNA expression.

**Conclusions:** The strong correlation between Weiss's protocol of prognostic evaluation and the microRNA profile of ACTs imply that both accurately identify malignant ACTs. However, in borderline tumors, the addition of microRNA evaluation may greatly assist in predicting the biologic behavior of a given tumor and, thereby, in selecting the appropriate treatment.

### 563 High Incidence of Immunohistochemical SSTR2a Expression in Gastro-Entero-Pancreatic Neuroendocrine Tumors (GEPNETs): Studies on Primary and Metastatic Diseases

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**Background:** It has been widely known that immunohistochemical expression of SSTR2a is directly related to the clinical response of somatostatin analogue(SA) in GEPNETs. This report is aimed at to elucidate the general incidence of SSTR2a positivity and that in the metastatic NEC in the liver. Staining intensity was compared with MIB-1 proliferative indices.

**Design:** Total 81 tumors were studied. 66 primary GEPNETs (46 pancreas, 10 duodenum, 7 rectum, 3 stomach) and 15 metastatic GEPNECs (11 cases of pancreatic NEC, 2 cases of rectal NEC and 1 case of duodenal NEC) were subjected to immunohistochemical staining for SSTR2a on formalin-fixed paraffin embedded(FFPE) sections. We used anti-SSTR2a antibody (Gramsch Co) and polimer method. MIB-1 was stained with antibody (DAKO Co) combined by polymer method.

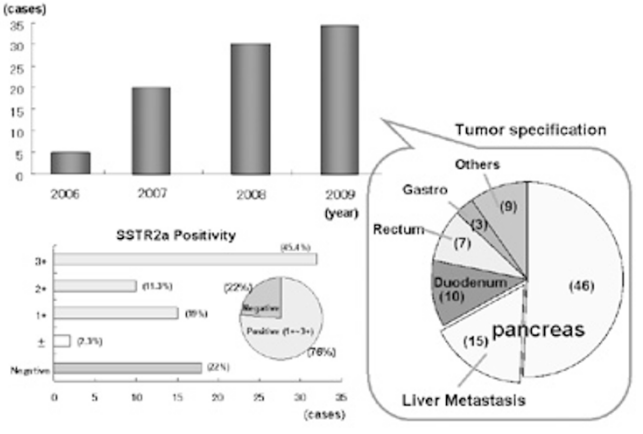


Figure1 Requested cases of SSTR2a staining at Department of Pathology, Tokai Univ.

**Results:** Immunohistochemical staining for SSTR2a on cell membrane of tumor cells was graded to 0:negative, 1+:weak, 2+:intermediate,3+:intense. In total numbers of cases, 76% of the cases showed 1+ to 3+ positive staining. 45.4% of cases revealed 3+ staining . 15 cases of metastatic GEPNECs in the liver showed the positive rate of SSTR2a 1+-3+ with 80% and 3+ with 67%. In 9 cases of metastases, MIB-1 index was higher than 10%(grade 2 by WHO) in 4 cases. There was no correlation between MIB-1 indices and SSTR2a staining.

**Conclusions:** In general, 76% of GEPNETs showed positive SSTR2a. Especially, the cases with liver metastases showed higher positive rate and more intense staining for SSTR2a and are expected to respond to SA.

### 564 IMP3 Expression in Thyroid Carcinomas

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**Background:** The majority of carcinomas of follicular cell origin are indolent, and undifferentiated thyroid carcinoma represents the most aggressive tumor of the thyroid gland. Poorly differentiated thyroid carcinoma lies in-between well-differentiated and undifferentiated carcinoma morphologically, immunohistochemically, and behaviorally. The insulin-like growth factor mRNA-binding protein 3 (IGF2BP3, IMP3) is a prognostic biomarker in renal clear cell carcinoma, low-stage urothelial carcinoma

of the bladder, ovarian clear cell carcinoma and hepatocellular carcinoma. Our aim is to evaluate the expression of IMP3 protein and its possible influence on prognosis in thyroid carcinomas.

**Design:** From the surgical pathological files compiled during 1988-2004, we selected 175 specimens of thyroid cancer, including 96 cases of papillary carcinoma, 47 cases of follicular carcinoma, 12 cases of poorly differentiated carcinoma and 20 cases of undifferentiated carcinoma. These specimens were obtained from a total of 130 patients; amongst these, 30 patients had multiple surgical specimens, including primary, metastatic and recurrent lesions. IMP3 protein expression was analyzed by immunohistochemistry.

**Results:** Negative IMP3 immunostaining was found in the majority of papillary carcinoma (83.3%), follicular carcinoma (85.1%) and poorly differentiated carcinoma (66.7%). In contrast, IMP3 protein was extensively expressed in most cases of undifferentiated carcinoma (95.0%). The background thyroid tissues in all specimens were negative for IMP3 staining.

| | Extent of IMP3 staining | | | | Total |
|---|---|---|---|---|---|
| | 0 | <10% | 10–50% | >50% | |
| Papillary carcinoma | 80 (83.3%) | 13 (13.5%) | 1 (1.0%) | 2 (2.1%) | 96 |
| Follicular carcinoma | 40 (85.1%) | 5 (10.6%) | 0 (0%) | 2 (4.3%) | 47 |
| Poorly differentiated carcinoma | 8 (66.7%) | 1 (8.3%) | 3 (25%) | 0 (0%) | 12 |
| Undifferentiated carcinoma | 1 (5.0%) | 0 (0%) | 0 (0%) | 19 (95.0%) | 20 |

The expression correlated with larger tumor size (≥4.0cm, p=0.003), presence of tumor necrosis (p<0.001), worse cancer type (p<0.001), and patient death (p<0.001). Whether the lesion was a primary, or a metastatic/recurrent one, did not influence the correlation between cancer type and IMP3 protein expression.

**Conclusions:** We found a significant association between undifferentiated thyroid carcinoma and IMP3 expression, regardless of primary or metastatic/recurrent tumor status. IMP3 protein expression correlates with the differentiation of thyroid carcinoma, which influences survival and prognosis.

### 565 Comparative Immunohistochemical Detection of Somatostatin Receptor (SSTR)2a and Dopamine Receptor (D2R) in Pituitary Adenomas for New Therapeutic Strategies

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**Background:** Pituitary adenomas are known to be invasive and sometimes aggressive in behavior. Somatostatin analogue(SA) and dopamine agonist(DA) bind to somatostatin receptor(SSTR)2a and dopamine receptor(D2R) respectively and suppress hormone hypersecretion and tumor cell proliferation. They have been used separately for selected cases of pituitary adenomas. Recent development of chimeric SA-DA compound which binds to both SSTR2 and D2R (Ferone D et al. 2009) is the rationale for our immunohistochemical study to clarify both receptors in the same tumors.

**Design:** Total 27 cases of pituitary adenomas(5 cases each for the groups of GHomas, PRLomas, TSHomas, ACTHomas, Gnomas and 2 cases of null cell adenomas) were subjected to immunohistochemical staining for SSTR2a and D2R on the same tumors on formalin-fixed paraffin embedded(FFPE) sections. Anti-SSTR2a antibody(Gramsch Co.) and anti-D2R antibody(Gene Tex, Inc.) and polimer method were used. Grading was done as follows; 0-negative, 1+-weakly positive, 2+-strongly positive.

**Results:** Immunohistochemical staining was interpreted as positive when it was observed on the cell membrane of the tumor cells. Staining results 0, 1+ and 2+ for SSTR2a and D2R were as follows for each tumor group: GHomas-SSTR2a 1+(2cases),2+(3cases), D2R 0(2cases),1+(2cases),2(1case), PRLomas-SSTR2a 0(5),D2R 1+(3cases), 2+(2cases), TSHomas-SSTR2a 2+(5cases), D2R 0(1case) 1+(1case),2+(3cases), ACTHomas-SSTR2a 0(4cases),1+(1case), D2R 0(2cases) 1+(3cases), Gnomas-SSTR2a 0(4cases), 1+(1case), Null adenoma-SSTR 1+(2cases),2+(1case), D2R 0(2cases) 1+(1case).

The scores for IHC of D2R and SSTR2a

| Adenomas | Case number | D2R | SSTR2a |
|---|---|---|---|
| GHoma | #1 | 1 | 1 |
| | #2 | 2 | 2 |
| | #3 | 1 | 1 |
| | #4 | 0 | 2 |
| | #5 | 0 | 2 |
| ACTHoma | #6 | 1 | 1 |
| | #7 | 0 | 0 |
| | #8 | 0 | 0 |
| | #9 | 1 | 0 |
| | #10 | 1 | 0 |
| PRLoma | #11 | 2 | 0 |
| | #12 | 1 | 0 |
| | #13 | 1 | 0 |
| | #14 | 1 | 0 |
| | #15 | 2 | 0 |
| TSHoma | #16 | 2 | 2 |
| | #17 | 2 | 2 |
| | #18 | 0 | 2 |
| | #19 | 1 | 2 |
| | #20 | 2 | 2 |
| GNoma | #21 | 0 | 0 |
| | #22 | 0 | 0 |
| | #23 | 0 | 0 |
| | #24 | 0 | 1 |
| | #25 | 0 | 0 |
| Null cell | #26 | 0 | 1 |
| | #27 | 0 | 2 |
| | #28 | 1 | 1 |

Scores: Negative:0, Weakly positive: 1, Strongly positive: 2

**Conclusions:** From our study, it is expected that TSHomas and GHomas can respond to chimeric SA-DA compound and that PRLomas respond better to DA only. Biside the exceptional cases, ACTHomas and Gnomas and Null adenoma should seek for other therapeutic approaches.

### 566 HMGA2 Nuclear Expression in Thyroid Tissue Is Restricted to Neoplastic Lesions and Is Associated with a Malignant Phenotype

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**Background:** HMGA2 gene is a member of high mobility group gene family and is upregulated in several malignant neoplasms including thyroid tumors. In this study we assessed HMGA2 expression in a comprehensive array of thyroid lesions and evaluate its potential clinical utility.

**Design:** A TMA was built using 1-mm diameter cores from 194 paraffin-embedded samples selected from 149 thyroid resection specimens. Sampled tissues included 7 unremarkable benign thyroid glands (BT), 19 nodular hyperplasia (NH), 15 adenomatoid nodules (AN), 14 follicular adenomas (FA), 10 follicular carcinomas (FC), 66 papillary carcinomas (PTC), 8 medullary carcinoma (MTC), 3 poorly differentiated carcinoma (PDC), and 7 undifferentiated carcinoma (UC). Tumors were sampled at least in duplicate and for 45 malignant tumors a core of the uninvolved thyroid was obtained as internal negative control (NC). Only nuclear expression was recorded and for each tissue core a final score was generated by multiplying the intensity score (1+ to 3+) with the percentage of positive cells, assigned as follows: 0 (0-5%), 1 (5-20%), 2 (20-50%), 3 (50-80%), and 4 (81%). A score of 2 was considered positive.

**Results:** Results are shown in table. HMGA2 was detected in 64 of 86 (74%) well differentiated thyroid carcinoma (WDTC), but only in 4% of benign thyroid samples. Two cases of FA had diffuse uniform staining, the remainder positive FA showing only weak focal staining. There were 2 positive cores in the PTC-NC group, which upon histologic review demonstrated incomplete and focal nuclear features of PTC. No other benign lesions exhibited nuclear HMGA2. HMGA2 expression was more frequent in PTC (82%) than in FC (30%) and rare in MTC (12%). HMGA2 is a good marker for malignant lesions with excellent specificity (96%) and positive predictive value (PPV) (94%) but only fair sensitivity (69%).

HMGA2 expression in thyroid tissue

| Lesion | HMGA2 positive | |
|---|---|---|
| BT | 0/7(0%) | Total benign |
| NC | 0/45(0%) | 4/100(4%) |
| NH/AN | 0/34(0%) | |
| FA | 4/14(29%) | |
| FC | 3/10(30%) | |
| PTC | 54/66(82%) | Total malignant |
| PDC | 2/3(67%) | 65/94(69%) |
| UC | 5/7 (71%) | |
| MTC | 1/8(13%) | |

**Conclusions:** HMGA2 nuclear expression is detected more frequently in WDTC, particularly PTC and was not seen in benign/hyperplastic lesions. This marker's promising positive predictive value and specificity for malignant phenotype is limited by a lack of discriminating value in the follicular neoplasms group and its modest sensitivity.

### 567 Sampling, Assessment, and Reporting of Thyroid Follicular Lesions: Survey of 165 Pathologists

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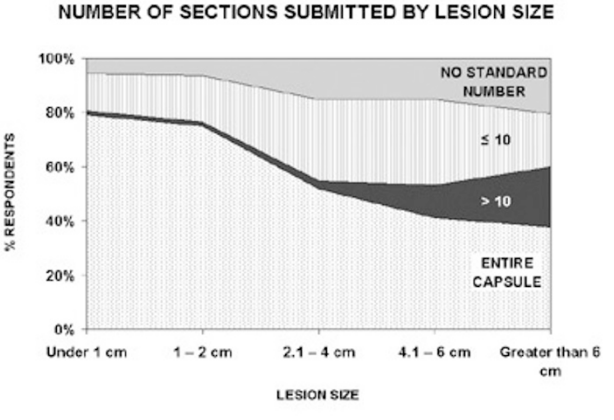
**Background:** Diagnosis of follicular thyroid carcinoma is based on identification of capsular or vascular invasion, which is subject to sampling. There is no standard of care for what constitutes "adequate" sampling of follicular lesions. We surveyed current practices in sampling, assessment, and reporting of follicular lesions.

**Design:** An anonymous electronic web-based survey was successfully distributed to 800 pathologists.

**Results:** 165 (21% response rate) survey respondents comprised a diverse group of head and neck and/or endocrine specialists (31%) and general pathologists (47%), in academic (38%) and private practice (54%), with practice size ranging from 2800 to 320000 specimens per year (median 25000). 73% had >10 years of experience. Sampling: For initial sampling of follicular lesions, 38–79% submit the tumor capsule entirely (FIGURE), with the number who submit entirely inversely proportional to lesion size (p=0.01).



Those who do not initially submit the entire tumor capsule report increasing the number of sections with solid, trabecular, or insular growth pattern (59%, p=.04), necrosis (69%, p<.01), increased mitoses (63%, p=.002), and thick capsule (53%). Sampling is unchanged for macrofollicular (63%, p<.001), microfollicular (61%, p<.01) or Hürthle cell histology (57%). The most frequent definition of a thick capsule is 0.5-1 mm (38%). Assessment: In cases where the initial sections are suspicious for capsular or vascular invasion, the majority (73% and 65%) report their initial action is to obtain deeper levels; ~70% obtain 3 levels. Reporting: 69% of specialists and 44% of non-specialists (p=.01) use a 3-tiered classification of minimally invasive, angio-invasive, and widely invasive follicular carcinoma. Over 60% do not specify the number of invasive foci, though 75% report invasion as "extensive" with 3 to 5 foci.

**Conclusions:** While significant variability exists in the current practice for sampling, assessment, and reporting of non-papillary follicular lesions, practice trends suggest submitting entire capsule of smaller lesions, selective additional sampling of larger lesions, and use of a 3-tiered classification system. Clinical research is required to establish a standard of care.

### 568 Growth Factor Receptor Expression and Microvascular Density in Adrenal and Extra-Adrenal Pheochromocytomas/Paragangliomas (PG)

*MB Kraemer, K Idrees, D Wang, S Liles, M Hesling, RD Chernock, JS Lewis, Jr, WE Grizzle, O Hameed.* University of Alabama, Birmingham, AL; Washington University, St. Louis, MO.

**Background:** Malignant PGs are rare and usually resistant to traditional chemotherapy. Recently, several reports have been published in which therapeutic responses were achieved with the multi-target tyrosine kinase inhibitor sunitinib. This prompted us to study the expression of different tyrosine kinase growth factor receptors in PGs.

**Design:** Fifty two benign and 14 malignant (metastatic) adrenal and extra-adrenal PGs were reviewed and punched in triplicate to produce a tissue microarray. Immunohistochemistry for VEGFR1, VEGFR2 and IGFR-β was performed and semiquantitatively evaluated utilizing an "H-score" ranging from 0-400. HER2 expression was also evaluated. Expression was correlated to the clinical and pathological features of the tumors, as well as to their microvascular density (MVD) which was calculated by BioquantR Image Analysis software (R&M Biometrics) following CD31 immunostaining.

**Results:** No tumor expressed HER2. VEGFR1 expression was seen in 14 (100%) malignant and 50 (96%) benign PGs with strong expression in 13 (93%) malignant vs. 20 (38%) benign cases (p<0.001). VEGFR2 expression was seen in 14 (100%) malignant and 48 (92%) benign PGs with strong expression in 12 (86%) malignant and 22 (42%) benign cases (p=0.01). IGFR expression was seen in 13 (92%) malignant and 44 (85%) benign PGs with strong expression in 10 (71%) malignant cases and 13 (25%) benign cases (p=0.003). The average H-scores of all receptors were significantly higher in malignant than benign PGs (336 vs. 193 for VEGFR1; 331 vs. 184 for VEGFR2; and 279 vs. 139 for IGFR) (all P values = or <0.001). The average MVD was also higher in malignant cases (263.8 vs. 179.2; P=0.08). Correlation between the different parameters is shown in the table.

Correlations

| | | VEGFR1 | VEGFR2 | IGFR | RFS | OS | Size | Weight |
|---|---|---|---|---|---|---|---|---|
| VEGFR1 | Pearson Correlation | | .596** | .700** | .198 | -.932* | .384** | .338* |
| | Sig. (2-tailed) | | .000 | .000 | .610 | .021 | .003 | .019 |
| VEGFR2 | Pearson Correlation | .596** | | .773** | .280 | -.717 | .191 | .206 |
| | Sig. (2-tailed) | .000 | | .000 | .466 | .172 | .159 | .159 |
| IGFR | Pearson Correlation | .700** | .773** | | .156 | -.928* | .241 | .107 |
| | Sig. (2-tailed) | .000 | .000 | | .689 | .023 | .073 | .470 |
| RFS | Pearson Correlation | .198 | .280 | .156 | | .996 | .927 | NC |
| | Sig. (2-tailed) | .610 | .466 | .689 | | .056 | .073 | |
| OS | Pearson Correlation | -.932* | -.717 | -.928* | .996 | | NC | NC |
| | Sig. (2-tailed) | .021 | .172 | .023 | .056 | | | |
| Size | Pearson Correlation | .384** | .191 | .241 | .927 | NC | | .803** |
| | Sig. (2-tailed) | .003 | .159 | .073 | .073 | | | .000 |
| Weight | Pearson Correlation | .338* | .206 | .107 | NC | NC | .803** | |
| | Sig. (2-tailed) | .019 | .159 | .470 | | | .000 | |

** Correlation is significant at the 0.01 level, * correlation is significant at the 0.05 level; NC, not calculated

**Conclusions:** Tyrosine kinase receptor expression is common in PGs, with more frequent and stronger expression in malignant cases. Moreover, expression correlated with size and weight, and negatively with overall survival. These findings support the rationale for considering tyrosine kinase inhibitors for the treatment of malignant PGs.

### 569 Carboxyl Ester Lipase (CEL) Expression in Normal Pituitary Gland and Pituitary Adenomas. Possible Role in the Modulation of Hormone Secretion Via Ceramide Pathway

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**Background:** CEL is an enzyme secreted by acinar cells of the pancreas, which hydrolyzes a wide variety of lipid substrates. It has also been identified in other tissues including lactating mammary gland, endothelial cells and liver. Recent findings have demonstrated a role of CEL in the regulation of ceramide functions through inactivation by hydrolysis. Ceramides are lipid second messengers with several biological roles. They are also known to be involved in the regulation of pituitary hormone secretion and particularly in the inhibition of GH release in experimental models. However, there are no studies about the expression of CEL in normal human pituitary and in pituitary adenomas. The expression of CEL in normal and adenomatous pituitary cells may have a regulation role on hormone secretion.

**Design:** CEL expression was investigated in 10 normal pituitary glands and 86 well characterized (12 FSH/LH, 18 a-SU/null cell, 6 TSH, 21 ACTH, 11 PRL, and 18 GH) adenomas using immunohistochemistry, western blotting, RT-PCR and electron microscopy immunocytochemistry.

**Results:** In normal adenohypophysis CEL was localized in TSH and ACTH cells, while among adenomas it was mainly found in functioning ACTH, GH, and TSH tumors, while its expression was poor in the corresponding silent adenomas and was lacking in FSH/LH-cell, null-cell and prolactin-cell adenomas. Ultrastructurally, CEL was localized in secretory granules, close to their membranes.

**Conclusions:** This is the first study demonstrating CEL expression in human normal pituitary gland and in functioning GH, ACTH and TSH adenomas. Considering that CEL hydrolizes ceramides inactivating their inhibitory function on pituitary hormone secretion, our findings suggest a possible role of CEL in the regulation of hormone secretion in both normal and adenomatous pituitary cells.

### 570 Cancer Stem Cells Markers in Human Thyroid Tumorigenesis: Role of p63 in Anaplastic Thyroid Carcinoma

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**Background:** The term stem cell describes cells capable of both prolonged self-renewal and differentiation into specialized cells. Evidence suggests that a small subpopulation of tumor cells has stem cell-like properties, and tumors may initiate and progress from these cells. A stem cell role for the cells of solid cell nests and the theory of a p63-positive thyroid stem-like cell has been proposed in differentiated thyroid tumors. Association with a stem cell phenotype has also been reported for CD44, CD133, and OCT3/4. The aim of this study is to evaluate a panel of potential cancer stem cell markers in order to identify cell subpopulations in normal, benign, differentiated tumors, and anaplastic thyroid carcinoma.

**Design:** 15 cases of anaplastic thyroid carcinoma (ATC) and 15 cases each of papillary thyroid carcinoma (PTC), follicular carcinoma (FC), and follicular adenoma (FA) were retrieved from the hospital files and consult archives. Immunohistochemical staining for p63, CD44, CD133, and OCT3/4 was performed on formalin fixed paraffin embedded tissue. The results were scored as weak, moderate, or strong and semiquantitatively as 1+ (<5%), 2+ (5-10%), 3+ (10-50%), and 4+ (>50%). Only nuclear staining was scored as positive for OCT3/4 and p63; cytoplasmic or membranous staining was scored as positive for CD44 and CD133.

**Results:** Nuclear positivity for p63 was diffusely present in 7 (47%) ATC cases, focally in 3 PTCs (in squamous metaplasia), and absent in FTC and FA. Nuclear positivity for OCT3/4 was present in 3 (27%) ATC, 6 (40%) PTC, 2 (14%) FC, and 1 (7%) FA. Diffuse membranous staining for CD44 was seen in all 15 ATC; cytoplasmic staining was also observed in 6 cases. All cases of FA, FC, PTC, benign, and normal follicular cells stained for CD44. Diffuse cytoplasmic staining for CD133 was seen in all 15 ATC, and was subjectively increased as compared to adjacent normal thyroid, FA, FC, and PTC.

**Conclusions:** Our findings support the theory of the existence of a p63-positive thyroid stem-like cell, and suggest that p63 may play an important role in tumor progression and anaplastic thyroid carcinoma. OCT3/4 may also play a role in these tumors. Stem cell markers CD44 and CD133 did not distinguish the cells. Further functional studies should be performed to better characterize and identify thyroid cancer stem cells. Finally, thyroid cancer stem cell identification will provide a specific target for therapy of advanced thyroid carcinomas.

### 571 Management and Outcome of 141 Patients with Thyroid Nodules with Cytological Diagnosis of Indeterminate Significance

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**Background:** $BRAF^{V600E}$ mutation analysis has been proposed as a valuable adjunctive tool for refining the cytological diagnosis of thyroid nodules. To define a clinical value of $BRAF^{V600E}$ mutation, the surgical outcome of 141 patients harboring thyroid nodules with equivocal cytologic diagnosis was analysed in according to the FNAB and mutation results.

**Design:** Fine needle aspiration biopsy (FNAB) and $BRAF^{V600E}$ mutation analysis were performed in the routine patient care at initial presentation. Patients were managed in according to the KUMC guideline. All patients with cytological diagnosis of indeterminate significance were managed by the $BRAF^{V600E}$ mutation analysis results. DNA was extracted after atypical follicular cells were scraped from the cytology slide and $BRAF^{V600E}$ mutation was analysed by pyrosequencing method.

**Results:** Among 141 cases with cytological diagnosis of indeterminate significance, 45 (31.9%) cases showed $BRAF^{V600E}$ mutation. All 45 patients with $BRAF^{V600E}$ positive and indeterminate cytologic features were recommended to have an operation. Among them 23 patients underwent surgery and 21 patients had papillary carcinoma and two patients had nodular goiter. Among 96 $BRAF^{V600E}$ mutation negative patients, 11 patients underwent surgery and turned out to have 3 papillary carcinomas, one follicular carcinoma, one follicular adenoma, and 6 nodular hyperplasias. The patients were recommended to have surgery when the nodules were large or ultrasonography findings were worrisome. Five patients removed their nodules by their own hope.

**Conclusions:** We found that BRAF analysis provide a great help to make a therapeutic decision when the FNAB results are equivocal.

### 572 Comparison of MicroRNA Expression in Thyroid Neoplasms: An Aid for Diagnostic Evaluation?

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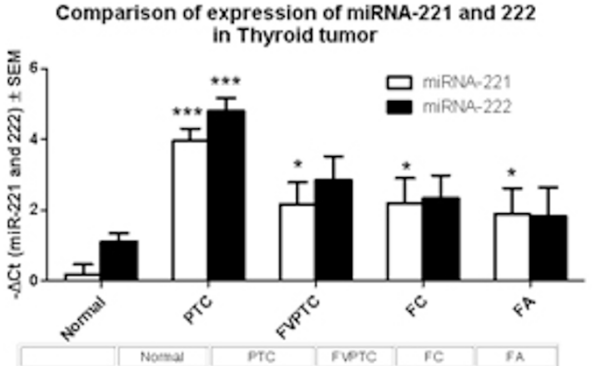
**Background:** MicroRNAs (miRNAs) is a class of small non-coding RNA molecules that regulate target gene expression. Aberrant expression of miRNAs plays a role as tumor suppressors or oncogenes in cancer. Thyroid cancer is the most common endocrine

malignancy, and miRNA deregulation has be reported in common human thyroid cancer. miRNA-221 and miRNA-222 are found upregulated in certain human cancers. We have previously reported marked overexpression of miRNA-221 and 222 in papillary thyroid carcinoma (PTC), and slight upregulation in other thyroid cancer including follicular carcinoma (FC), follicular variant of papillary thyroid carcinoma (FVPTC). To compare the miRNA regulation in benign and malignant thyroid neoplasms, we extended our study to include follicular adenoma (FA).

**Design:** An electronic database search was performed from 1999 to 2008 in our institution for thyroid neoplasms including FA. The surgical slides were examined, neoplastic and normal thyroid tissue was taken as paired samples from the same specimen. miRNA was extracted from paraffin-embedded thyroid tissue, and was quantified by RT-PCR (Taqman assay). miRNA-RNU was used as an internal control for calculation of Ct (cycle threshold) values. Unpaired t-test was used for statistical analysis.

**Results:** The expression of miRNA-221 and 222 in the studied thyroid tumors are illustrated in Figure 1. Our results showed miRNA-221 and 222 are both significantly upregulated in PTC compared to normal thyroid and other thyroid tumors. miRNA-221is also slightly overexpressed in FVPTC, FC and FA. However, miRNA-222 expression did not show statistical difference in FVPTC or FC or FA. There is no significant difference in expression levels of miRNA-221 and miRNA-222 among FVPTC, FC, or FA.

**Conclusions:** We studied expression levels of miRNA-221 and 222 in thyroid tumors (PTC, FVPTC, FC and FA). Both miRNAs remain as highly sensitive diagnostic markers for PTC. However, other thyroid tumors, FVPTC, FC and FA, also showed certain levels of upregulation of both miRNAs, especially miRNA-221. Further investigation is required to understand the biology and to apply biomarker signatures in diagnostic evaluation of thyroid neoplasms.




| | Normal | PTC | FVPTC | FC | FA |
|---|---|---|---|---|---|
| n | 9 | 10 | 16 | 10 | 9 |
| -ΔCt (miR-221) | 0.18 ±0.28 | 3.96 ±0.36*** | 2.16 ±0.63* | 2.20 ±0.71* | 1.88 ±0.74* |
| -ΔCt (miR-222) | 1.11 ±0.24 | 4.81 ±0.37*** | 2.85 ±0.68 | 2.34 ±0.64 | 1.82 ±0.82 |

mean ± SEM is shown.
t-test was used for statistical analysis. *** p<0.0005, * p<0.05

### 573 PAX-8 Is a Sensitive Marker for Thyroid Differentiation. Comparison with PAX-2, TTF-1, and Thyroglobulin

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**Background:** Diagnostic markers for thyroid differentiation remain in development. PAX-8 and PAX-2 are members of a transcription factor family instrumental for fetal development and probably neoplastic transformation of kidney, Müllerian organs, and thyroid. Expression of PAX-8 and PAX-2 in thyroid tissue is evaluated and compared with that of thyroglobulin and TTF-1, the two traditional thyroid markers.

**Design:** Consecutive tissue sections of non-neoplastic thyroid tissue (n= 40), adenomatous nodule (n= 26), follicular neoplasms (n=10), papillary carcinoma (n=10), medullary carcinoma (n=4), and benign parathyroid tissue (n=6) were submitted for PAX-8, PAX-2, TTF-1, and thyroglobulin immunostain.

**Results: PAX-8**: 86/86 thyroid tissue samples regardless of diagnoses was stained (nuclear, 3/3+, in virtually 100% of tumor or non-neoplastic cells); 0/4 medullary carcinomas; 6/6 parathyroid (nuclear, 1-2/3+; about 30% of parenchymal cells). **PAX-2**: No staining for any thyroid, parathyroid, or medullary carcinoma specimens. **TTF-1**: 86/86 thyroid tissue samples regardless of diagnoses (nuclear, 3/3+, in virtually 100% tumor or non-neoplatic cells); 2/4 medullary carcinomas (nuclear, 1-2/3 +; about 20% of tumor cells); 0/6 parathyroid. **Thyroglobulin:** 86/86 thyroid tissue samples regardless of diagnoses (colloid and cytoplasm, 1-3/3+, in virtually 100% of tumor or non-neoplatic cells); 2/4 medullary carcinoma (cytoplasm, 3/3+, about 20% of tumor cells); 0/6 parathyroid; there was also staining for lymphoid cell cytoplasm and a constant heavy background.

**Conclusions:** 1) PAX-8 is a very sensitive marker for thyroid differentiation regardless of diagnoses. 2) In spite of ontogenic similarity with PAX-8, PAX-2 is not expressed by thyroid tissue; 3) PAX-8 is as sensitive for thyroid differentiation as TTF-1; however, there is overlapping staining patterns of PAX-8 and TTF-1 in parathyroid tissue and medullary carcinoma, suggesting a complementary diagnostic role between them; 4) PAX-8 is a better marker than thyroglobulin for detecting thyroid differentiation.

### 574 Increased Expression of Ribonucleotide Reductase Subunit M2 in Adrenal Cortical Carcinoma: Possible Mechanism of Chemoresistance

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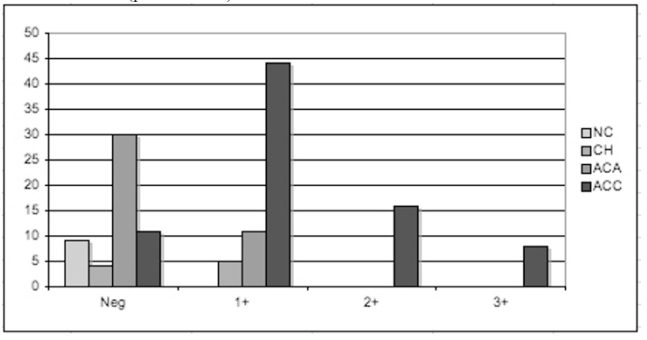
**Background:** Ribonucleotide reductase small subunit M2 (RRM2) plays an essential role in DNA synthesis and repair. Increased expression levels of RRM2 have increasingly been associated with tumor progression, resistance to chemotherapy, and enhanced potential of invasion and metastasis for a wide variety of solid tumor types. In a previously published transcriptional profiling study, we showed that RRM2 transcripts were more abundant in adrenal cortical carcinomas (ACC) compared to adenoma and normal cortex. Given the chemoresistance commonly seen in patients with ACC, we further explored the expression of RRM2 protein in a large cohort of benign and malignant adrenocortical tumors as well as normal cortex and cortical hyperplasia.

**Design:** RRM2 protein was examined using an adrenocortical-specific tissue array with 138 tissues and immunohistochemistry using an anti-RRM2 antibody. Stained tissue arrays were scored using as 0, 1+, 2+, and 3+ based on the intensity and distrubution of immunoreactivity. Chi-square statistics were used to assess the significance of the results.

**Results:** The RRM2 IHC results are shown in the chart below, which represents the number of cases of normal cortex (NC), cortical hyperplasia (CH), adrenocortical adenoma (ACA), and adrenocortical carcinoma (ACC) according to their staining results. The ACC cohort clearly shows higher levels of RRM2 immunoreactivity compared to the other tissues (p=8.84 E-10).




**Conclusions:** Our results validate increased expression of RRM2 protein in a statistically-significant subset of adrenocortical carcinomas, compared to adrenocortical adenoma and other cortical tissues. These results provide a possible explanation why some ACCs exhibit resistance to cytotoxic chemotherapy. Routine immunohistochemical assessment of individual ACC cases for RRM2 protein expression may become clinically usefel and play a role in the selection of the most appropriate therapy for patients with ACC.

### 575 MicroRNA Profiling of Differentiated, Poorly Differentiated and Anaplastic Thyroid Carcinoma, a Comparative Approach

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**Background:** MicroRNAs (miRNAs) belong to a class of small non-coding RNA species that are important regulators of a variety of biological processes including oncogenesis. Thyroid carcinomas encompass a wide spectrum ranging from differentiated thyroid carcinoma (DTC) to poorly differentiated (PDTC) and anaplastic thyroid carcinoma (ATC). DTC of both follicular and papillary types can progress to PDC and AC. The aim of our study was to evaluate if there is differential miRNA expression in various tumor subtypes during this progression.

**Design:** A search of the pathology archives over an 18-year period (1990-2008) retrieved 28 cases of PDTC. Tissue was available on 23 cases for the study, which included 15 PDTC with DTC, and 3 also had ATC. The DTC component on these 15 cases included follicular carcinoma FTC (7), follicular variant of papillary carcinoma FVPC (6) and papillary thyroid carcinoma PTC (2). In addition 6 cases were pure PDTC and 2 pure ATC. Seventeen cases of DTC (9 FTC, and 8 PTC) only were also selected from the same period. Sections were deparaffinized and individual tumor areas were retrieved by laser capture microdissection. Total RNA was isolated using the RecoverAll Total Nucleic Acid Isolation Kit and reverse transcribed into cDNA using miRNA-specific stem-looped primers and preamplified. miRNA profiling was done by qRT-PCR with the TaqMan Human MicroRNA A Array v2.0. Data analysis was done using Statminer and MeV software.

**Results:** Unsupervised hierarchical clustering analysis revealed that FTC and PDC tend to cluster together in the absence of ATC, with few differentially expressed miRNAs e.g. miR-891a. Consequently, ATC has a distinct miRNA signature significantly different from FTC and PDTC (which cluster together). Interestingly, in samples with evidence of all components i.e. FTC, PDTC and ATC, miRNA expression is very similar within individual components. Similar observations were made for PTC. However, FVPC has a distinct miRNA signature as compared to PDTC (c.f. FTC).

**Conclusions:** We present some interesting data generated from preliminary analysis of miRNA expression profile, especially with respect to PDTC. In tumors with all three components (i.e. DTC, PDTC and ATC), the expression profiles tend to be similar indicating a fundamental miRNA programming change at the level of DTC putting it at an increased risk for ATC. Several miRNAs were found to be differentially expressed between the two major groups (FTC and PDTC without ATC) and (FTC and PDTC with ATC) and will be validated by miRNA-specific qRT-PCR.

### 576 Definition and Clinical Significance of Vascular Invasion in Thyroid Carcinomas Derived from Follicular Epithelium

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**Background:** The diagnosis of well differentiated thyroid carcinoma (WDTC) is based on the presence of nuclear features of papillary thyroid carcinoma or capsular and/or vascular invasion by a follicular neoplasm. The criteria for vascular invasion are controversial. Several reports have suggested that angioinvasion is not a predictor of bad prognosis, but these results may be attributed to application of inappropriate criteria. We reviewed retrospectively the clinicopathological features of a series of angioinvasive thyroid carcinomas derived from follicular epithelium as defined by the most rigid criteria in order to determine whether rigid criteria for vascular invasion have clinical significance.

**Design:** A series of 4000 thyroid carcinomas derived from follicular epithelium were obtained from the files of University Health Network between September 2001 and August 2009. Using the criteria of tumor cells invading through a vessel wall associated with thrombus adherent to intravascular tumor, 116 angioinvasive carcinomas were identified; anaplastic thyroid carcinomas were excluded. Patient age and gender, tumor size, histological tumor type and variant, the presence of multifocal disease, extra-thyroidal extension (ETE), lymph node and surgical margin status were collected. The clinical charts of the patients were reviewed to determine the presence of distant metastasis and/or local tumor recurrence during follow-up.

**Results:** Tumors from 116 patients (35 males, 81 females, age 22-84, mean 52.39 years) ranged from 0.8 cm to 11.5 cm (mean 5.08 cm) and included 82 WDTCs (71%) 22 poorly differentiated thyroid carcinomas (PDTCs) (19%) and 12 WDTCs with focal dedifferentiation (10%). Multifocal disease and ETE were present in 38 (33%) and 25 (22%), respectively. Surgical margins were involved in 56 (50%). Lymph node metastases were found in 42 of 70 patients (60%) that had undergone lymph node dissection. Follow-up information was available for 73 cases; follow-up time ranged from 1 month to 8 years. 24 patients developed distant metastasis (33%) and 27 patients had local tumor recurrence (37%). The most common metastatic sites were lungs (23 patients), bone (11 patients), brain (2 patients) and liver (1 patient). Distant metastasis originated from 14 WDTCs (58%), 6 PDTCs (25%) and 4 WDTCs with focal dedifferentiation (17%).

**Conclusions:** Using rigid criteria, the identification of vascular invasion predicts distant metastasis in patients with thyroid carcinomas, especially in WDTCs.

### 577 The Use of Genetic Programming for Diagnostic Texture Analysis in the Assessment of Follicular Variant Papillary Thyroid Carcinoma: A Feasibility Study

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**Background:** There is a considerable inter- and intra-observer variability in the diagnosis of follicular variant of papillary thyroid carcinoma (FVPTC) even among experts. Light microscopy is the current gold standard for this diagnosis, but the recognition of minimal features of FVPTC is highly subjective. We analyzed the texture pattern of FVPTC and benign conditions using genetic programming (GP) software. GENIE© (GENetic Imagery Exploration) is a GP software system that builds automatic feature extraction algorithms for image analysis, using spectral and spatial signatures of the images.

**Design:** Five H&E-stained slides of normal thyroid parenchyma, five slides of benign thyroid lesions, and 10 FVPTCs were scanned to digital images. A training set was selected from a subset of the digital slides to include normal parenchyma, benign follicular lesions and FVPTC with florid diagnostic nuclear features. We defined benign, malignant and background samples by marking regions in the training slides. We trained GENIE using these selected and defined areas. Initially, we created two classifiers, one that runs analyses at 5x magnification, and another at 20x. The remaining digital slides were then analyzed with these classifiers.

**Results:** GENIE provided consistent percentage measures of the selected regions in terms of background, benign and malignant in repeated challenges. In most cases, GENIE was impressive in identifying malignant and benign regions. In some cases, reactive atypia in adenomas or thyroiditis was classified in the malignant category. Macrofollicular areas of FVPTC and areas with subtle nuclear irregularities were classified as benign.

**Conclusions:** Our preliminary results indicate that GENIE can recognize benign and malignant thyroid without intra-observer variation and without the need for image analysis expertise. The major weakness of GENIE is that the amount of image capacity for a classifier training set is limited. Several different classifiers must be created; it appears that a single classifier will have not high sensitivity and/or specificity. We expect that retraining and the application of feature interference technology and deep belief nets will reduce false positivity and negativity rates. This application will require validation on a larger series.

### 578 A Combined Molecular-Pathological Score To Predict Aggressiveness of Thyroid Papillary Microcarcinoma

*LA Niemeier, HA Kuffner, S Carty, AF Stewart, YE Nikiforov.* UPMC, Pittsburgh, PA.

**Background:** Papillary thyroid microcarcinoma (TPM) is an incidentally discovered papillary carcinoma of the thyroid that is less than 1.0 cm in greatest dimension. Most of these tumors are indolent and are cured by lobectomy, although some of them are aggressive and result in local and distant metastases and may lead to patient death. Currently, there is no standardized clinical management for patients with these lesions. The aim of our study was to evaluate the utility of a combination of molecular and histological criteria to differentiate those TPM that behave more aggressively and need more expensive treatment.

**Design:** TPM cases were selected from the University of Pittsburgh Thyroid Cancer Database from 1991-2004. Two groups were selected. The aggressive tumor group (AG) consisted of microcarcinomas with lymph node metastasis, distant metastasis, or tumor recurrence after surgery (n=29). The non-aggressive tumor group (NAG) was selected from tumors with no such complications that were matched for tumor size, patient gender, and length of follow-up (n=30). Histologic slides were reviewed and scored for multiple microscopic criteria and molecular analysis for BRAF and RAS mutations was performed using DNA isolated from tumor sections after microdissection.

**Results:** BRAF mutations were detected in 20/26 (77%) of AG tumors and in 8/25 (32%) of NAG ($p<0.002$). In addition, the following histological features were found with statistically significant difference between the two groups: extrathyroidal extension, superficial tumor location (less than 1.0 mm from thyroid capsule), infiltrative border, tall cell variant, intrathyroidal tumor spread, tumor encapsulation, and significant tumor fibrosis. Based on these findings, a molecular-pathological sore (MPS) was developed with 2 points given for a BRAF mutation and 1 point for presence of each of the following five features: extrathyroidal extension, superficial location, intrathyroidal spread, tumor fibrosis, and tall cell variant. Based on this, tumors with an MPS of 0-2 were all NAG, whereas an MPS of 3 correlated with the 75% of AG tumors and an MPS of 4 or more with 83% of AG tumors.

**Conclusions:** We developed a diagnostically simple and practical scoring system based on the combination of one molecular and 5 histopathologic parameters that can reliably separate TPM into high-risk (score 3 and higher) and low-risk (score 0-2) groups.

### 579 A Study of Parathyroid Transcription Factor GCM2 Expression in Parathyroid Lesions

*D Nonaka.* New York University School of Medicine, New York, NY.

**Background:** The parathyroid glands and thymus arise from a common primordium of the third and fourth pharyngeal pouch endoderm. The expression of the transcription factors *Gcm2* and *Foxn1* divides the primordium into parathyroid- and thymus-specific domains. *Gcm2* is exclusively expressed in the parathyroid gland in humans, and deletion of this gene in mice results in absence of the parathyroid glands. There is no study on Gcm2 immunohistochemistry application in surgical pathology.

**Design:** A total of 40 cases of parathyroid lesions including 27 adenomas, 2 atypical adenomas, 2 carcinomas, 8 hyperplastic lesions, and 1 case of recurrent hyperplasia of autograft gland were stained with anti-Gcm2 antibody. Anti-Gcm2 was also applied to 70 thyroid tumors (follicular adenoma/carcinoma including Hürthle cell type, and papillary, insular, medullary and anaplastic carcinomas), 27 adrenocortical tumors (adenomas and carcinomas), 43 pheochromocytomas and paragangliomas, 32 carcinoids from the lung and GI tract, and 28 Merkel cell carcinomas, 116 pulmonary adenocarcinomas, 29 pulmonary squamous cell carcinomas, 50 carcinomas from a variety of organs, and 34 melanomas as well as tissues from a variety of organs including parathyroid. The extent of staining was graded as focal (5-50%) and diffuse (50-100%).

**Results:** Gcm2 nuclear expression was seen in all normal, hyperplastic and neoplastic parathyroid lesions in diffuse fashion while no Gcm2 expression was seen in any other normal tissues and tumors including thymus and thyroid.

**Conclusions:** Parathyroid transcription factor, Gcm2, is a highly sensitive and specific marker for parathyroid lesions. Although parathyroid hormone (PTH) immunohistochemistry stain is a useful marker, its reaction tends to be variable in extent and intensity in parathyroid neoplasia, and Gcm2 would serve as a useful adjunct marker.

### 580 Oxyphilic Hypercellular Follicular Nodule (OHCFN) of the Thyroid: A 10 Year Experience

*AL Patton, SP Gmitro, JA Brainard.* Cleveland Clinic, Cleveland, OH.

**Background:** The term oxyphilic hypercellular follicular nodule (OHCFN) is used in thyroid FNA to describe cellular aspirate samples comprised nearly exclusively of Hurthle cells. Associated surgical pathology diagnoses commonly include benign hyperplastic nodule in the setting of lymphocytic thyroiditis, as well as Hurthle cell adenoma or carcinoma. Prior authors have suggested an increased rate of neoplasms in this patient group when compared with cellular non-Hurthle follicular nodules. Recently, measurement of preoperative TSHR mRNA has proven helpful in identifying patients with thyroid malignancy. The goal of our study is to correlate FNA interpretation with surgical diagnosis and results of preoperative TSHR mRNA testing when available.

**Design:** The electronic pathology database was searched for thyroid FNA samples interpreted as OHCFN. These samples share in common a cellular Hurthle cell sample with minimal colloid with cells arranged in a predominantly microfollicular pattern. Surgical pathology diagnoses were collated. Preoperative TSHR mRNA elevation (>1.0 ng/ug) was assessed in a subset.

**Results:** 182 FNA's interpreted as OHCFN over a 10 year period were studied (138 female, 44 male, median age 55.5 years). Histologic follow-up was available for 137 cases (Table 1).

Follow-up histology of 137 surgical cases with the cytologic diagnosis "Oxyphilic Hypercellular Follicular Nodule"

| Surgical Diagnosis | Number | Percentage |
|---|---|---|
| Hurthle Cell Carcinoma | 26 | 19 |
| Papillary Thyroid Carcinoma | 9 | 6.6 |
| Hurthle Cell Adenoma | 43 | 31.4 |
| Benign Thyroid Nodule | 51 | 37.2 |
| Chronic Lymphocytic Thyroiditis | 8 | 5.8 |
| Neoplatic | 78 | 56.9 |
| Non-neoplastic | 59 | 43.1 |

Of these, 36 pts had chronic lymphocytic thyroiditis, 24 non-neoplastic cases and 12 neoplasms. Preoperative TSHR mRNA was tested in 16 cases (Table 2).

Final histologic diagnosis of cases having preoperative TSHR mRNA evaluation (n=16)

| Final Histologic Diagnosis | # of patients having normal TSHR mRNA (<1.0 ng/ug) | # of patients having elevated TSHR mRNA (>1.0 ng/ug) |
|---|---|---|
| Hurthle Cell Carcinoma | 3† | 2 |
| Papillary Thryoid Carcinoma | 2 | 0 |
| Hurthle Cell Adenoma | 4† | 0 |
| Benign Thyroid Nodule | 3 | 2† |

There was 1 case from each of these categories having an incidental microscopic papillary thryoid carcinoma.†

**Conclusions:** The majority of FNA's interpreted as OHCFN are neoplasms (59.6%) and nearly half of these are malignant . Chronic lymphocytic thyroiditis is identified in nearly one-third of patients. Elevated preoperative TSHR mRNA values did not correlate with histologic follow-up of malignancy in our study.

### 581 Characterization of Estrogen Receptor beta 1 in Human Pituitary Adenomas

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**Background:** ER-β, as ER-alpha, is an extremely important component of the complex signal transduction pathway that specifically regulates the growth and development of target tissues and tumors. Functional studies have shown that overexpression of ER-β1 has antiproliferative and proapoptotic effects. ER-β1 has been reported as a potent inhibitor of cell proliferation, invasion and motility in the human breast and colon cancer cell line. Methylation of ER-β1 has been detected in breast and prostate cancers. However, little is known about ER-β1 expression pattern in human normal pituitary tissues and pituitary tumors.

**Design:** In this study, the expression and localization of ER-β1 protein was examined in 4 normal pituitary tissues by a double-staining technique and in 91 pituitary adenomas using immunohistochemistry. In addition, ER-β 1 DNA promoter hypermethylation status was investigated by MS-PCR in 43 pituitary adenomas. Furthermore, function analysis of ER-β 1 has been done *in vitro*.

**Results:** In 4 normal adenohypophyseal samples, ER-β1exhibited nuclear and cytoplasmic reactivity with moderate density (20-30%). Using double-staining, ER-β1 co-localized with each of the adenohypophysial hormones including GH, PRL, ACTH, FSH, TSH and LH. In pituitary adenomas, ER-β1 expression was lost in 37% (33 of 91) tumors, was significantly reduced in 20% (19 of 91) tumors. Loss expression of ER-β1 was frequently observed in GH cell adenomas (20 of 23 cases), PRL cell adenomas (11 of 15 cases) and ACTH cell adenomas (6 of 7 cases). Loss or reduced expression of ER-β1 was rarely observed in FSH/LH cell adenomas and null cell adenomas. Furthermore, ER-β1 DNA promoter hypermethylation was detected in adenomas (10 of 43, 23.2%) but not in normal tissues. Methylation status correlated with significant reduction of ER-β1 expression.

**Conclusions:** These data indicate that ER-β1 inactivation through CpG hypermethylation is common in pituitary adenomas. Differential expression of ER-β1 in different tumor types might have physiological or therapeutic significance.

### 582 MicroRNA Expression Abnormalities in Pituitary Adenomas Are Associated with Distinctive Pathologic Features and May Contribute to Tumorigenesis

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**Background:** MicroRNAs are small noncoding RNAs that regulate gene expression by targeting specific mRNAs for degradation or translation inhibition. Recent evidence indicates that microRNAs can contribute to tumorigenesis and tumor progression and may have diagnostic and prognostic value in several human malignancies. We investigated the global microRNA expression patterns in normal human pituitary and adenomas to evaluate their involvement in tumorigenesis and clinicopathologic features of these tumor types.

**Design:** Using the most new miRNA microarray, we studied about 700 microRNA expression in 5 human adenohypophyses and 53 pituitary adenomas, including 11 somatotroph, 6 lactotroph, 13 corticotroph (8 associated with Cushing's disease and 5 silent), 12 gonadotroph adenomas, 2 TSHomas, 8 Null cell adenomas and 1 familial isolated pituitary adenoma. Real-time RT-PCR analysis was done to evaluate the expression of some miRNA. Web-based computer programs were used to predict potential miRNA-target gene. Pituitary tumor cell lines has been used to examine the relation between miRNAs and target genes.

**Results:** Our data showed that a common pattern of microRNA expression distinguishes any tumor type from normal pituitary tissues: Forty-seven miRNA were differentially expressed between normal pituitary and pituitary adenomas. 32 were up-regulated and 15 down-regulated in adenomas compared to normal pituitaries. In addition, some microRNAs expression can distinguish among each tumor type. Moreover, several of the identified miRNAs are involved in tumor invasion and tumor size. Furthermore, we identified several miRNA targeted genes that may related to pituitary tumorigenesis and tumor cell proliferation *in vitro.*

**Conclusions:** These results suggest that alteration in microRNA expression and target genes is related to pituitary tumorigenesis and tumor progression. Some of them may prove useful in distinguishing tumors with different clinical behavior and may to be possible therapeutic targets for the treatment.

### 583 Molecular Genotyping of Papillary Thyroid Carcinoma Follicular Variant According to Its Histologic Subtypes (Encapsulated vs Non-Encapsulated Infiltrative) Reveals Distinct BRAF and RAS Mutation Patterns

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**Background:** Papillary thyroid carcinoma, follicular variant (FVPTC) presents usually as an encapsulated tumor and less commonly as a partially/non-encapsulated infiltrative neoplasm. The encapsulated form rarely metastasize to lymph node (LN) (5% of cases) while infiltrative FVPTC often harbors LN metastases (65% of patients) (*Cancer* 2006;107:1255). The molecular profile of FVPTC was shown to be close to the follicular adenoma/carcinoma (FA/FC) group of tumors with a high RAS and very low BRAF mutation rates (*Am J Surg Pathol* 2006;30:216). A comprehensive survey of oncogenic mutations in FVPTC according to its encapsulated and non-encapsulated forms has not been done.

**Design:** Paraffin tissue from 28 patients with encapsulated FVPTC and 19 with infiltrative FVPTC were subjected to mass spectrometry genotyping encompassing the most significant oncogenes in thyroid carcinomas: 111 mutations in RET, BRAF, NRAS, HRAS, KRAS, PIK3CA, AKT1, and other related genes.

**Results:** There was no significant age or gender difference between encapsulated and infiltrative FVPTC. Encapsulated FVPTC were larger than their infiltrative counterpart (median size:2.9 and 2 cm respectively) but this was not significant. There was no significant differences in vascular invasion between both groups. Margins were more often positive in the infiltrative FVPTC (5/19, 26%) than in the encapsulated tumors (1/28, 4%-p=0.03). Extra-thyroid extension was markedly increased in infiltrative FVPTC (10/19, 53%) compared to encapsulated tumors (1/28, 4%-p=0.0001). The LN metastatic rate was much higher in infiltrative (10/19, 53%) than in encapsulated FVPTC (2/28, 7%-p=0.001). BRAFV600E mutation were found in 5 of 19 (26%) of the infiltrative tumor and in none of the encapsulated FVPTC (p=0.007). RAS mutations were seen in 10 of 28 (36%) of the encapsulated group (5 *NRAS*_Q61R, 3 *HRAS*_Q61, 1 *HRAS*_G13C and 1 *KRAS*_Q61R) and in only 3 of 19 (16%) of infiltrative FVPTC (2 *NRAS*_Q61R, 1*HRAS*G12V) (p=0.17). No other mutations were detected.

**Conclusions:** 1) Encapsulated FVPTC have a molecular profile similar to FA/FC (significant rate of RAS mutation and no BRAF mutations). 2) Infiltrative FVPTC have an opposite molecular profile closer to classical PTC than to FA/FC (more BRAF and less RAS mutations than encapsulated FVPTC). 3) The molecular profile of encapsulated and infiltrative FVPTC parallels their behavior (i.e metastatic LN pattern).

### 584 Molecular and Morphologic Characterization of Thyroid Carcinomas According to Extra-Thyroid Extension Status

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**Background:** The relationships between mutational profile, histologic subtype, extra-thyroid extension (ETE) status and their impact on survival in thyroid carcinomas (TC) have not been well characterized

**Design:** All cases of TC with ETE but without nodal metastases at presentation (NMP) were identified over a 20 year period and grouped into gross and microscopic ETE (METE). METE was subdivided into focal (1-2 foci of 1mm in size each) and established (>2 foci or any focus >1 mm). A group of 14 papillary thyroid carcinomas (PTC) without ETE and NMP was also analyzed. Cases with paraffin tissues were subjected to mass spectrometry genotyping encompassing the most significant oncogenes in TC: 111 mutations in RET, BRAF, NRAS, HRAS, KRAS, PIK3CA, AKT1, and other related genes.

**Results:** 81 (10%) of 829 patients in the database had ETE and no NMP. Histologic subtype in TC with METE (n=52) was as follows: Classical PTC- 24 (46%), Tall cell PTC- 15 (29%), PTC microcarcinoma- 7 (13%), PTC follicular variant (FV) infiltrative- 2 (4%), solid variant PTC- 1(2%), poorly differentiated TC (PDTC)- 2 (4%),anaplastic- 1(2%). In cases with gross ETE (n=29), there were 10 classical PTC (34.5%), 6 Tall cell PTC (20.7%), 1 PTC FV infiltrative (3.5%), 9 PDTC (31%), 3 anaplastic (10.3%). There was a higher disease specific survival (DSS) in patients with METE compared to those with gross ETE (p<0.0001). Except for the anaplastic case, all patients with METE did not recur including 23 without radioactive iodine therapy (median follow up 9 years). Within patients with gross ETE into trachea/esophagus, higher grade (defined by high mitotic activity and/or tumor necrosis) correlated with worse DSS (p<0.05). There was no survival difference within METE (focal vs established). 56 cases with ETE were genotyped as follows: 39 BRAFV600E (69.6%), 1 BRAFV600E-AKT1 (1.8%), 1 N-RAS (1.8%), 1 K-RAS (1.8%), 14 wild type (25%). Within PTC, BRAF positivity rate according to ETE status was as follows: 13+/15 (87%) in gross ETE, 25+/34 (73%) in METE, 5+/13 (38%) in no ETE. (No ETE vs ETE,p=0.01). If PTC FV are excluded, BRAF positivity does not correlate with ETE status in classical/tall cell PTC.

**Conclusions:** 1) PTC with METE without NMP recur very rarely. 2) High mitotic activity/tumor necrosis confers worse DSS even in patients stratified for gross ETE in trachea/esophagus. 3) BRAF positivity correlates with the presence of ETE in PTC but this relationship is lost within classical/tall cell PTC if PTC FV are excluded from the analysis.

### 585 Lipid-Rich Neuroendocrine Tumors ("Carcinoids") of the Appendix: Potential Confusion with Goblet Cell Carcinoid

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**Background:** So-called clear cell change has been described in neuroendocrine tumors at several locations. Those associated with von Hippel Lindau disease are pathognomonically "clear" and the cytoplasmic appearance has been ascribed to intracytoplasmic lipid. However, lipid has not been demonstrated in all cases of clear cell carcinoid tumors. Such variants have not been described in carcinoid tumors of the

appendix and cases with a prominent proportion of clear or more correctly, lipid-rich cytoplasm may bear a superficial resemblance to goblet cell carcinoid and/or signet ring adenocarcinoma.
**Design:** The computer records of the Department of Pathology, University Health Network, Toronto were searched from 2001 to 2009 for cases coded as appendiceal carcinoid tumors. Goblet cell carcinoid tumors were excluded for the purposes of this study.
**Results:** Seven of the 43 cases retrieved fulfilled the aforementioned criterion and were included for the purposes of this study. Seven cases, in 5 females and 2 males ranging in age from 22 to 65 years, were noted to have a population of lipid-rich and vacuolated clear cells accounting for 25% or more of the tumor population. The carcinoid tumors were incidental in all cases with 4 of patients presenting with appendicitis, 2 with concomitant mucinous cystadenocarcinomas of the appendix and 1 with an adenocarcinoma of the ascending colon. Morphologically, the tumors had a nested and trabecular pattern and were composed of an admixture of microvesicular and clear lipid-rich cells. There were no mitoses, areas of necrosis of lymphovascular invasion and all cases extended to the mesoappendix. All cases were positive for synaptophysin, chromogranin and serotonin but negative for inhibin. Three cases were examined ultrastructurally, and showed the presence of intracytoplasmic lipid and neurosecretory granules. None of the patients have shown evidence of recurrent disease.
**Conclusions:** The importance of recognizing this variant of carcinoid tumor in the appendix is to avoid confusion with goblet cell carcinoid tumors with or without a signet ring adenocarcinoma. The presence of multi-vacuolated, foamy and clear cells, some resembling signet ring or goblet cells, in otherwise classical carcinoid tumors is rare but should considered in this context in the appendix.

### 586 Is Multifocality of Papillary Carcinoma of Thyroid (PTC) Explained by Intra-Thyroidal Lymphatic Metastasis?

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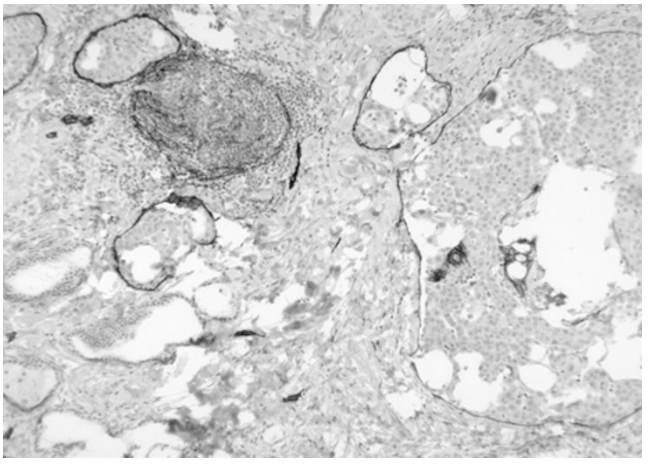
**Background:** Papillary carcinomas of thyroid are managed by total thyroidectomy based on their high incidence of multifocality and bilaterality. Whether these multiple foci actually represent synchronous development of independent clones or represent intra-thyroidal metastatic foci from a single clone is debatable. Recent molecular studies suggest that 15-35% multifocal (MF) tumors are multi-clonal; the remainder represent a single clone. The aim of this study is to determine the frequency of intralymphatic tumor foci and the vaule of intratumoral (IT) and peritumoral (PT) lymphatic density (LD) assessment in predicting multifocality and lymph node metastases in PTC.
**Design:** Consecutive cases of MF and a matching number of unifocal (UF) PTC diagnosed in our institution between 2003-2009 were selected for the study. Blocks containing the interface of tumor and normal tissue were immunostained with D-2-40 (Biocare Medical) using Ventana Benchmark XT. IT and PT (within 10mm around the tumor) LD was quantified by 2 reviewers by averaging the number of lymphatics in 4 high power fields in "hot spots" identified at low power. Intralymphatic tumor (ILT) nests were also noted.
**Results:** PT LD was significantly higher than IT LD in both uni and multifocal PTC.

| Tumor(n=61) | Age-range (mean) | M/F | IT LD (mean+/-Std) | PT LD (mean+/-Std) | t(p-value) |
|---|---|---|---|---|---|
| Multifocal(n=29) | 10-73 (44) | 8/21 | 0.41+/-0.68 | 4.18+/-1.77 | 10.28(<0.0001) |
| Unifocal(n=32) | 17-68 (44) | 9/23 | 0.18+/-0.39 | 2.89+/-1.26 | 11.35(<0.0001) |

IT LD did not differ between UF and MF groups (0.18±0.39, 0.41±0.68; p=0.114) or between cases with lymph node metastases (LN+) and those without (LN-). However, PT LD was higher for multifocal compared to UF PTC (2.89±1.26, 4.18±1.77; p=0.003) and between LN+ and LN- cases (p=0.05) . Four cases with ILT were identified in MF PTC.



**Conclusions:** We found peritumoral but not intratumoral lymphangiogenesis to correlate with both multifocality and nodal metastases in PTC. Only 4/29 cases of multifocal PTC showed intra-lymphatic tumor suggesting that plurality of tumor foci cannot be explained only on the basis of intra-lymphatic spread within thyroid.

### 587 Grade Progression of Well Differentiated Neuroendocrine Tumors (WDNETs) of the Enteropancreatic System Is Distinct from Poorly Differentiated (PD) High Grade Neuroendocrine Carcinomas (PDHGNECas)

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**Background:** The grade of NETs is generally assessed by proliferative activity. High mitotic rate defines the group of HG and aggressive tumors. Often designated HGNECa, these tumors exhibit poorly differentiated (PD-) and HG histopathology and poor outcome, and they are regarded as distinct from well differentiated (WD) NETs. However, in addition to PD-HGNECas, there exist tumors that display features of both a WD-NET and high proliferative activity. We investigated clinicopathological features and the status of *RB1* gene mutation and loss of Rb expression in WD-NETs with HG features and PD-HGNECas.
**Design:** High grade was defined using WHO classification of lung NETs (>10 mitoses/10 HPF). Clinicopathological features were compared between each grade and differentiation group. Immunostain for Rb was performed. Tumors of relevant component were micro-dissected followed by RNA and genomic DNA extraction. *RB1* gene expression was evaluated by real time PCR and *RB1* mutation was carried out by whole genome amplification, *RB1* exon-specific PCR and subsequent sequencing.
**Results:** 49 NETs satisfied the criteria of >10 mitoses/10 HPF. Thirty-nine cases (80%) revealed features of PD-NECa, and ten tumors (20%) exhibited foci of HG features, but had >50% component of low grade WDNET. The component of an adenocarcinoma was not identified in WD-NETs of any grade; in contrast it was present in 29/39 (74%) cases of PD-HGNECa. Immunostains demonstrated Rb protein loss in 74% PD-NECas and retained all WD-NETs regardless the grade. There was a complete concordance between Rb protein loss and *RB1* gene expression by RT-PCR and *RB1* mutation.

Table 1. Clinicopathologic features and RB1 Status of Subtypes of NETs

| NET Subtype | Adenocarcinom Component | Mitoses/50 HPF | Ki67 | Metastasis | Nen FU (Mons) | RB1 Mutation/ Rb loss | NED/AWD/ DOD% |
|---|---|---|---|---|---|---|---|
| WE-LG | 0 | 0-36 | 1-10% | 28% | 44 | 0 | 63/17/20 |
| WD-HG | 0 | >50 | 20-50% | 100% | 30 | 0 | 14/80/6 |
| PD-HG | 29/39 (74%) | >100 | 50-90% | 100% | 14 | 30/39 (77%) | 25/unk/50 |

NED=no evidence of disease; AWD=alive with disease; DOD=died of disease

The most common *RB1*mutations occurred at exon 8, followed by additional mutation of exon 2, 4, 6, 7, 18, and 21.
**Conclusions:** WD- NETs may rarely exhibit high proliferative activity, but they can be distinguished from PD-HGNECas by their clinical behaviors and by their status of *RB1* gene expression and mutation.

### 588 Adrenocortical Tumors (ACT): Evaluation and Harmonization of the Reading of the Weiss System Criteria at the French Level

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**Background:** ACT diagnosis of malignancy is based on a Weiss score ≥3. This score has 9 criteria and some of them lack of reproducibility. A project of evaluation and harmonization of the reading has been carried out at the French level.
**Design:** 5 slides of 50 ACT (Weiss score 0 to 9) were scanned and accessible via the internet. 12 pathologists have examined 25 ACT once and 25 ACT twice. Intra-observer reproducibility (IaOR) and inter-observer reproducibility (IrOR) have been evaluated by the Kappa (K) coefficient considered as almost perfect if >0,8.
**Results:** For Weiss ≥3 vs <3, IaOR was almost perfect for 10/12 pathologists and IrOR was substantial (K=0,7). Necrosis was the only criterion almost perfect (K IaO>0,8 for 9/12 pathologists, K IrO=0,78). It was followed by clear cells percentage, venous invasion and mitoses. The least reproducible criteria were sinusoidal invasion (K IaO>0,8 for 0/12 pathologists, K IrO=0,40) and atypical mitoses (K IaO>0,8 for 3/12 pathologists, K IrO=0,29) followed by capsular invasion, diffuse architecture and nuclear grade.
**Conclusions:** The insufficient reproductibility has led to a work of harmonization of the reading of the Weiss criteria. A second examination of the same 50 ACT was performed allowing a significant improvement of the IrOR (K=0,75). This work has shown that virtual slides system is adequate for conducting such project. We plan to compare the results of the classical Weiss system to those of Weiss system modified by Aubert et al. FT SA EL, AA MP, MD LD FG CH CM GM NS KR, EB XB JC RL contributed equally to the work.

### 589 Epidermal Growth Factor Receptor Over-Expression in Thyroid Neoplasia Associates with Chromosome 7 Polysomy

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**Background:** Epidermal growth factor receptor (EGFR) activation is a prominent contributor to tumor proliferation and angiogenesis. Alterations in the EGFR gene including copy number changes have correlated with response to some tyrosine kinase inhibitors in other tumor types. Understanding mechanisms of EGFR activation in thyroid neoplasia may provide additional pathways for targeted therapy in advanced patients.
**Design:** Forty-nine thyroid tumors including 20 follicular carcinomas (FC),15 medullary carcinomas (MTC), 9 papillary carcinomas (PTC), and 5 anaplastic carcinomas(ATC) comprised a tissue microarray used for the following studies. EGFR expression was evaluated by immunohistochemistry (Clone 31G7, 1:50, Zymed) and scored 0-3+. The

EGFR gene and Chromosome 7 copy number were assessed by fluorescence in situ hybridization (FISH); polysomy was defined as 3 or more copies in >=10% of cells.
**Results:** EGFR was over-expressed (3+) in 11 of 49 (22.4%) tumors (4/20 FC, 3/15 MTC, 1/9 PTC, 2/5 ATC). Ten of the 11 (90.9%) tumors with high EGFR expression also showed chromosome 7 polysomy (p=0.0002). Chromosome 7 polysomy was present in 20 of 49 (40.8%) tumors (8/20 FC, 7/15 MTC, 3/9 PTC, 2/5 ATC). High polysomy (>40% of cells with 3+ copies) was present in 9 of the 20 (45.0%) polysomic tumors. EGFR gene amplification was not identified.
**Conclusions:** EGFR is 1) over-expressed in a subset of thyroid neoplasms (follicular and c-cell origin), and correlates with chromosome 7 polysomy. 2) Polysomy was not limited to high EGFR expressing tumors. 3) As polysomy maybe a marker of response to targeted therapies, further evaluation of EGFR and polysomy by FISH in advanced thyroid patients is warranted.

### 590 The Value of Thyroid Atypia of Undetermined Significance (AUS) Terminology and Its Follow-Up by Repeat FNA

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**Background:** Since 2001, we have used a 6-tier system to report thyroid FNA with 3 "indeterminate" categories and repeat FNA (rFNA) for follow-up of the Atypia of Undetermined significance (AUS) category. We further sub-categorize AUS as "morphologic" (AUS-M) and "adequacy related" (AUS-A). With increasing acceptance of rFNA for AUS, we assessed compliance with follow up and the malignancy outcomes of AUS, Neoplasm and Suspicious.
**Design:** All thyroid biopsy reports between June 2006 and June 2009 were retrieved from our files. Data was analyzed and correlated with surgical outcomes.
**Results:** 4242 adequate thyroid FNA's were categorized as shown in the Table. Follow-up FNA was done in 319 (37%) of patients first diagnosed as AUS. A definitive diagnosis was made in 192 (60%) with rFNA. On rFNA, 113 (35%) remained as AUS of which 46 had surgery and 12 (26%) were malignant. In comparison, 66% Neoplasm and 84% Suspicious cases underwent surgery. Malignancy outcomes were AUS-overall 7%, Neoplasm 17% and SUSP 63%. When AUS outcomes were assessed by subtype and whether rFNA was done prior to surgery, the malignancy rate was 21% in AUS-A versus 6% in AUS-M cases, and 26% in AUS resected after an AUS rFNA versus 2% in AUS cases that went directly to surgery after the first AUS diagnosis.

Distribution of Thyroid Biopsies from June 2006 through June 2009

| | Total Number of Cases | Number of resected cases | Negative for Malignancy | Neoplasm | Positive for Malignancy |
|---|---|---|---|---|---|
| Negative | 2940 (70%) | 190 (65%) | 153 (80%) | 33 (17%) | 3 (2%) |
| Other | 855 (20%) | 332 (39%) | 189 (57%) | 119 (36%) | 24 (7%) |
| "Morphologic" | 695 | 298 (43%) | 168 (57%) | 113 (38%) | 17 (6%) |
| "Adequacy" | 160 | 34 (21%) | 21 (62%) | 6 (18%) | 7 (21%) |
| Neoplasm | 212 (5%) | 140 (66%) | 43 (20%) | 68 (32%) | 25 (17%) |
| Suspicious | 49 (1%) | 41 (84%) | 5 (12%) | 10 (24%) | 26 (63%) |
| Positive | 186 (4%) | 152 (82%) | 0 | 3 (2%) | 153 (98%) |

**Conclusions:** (1) AUS is a valuable subcategory in thyroid FNA reporting with a lower malignancy outcome (7%) than Neoplasm (17%) and Suspicious (63%). (2) Repeat FNA for AUS definitively categorizes over half of cases first interpreted as AUS (59%) (3) Over half (53%) of AUS on repeat FNA were Negative and did not need surgery (4) The malignancy rate in cases diagnosed as AUS on repeat FNA (26%) is higher than that of the Neoplasm category (17%) and thus these cases need resection (5) "Adequacy related" AUS cases have a higher malignancy rate than "morphologic" AUS (21% versus 6%), emphasizing the importance of not downgrading suboptimal cases to Negative.

# Gastrointestinal

### 591 Histologic Subtypes of Microsatellite Instability-High (MSI-H) Colorectal Adenocarcinomas (CRCs) and Their Association with Clinicopathologic Features and Prognosis

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**Background:** MSI-H CRCs are seen in hereditary non polyposis colon cancers and have better patient outcome in sporadic CRC. Mucinous/variegated/undifferentiated histology is associated with MSI. Small number of MSI-H CRCs have histology similar to conventional MSI-stable moderately differentiated adenocarcinoma, significance of which is unclear. We studied the histologic subtypes of MSI-H CRC and correlated them with clinicopathologic features and disease-free survival (DFS).
**Design:** Patients with CRC who underwent resection without neo-adjuvant therapy (1998-2008) and had MSI-H status confirmed by immunohistochemistry or molecular assay were included. All tumors were analyzed for known histologic features of MSI-H CRC including intraepithelial lymphocytosis (IEL)/high power field (hpf).

| **Classic MSI-H (n=89)** | |
|---|---|
| Undifferentiated carcinoma (n=17) | > 50% undifferentiated component |
| Mucinous carcinoma (n=8) | > 50% mucinous component |
| Signet ring cell carcinoma (n=8) | > 10% signet ring component |
| Variegated (n=48) | Two or more components each >5% |
| Cribriform/poorly differentiated (n=8) | Cribriform architecture > 20% or any focus of sheets of cells |
| **Conventional histology with/without IEL (n=35)** | |
| Moderately differentiated adenocarcinoma with absence of classic MSI-H features, neuroendocrine differentiation and tumor budding | |

The IEL/hpf in classic MSI-H histology group were >4 in 58 tumors, 2-4 in 30 and <2 in 1. The IEL/hpf in conventional histology group were >4 in 8 tumors, 2-4 in 12 and <2 in 15. Details of adjuvant therapy, recurrence status, and DFS were obtained from the medical record. The statistical analysis was performed with Chi-square/Fisher's exact test and Cox proportional hazards models.
**Results:** CRCs with classic MSI-H histology were right sided (P=0.03), had higher T(P<0.01), N(P<0.01),and M(P=0.01) stage at time of surgery and higher 5 year recurrence with stage I-III patients (P=0.04) than did CRCs with conventional histology with or without IEL. In the univariate analysis, classic MSI-H histology (P=0.02), higher N stage (P=0.02) and age <40 years were associated with worse DFS in stage I-III patients. Using the multivariate Cox proportional hazards model, after adjusting for age, classic MSI-H histology was associated with worse DFS (P=0.02).
**Conclusions:** This study describes a subset of MSI-H CRC with histology similar to MSI-stable CRC, with lower TNM stage, and longer DFS than MSI-H CRC with classic MSI-H histology.

### 592 Tumor Thickness at Tumor-Normal Interface (TNI): A Novel Pathologic Indicator of Chemotherapy Response in Hepatic Colorectal Metastases (HCRM)

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**Background:** Progress in treatment of HCRM demands pathologic indicators of therapy response. Pathologic response;one of the best predictor of disease free survival (DFS) has limitation of low reproducibly among pathologists. Based on observation of majority of residual tumor cells seen at the TNI, we hypothesized that the tumor thickness at the TNI correlate with radiologic and pathologic response and DFS.
**Design:** This study included 119 patients (M/F= 1.8, median age 56 years) with resected HCRM (moderately differentiated adenocarcinoma) following preoperative chemotherapy ± Bevacizumab. Imaging response was assessed by the RECIST criteriae in 50 patients. The pathologic response was categorized as complete (no tumor cells), major (<50% residual tumor cells) and minor (>50% residual tumor cells), as published previously. All H&E sections from the tumors were reviewed by two pathologists and maximum tumor thickness comprised of uninterrupted layers of tumor cells was measured perpendicular to the TNI in millimeter. In tumors where entire section has tumor without stroma, the highest thickness measured on a glass slide was utilized for analysis. The maximum tumor thickness <0.5mm was considered as <0.5mm without additional measurement. For specimen with >1 tumor, average residual tumor and maximum thickness at TNI were used.
**Results:** Seventy-six received oxaliplatin based chemotherapy, 43 received irinotecan based chemotherapy and 90 received Bevacizumab. The imaging response was complete in 14, partial in 32 and progression in 4. The complete pathologic response was seen in 9, major response in 52 and minor response in 58. Median tumor thickness at TNI was 2mm (IQR 0.5 to 6mm). The tumor thickness correlated with pathologic (Spearman r=0.81, p<0.01) and radiologic response (Spearman r=0.37, p<0.01). Cut of thickness of 3mm differentiated minor vs. major/complete pathologic response (sensitivity 0.85, specificity 0.89). Tumor thickness correlated with the DFS as continuous variable in log-transformed analysis and lower thickness predicted better DFS (p=0.02). In the Cox regression analysis tumor thickness was a better predictor of DFS than pathologic response. The tumor thickness did not correlate with the type of cytotoxic chemotherapy, but was smaller in patients treated with bevacizumab (p=0.04).
**Conclusions:** Tumor thickness measured at TNI is potentially a new prognostic factor for therapy response and survival outcome in patients with HCRM.

### 593 Evidence That Dysplasia Begins in the Bases of the Pits in the Pathogenesis of Gastric Cancer

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**Background:** Intestinal-type gastric cancer is believed to develop via an intestinal metaplasia-dysplasia-carcinoma pathway. Anecdotally, we have noted that dysplasia-like atypia may be limited to the deep pit epithelium, without surface epithelium involvement, particularly in the stomach of patients with gastric cancer. We hypothesized that this type of epithelial alteration may represent an early form of dysplasia [Pit Dysplasia (PD)]. The aim of this study was to evaluate the clinical, pathologic, and biologic features of PD in an attempt to determine if it is a significant precursor to gastric cancer.
**Design:** Routinely processed tissue sections from 102 randomly selected resection specimens from patients with gastric cancer (mean age 67; M/F ratio 1.6), and from 22 patients with chronic gastritis (mean age 56.8; M/F ratio 1.8) but without cancer (controls), were evaluated for a wide variety of gross and microscopic features. A subset of 30 study cases were also immunostained for Ki67, E-Cadherin, and p53, and evaluated for the presence and degree of positivity in areas of intestinal metaplasia (IM), PD, and carcinoma.
**Results:** PD was present in 50/102 (49%) study patients compared to only 9% of controls (p<0.05). Patients with PD showed an older mean age at diagnosis (71 vs. 64 years, p=0.02), but a similar M/F ratio compared to patients without PD. Pathologically, gastric cancers with PD showed a significantly increased proportion of intestinal-type adenocarcinomas (82% vs. 37%, p<0.01), a higher degree of tumor differentiation (p<0.01), lower overall pathologic stage (p=0.04), an increased association with chronic gastritis (p<0.01) and a significantly higher proportion of cases with IM (40% vs. 13%, p<0.01) and conventional (full pit) dysplasia (44% vs. 4%, p<0.01) compared to study cases without PD. PD was situated adjacent to neoplasia in 72% of cases (low-grade: 24%, high-grade dysplasia: 26%, carcinoma: 66%) and distant from the neoplasia in 28%. The degree of Ki67 and p53 staining increased progressively, and significantly, in areas of IM, PD, and cancer, whereas E-Cadherin staining decreased.
**Conclusions:** Dysplasia-like changes limited to the deep portions of the pits, without surface epithelium involvement, probably represents an important histologically identifiable precursor to gastric cancer. Further prospective biopsy studies are needed to determine the risk of neoplastic progression in patients with PD detected in mucosal biopsy specimens.