1757 Identification of Ocular Sebaceous Neoplasia with Evaluation for Mismatch Repair Proteins

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Background: Recognizing sebaceous carcinoma is important because of its risk for metastasis and tumor death. Histopathology alone is often difficult because fresh tissue for oil red-O stain is not always available. Several immunohistochemical stains (IHC) have been evaluated but often the diagnosis is elusive. Furthermore, the diagnosis of a sebaceous adenoma or carcinoma raises the possibility of Lynch syndrome, a cancer predisposition syndrome. Mismatch repair protein(MMR) abnormalities are a feature of Lynch syndrome, and they can be detected with IHC for the MLH1, MSH2, PMS2, and MSH6 proteins.

Design: 14 sebaceous carcinomas and 3 adenomas from the eyelid (17 specimens) from 14 patients were evaluated for oil red-O when possible as well as IHC for AR, EMA, BER-EP4, CAM5.2 for diagnosis. MMR for MLH1, MSH2, PMS2, and MSH6 by IHC were evaluated as a possible screening panel for Lynch syndrome. Two patients already had other tissue tested by PCR for microsatellite instability (MSI) for Lynch syndrome.

Results: Oil red-O was done on 6 of 17 specimens and positive in 5 of the 6 (83%). AR was positive in 2 of 3 adenomas (67%) and 5 of 14 carcinomas (36%). EMA was positive in 2 of 3 adenomas (67%) and 11 of 14 carcinomas (79%). BER-EP4 was negative in all 3 adenomas and positive in 7 of 14 carcinomas (50%). CAM5.2 was positive in 1 of 3 adenomas (33%) and 8 of 14 carcinomas (57%). All 17 specimens(100%) showed positive nuclear staining for MLH1 and PMS2, indicative of normal protein expression. In 14 of 17 specimens from 12 patients, MSH2 and MSH6 were also normal. 3 specimens from 2 patients that had rare positive cells for MSH2 and MSH6. Both patients had previous colonic tumors tested for MSI and were found to be MSI-H, indicative of possible Lynch syndrome.

Conclusions: Diagnosing sebaceous carcinoma is important prognostically but sometimes challenging with routine histopathology. In our experience androgen receptor (AR) was found to be confirmatory in only 36% of cases and not dependent on the size of the tumor available. The other IHC stains were of marginal benefit and not specific. While none of the cases showed complete loss of expression of a MMR protein, there were 2 patients in with only rare positive cells who had known MSI-H tumors in the colon, suggestive of Lynch syndrome. Additional studies of these eyelid tumors may elucidate the possible role of using them for screening for Lynch syndrome.

Pancreas

1758 International Consensus Study on the Terminology and Diagnosis of Tumoral Intraepithelial Neoplasms ("Adenomas" and "Intracystic Papillary Neoplasms" of WHO-2010) of the Gallbladder

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Background: The 7 types of tumoral intraepithelial neoplasms (TINs) of the gallbladder (GB) originally recognized by Dr.Albores-Saavedra in the AFIP fascicle, were recently regrouped in the WHO-2010 under 2 major categories of adenoma vs. intracystic papillary neoplasm.

Design: 28 selected examples of TINs were evaluated on a digital platform by 18 authors from 9 countries.

Results: There was overwhelming consensus that these were neoplastic lesions (except 1 case), and that they were distinct from conventional (non-tumoral) dysplasia. There was fair agreement on the degree of dysplasia and the presence of invasive carcinoma (κ: 0.36 and 0.35, respectively). However, the terminology was highly problematic. The number of different diagnostic names used per case ranged 4-13 (median 8), and in each case at least half of the authors did not use the WHO terminology, some citing the potential misunderstanding of the term intracystic giving the impression of a tumor arising within a cyst. Furthermore, when forced to use the WHO, there was no agreement on specific subcategories (κ: 0.01-0.19), and even more importantly, agreement on placing the cases into adenoma vs. intracystic papillary neoplasm categories was fairly poor (κ: 0.23), with >25% of the evaluators disagreeing with the others in 43% of the cases. The cell lineage, one of the bases of the WHO classification, also had poor agreement (κ: 0.17) as assessed in routine histology alone.

Conclusions: There is consensus on the neoplastic nature of GB-TINs, and fair agreement on the grade of dysplasia and invasiveness. However, as in TINs of pancreas and bile ducts, the growth patterns and cell lineages show significant overlaps, leading to great subjectivity and frequently precluding the reproducible application of WHO classification. A unifying terminology is needed, emphasizing their analogy with pancreatobiliary counterparts.

1759 Growth Patterns of High-Grade Gallbladder Dysplasia: Clinicopathologic Associations and Diagnostic Implications in an Analysis of 318 Cases

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Background: The diagnosis of dysplasia in the gallbladder (GB) is a well known challenge. Meanwhile, there is virtually no data on the growth patterns of high-grade dysplasia (HGD) of GB, and their diagnostic and clinical significance.

Design: 318 cases with unequivocal conventional (non mass-forming) HGD, 189 with accompanying invasion, were analyzed.

Results: Various growth patterns were recognized and often occurred in a mixture: Flat/ondulating in 82%, tubular- 66%, micropapillary/tufting-52%, denuding/clinging-19%, tall-papillary-22%, urothelial-like 1%, acantolytic-1%. When analyzed based on the predominant pattern(**also see table**): Tall papillary was commonly associated with invasion (88%; p= 0.001) and the worst clinical outcome, with 5-yr survival of 25%. Denuding/clinging had a similar trend but not statistically significant. In contrast, flat type had the best survival (66%, p=0.002). Hyalinizing cholecystitis ("incomplete" porcelain) cases (n=35) typically had either denuding/clinging or flat patterns, and almost never tall papillary or tubular. Tubular pattern was often difficult to distinguish from invasion, in particular, the foamy-gland, foveolar and attenuated cell variants.

Variables (Units)	Denuding (n=12)	Flat (n=190)	Glandular/ Tubular (n=51)	Micro-papillary (n=32)	Tall Papillary (n=33)	p-value
Age, mean (±SD), year	68.2 (7.4)	59.7 (14.9)	66.7 (12.9)	62.6 (11.4)	68.0 (9.8)	**0.001**
Male / Female	2 (17%) / 10 (83%)	37 (21%) / 142 (79%)	8 (18%) / 35 (78%)	8 (27%) / 22 (73%)	9 (27%) / 24 (73%)	0.1139
Presence of Invasion	10 (83 %)	91 (48%)	38 (75%)	21 (66%)	29 (88%)	**<0.0001**
Invasion Size, mean (±SD), mm	28.9 (16.1)	32.0 (23.0)	15.7 (14.4)	24.8 (19.2)	22.4 (17.0)	**0.0053**
Stage of Invasive Tumor						
T1	0	10 (12%)	4 (12%)	2 (10%)	3 (11%)	0.9223
T2	8 (80%)	41 (50%)	20 (59%)	10 (48%)	14 (50%)	
T3	2 (20%)	31 (38%)	10 (29%)	9 (43%)	11 (39%)	
Survival Rates						
1-year	50%	76 %	88%	69%	68%	**0.002**
3-year	25%	70%	65%	54%	30%	
5-year	25%	66%	58%	54%	25%	
Median Survival (mos)	18	N/A	N/A	N/A	19%	

Conclusions: HGD of GB occurs in various growth patterns. Tall papillary examples are significantly more commonly associated with higher frequency of invasive carcinoma and adverse outcome. Clinging/denuding pattern also appears to be aggressive, is common in hyalinizing cholecystitis and needs to be carefully searched for. Tubular examples often mimic invasive carcinoma but are more indolent in behavior. Recognition of these patterns would allow accurate diagnosis and guide more targeted pathologic examination and prognostication.

1760 High-Grade Neuroendocrine Carcinomas of the Pancreas: A Clinicopathologic Analysis of 60 Cases

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Background: In the pancreas, data regarding the types and significance of high-grade neuroendocrine carcinoma (HGNEC) has been very limited.

Design: Ninety-three cases, biopsied or surgically resected at 12 different institutions from 1988-2012, that had been diagnosed as HGNEC were reassessed with the morphologic criteria employed in the lung as well as IHC labeling with NE (chromogranin/synaptophysin) and acinar (trypsin/chymotrypsin) markers. Thirty-three were excluded, most being reclassified as acinar cell carcinoma (ACC). Remaining 60 were assessed as HGNEC for clinicopathologic features and survival outcomes.

Results: The mean age of patients qualified as HGNEC was 58 (range, 27-84). M/F ratio was 1.2. Increased serum hormone levels were present in only 3 (5%) patients (insulin in 2, VIP in 1). Sixty percent of the tumors were localized in the head of the gland, 10% in the body and 30% in the tail. Median size was 4.5 cm (range, 2.3-20). The majority (92%) of the tumors was pure NEC, 38% of which were small cell type. There was an associated adenocarcinoma component in 4, and an IPMN in 1. The incidence of vascular and perineural invasion were 71% and 62%, respectively. In 55%, one or more, usually the retroperitoneal, surgical margin was positive. Sixty-three percent of the patients had metastatic disease at presentation and an additional 28% subsequently developed metastases, usually to the regional lymph nodes and liver. Follow-up (F/U) information was available in 37 (62%) patients. 26 died of disease, with a median survival of 11 mos (range, 0-77); 11 patients were alive with disease, with a median F/U of 17 mos (range, 0-50). The median survival for all cases was 15.1 mos, with 5-yr survival of 17%.

Conclusions: Our data demonstrates that ACC often get misdiagnosed as HGNEC and strongly suggests that thorough immunohistochemical evaluation be performed before HGNEC diagnosis is rendered. HGNECs of the pancreas is a highly aggressive neoplasm, with frequent metastases and poor survival. Most patients die within less than a year. About 40% of the tumors are of small cell type and the majority of the tumors are non-functional.

1761 Increased (>20%) Ki67 Proliferation Index in Morphologically Well Differentiated Pancreatic Neuroendocrine Tumors (PanNETs) Correlates with Decreased Overall Survival

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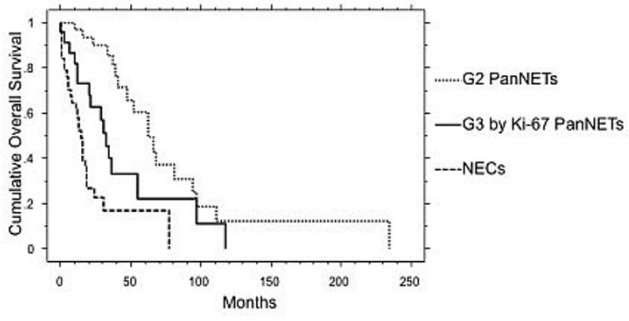
Background: By WHO classification, PanNETs should be graded by mitotic rate and Ki67 proliferation index, with grade (G)2 defined as a mitotic rate of 2-20 mitoses/10 HPF or a Ki67 index of 3-20%. G3 is defined as a >20 mitoses/10 HPF or a Ki67 index of >20%. Given the fact that some PanNETs show discordance between mitotic rate and Ki67 index, we performed Ki-67 immunohistochemical labeling on well differentiated (WD) mitotic G2 PanNETs in our institutional databases and examined clinicopathologic features and survival outcomes.

Design: Morphologically WD PanNETs biopsied or surgically resected at our institutions from 1999-2012 were reviewed. The percentage of Ki67 positive cells was quantified either using image analysis or manual counting on camera-captured/printed images of Ki67 hot spots. Survival outcome was determined using the Social Security Death Index. For comparison, 39 morphologically poorly differentiated (either small cell or large cell type) neuroendocrine carcinomas (NECs) were retrieved, and similarly assessed for proliferation and survival.

Results: Of 65 mitotic G2 PanNETs, 23 were G3 based on Ki67 index (median=30%, 21%-65%). Overall, patients with grade-discordant PanNETs had a survival time shorter than grade-concordant G2 PanNETs (p=0.01) but longer than patients with poorly differentiated NECs (p=0.017), with grade-discordant tumors showing median survival of 32.3 mos and 5-yr survival of 22.1% (vs 62.7 mos and 60.5% for grade-concordant G2 PanNET, and 15.1 mos and 16.8% for NEC).



Conclusions: Our data support the notion that the mitotic rate and Ki67 index-based grades of PanNETs can be discordant, and when the Ki67 index is greater, the clinical outcome is significantly worse. We further demonstrate that WD PanNETs that are G3 by Ki-67 are still different from bona fide NECs, suggesting the current G3 can be further separated into G3 (WD PanNET with increased proliferation index) and poorly differentiated NEC.

1762 Histologic Changes in Non-Neoplastic Pancreas after Neoadjuvant Therapy for Pancreatic Ductal Adenocarcinoma

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Background: Neoadjuvant chemoradiation therapy (NCRT) has been increasingly used to treat patients with pancreatic ductal adenocarcinoma (PDAC). However, the histologic changes in non-neoplastic pancreatic parenchyma after NCRT have not been examined in detail.

Design: We retrospectively reviewed the archival H & E slides from 218 patients with PDAC who completed NCRT and underwent pancreatectomy at our institution. Seventy-five patients who underwent pancreatectomy for PDAC without NCRT were used as the control group. All the cases were reviewed by two pathologists for the presence of pancreatic intraepithelial neoplasia (PanIN) of different histologic grades; "neuroma-like" nerve proliferation; islet cell hyperplasia; pancreatic fibrosis (none, mild, moderate or severe) and inflammation (none, mild or moderate to severe). The neuroma-like nerve proliferation was defined as haphazardly dispersed, hypertrophic nerve bundles within fibrous stroma in the peripancreatic tissue, unrelated to perineural invasion. The islet cell hyperplasia was defined as increase in the number and size of the existing islets, as well as linear and anastomosing cords segueing into ductal formations. Data analyses were performed using Chi-square analysis or Fisher's exact tests with two-sided significance level of 0.05.

Results: Among the 218 patients who received NCRT, none, mild, moderate and severe fibrosis were present in none, 4.6%, 68.4% and 27.0% patients respectively compared to 18.7%, 40.0%, 32.0% and 9.3% patients in the control group (p=0.01). Neuroma-like nerve proliferation and islet cell hyperplasia were present in 34 (15.6%) and 79 (36.2%) patients respectively in the NCRT group, compared to 5.3% and 12.0% respectively in the control group (P=0.02 and P<0.01, respectively). Moderate to severe inflammation were present in 15 (20%) patients in control group compared to 12 (5.5%) patients in the NCRT group (p=0.01). However, we did not observe significant difference in the frequencies of PanIN lesions of any histologic grade (grades I, II and III in 84%, 72.5% and 37% respectively in the NCRT group, and 85%, 76% and 39% respectively in the control group; p>0.05).

Conclusions: This study demonstrates that patients who received NCRT have more pancreatic fibrosis, higher frequencies of neuroma-like nerve proliferation and islet cell hyperplasia, but less frequent moderate to severe inflammatory infiltrates than those who did not received NCRT. However there is no significant difference in the incidence of PanIN lesions between these two groups.

1763 Nuclear Accumulation of ß-Catenin in Well-Differentiated Pancreatic Neuroendocrine Tumors from Patients with Familial Adenomatous Polyposis

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Background: Although rare, pancreatic neuroendocrine tumors (PanNETs) are the second most common malignancy in the pancreas. Aberrant expression of β-catenin has been described in a number of solid tumors, including solid pseudopapillary tumor of the pancreas. In the current study, we examined the expression of β-catenin in PanNETs.

Design: Two tissue microarrays (TMAs) were constructed using well-differentiated PanNETs from 54 patients who received a Whipple procedure or distal pancreatectomy and/or hepatectomy for primary and metastatic PanNETs during 01/2002-12/2009. Immunohistochemical stains for β-catenin, E-cadherin, chromogranin and synaptophysin were performed on the TMAs. Pathology reports and clinical history were also reviewed.

Results: A genetic syndrome was identified in 11 of 54 (24%) patients, including 6 with multiple endocrine neoplasia type 1, 3 with von Hippel-Lindau disease, and 2 with familial adenomatous polyposis (FAP). Fifteen of them (28%) were functional tumors, including 9 insulinomas, 4 gastrinomas, 1 glucagonoma, and 1 adrenocorticotropic hormone-secreting tumor. The majority of the tumors had moderate to strong membranous β-catenin labeling (48/54, 89%) of which two (4%) also had strong nuclear staining. Of the six that had loss of membranous staining, one showed focal nuclear accumulation. All six cases with loss of membranous staining also displayed loss of E-cadherin expression, whereas all other tumors had intact E-cadherin labeling, including the 2 cases with β-catenin nuclear accumulation. Both patients with FAP had a PanNET showing nuclear accumulation of β-catenin, and liver metastasis was their first presentation. The primary tumor was unresectable for one of the FAP cases, who also had numerous colonic adenomas and multiple colon cancers discovered soon after his diagnosis of PanNET at 61 years of age. The resected liver metastasis showed a well-differentiated neuroendocrine tumor with <2 mitoses/10 HPFs. The second patient was a 44 year old female, who had a known history of FAP and had previously received a total colectomy. The primary was resected which showed a 2 cm well-differentiated PanNETs but with frequent mitoses (focally >20 mitoses/10 HPFs).

Conclusions: Nuclear accumulation of β-catenin can be seen in a small set of PanNETs, which may be related to FAP. PanNETs may be one of the extracolonic presentations in patients with FAP.

1764 Depletion of Pancreatic Intranuclear Rodlets in Patients with Type 2 Diabetes

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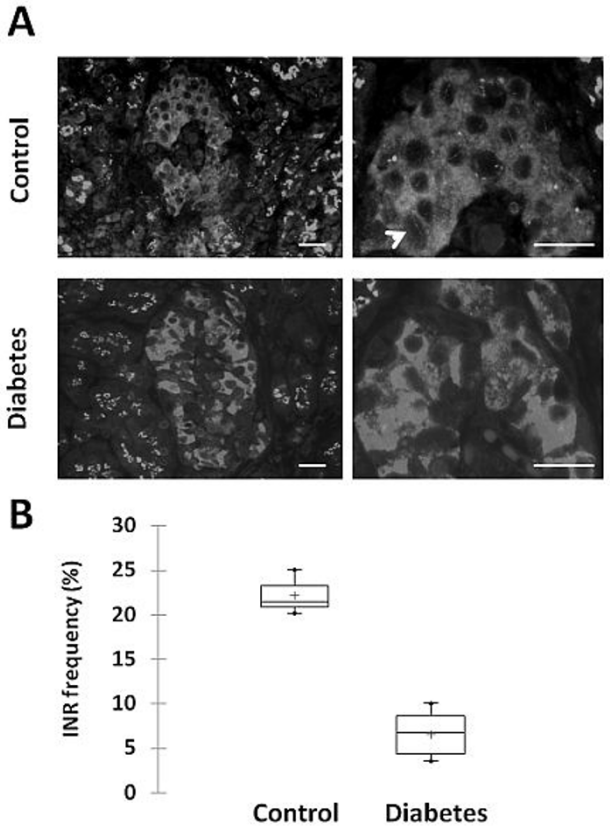
Background: Intranuclear rodlets (INRs) are rod-shaped intranuclear inclusions present in neurons of the human brain. We have demonstrated that neuronal INRs are markedly depleted in the Alzheimer Disease and current investigations are exploring the role of INRs in the pathogenesis of neurodegeneration. We also identified these structures in B cells of the pancreatic islets and recently demonstrated the depletion of pancreatic INRs in the mouse models of type 2 diabetes. The objective of this study is to compare the frequency of pancreatic B cells INRs in diabetic and control patients.

Design: Formalin-fixed, paraffin-embedded blocks of normal human pancreas from twelve patients who underwent Whipple procedure were selected as follows: seven cases from confirmed type 2 diabetes patients and five cases from non-diabetic patients as controls. Double immunofluorescence staining was performed on 5um pancreatic tissue sections with anti-class III A tubulin (C3T) to detect INRs, and anti-insulin to detect the pancreatic B cells. Fluorescent images of pancreatic islets (10 per each section) were acquired and manual counts of B cell nuclei and INRs containing nuclei were performed.

Results: As represented in Figure 1A, the proportion of B cell containing INRs was significantly reduced in diabetic patients (median 6.8%; SD 2.6; range [3.6% – 9.8%]) compared with control patients (median 21.5%; SD 2.5; range [20.2% – 25.1%]); scal bar at 20um. The box plot diagram (Figure 1B) shows the differential of INRs frequency in diabetes and control groups (p<0.0001).



Conclusions: The results of the present study represent the first report of INRs depletion in human pancreatic B cells of type 2 diabetes patients. In light of the considerable evidence for B cell dysfunction in the pathogenesis of type 2 diabetes, it will be of interest to investigate the role of INR depletion in the "functional degeneration" of pancreatic B cells.

1765 *GNAS* Mutations in Concomitant Pancreatic Ductal Adenocarcinoma: A Pilot Study

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Background: Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a cystic precursor lesion of infiltrating pancreatic adenocarcinoma. *KRAS* mutations occur frequently in both IPMN and pancreatic intraepithelial neoplasia (PanIN). Recent reports demonstrated that *GNAS* mutations are prevalent in IPMN, especially in the intestinal type (IPMN-I), as well as invasive carcinoma derived from IPMN (invasive IPMN), but *GNAS* mutations are rare (up to 7%) in PanIN lesions and are absent in conventional pancreatic ductal adenocarcinoma (PDAC) that does not have any association with IPMN. However, *GNAS* mutations have not been evaluated in PDAC that is seen as a separate focus in the pancreas with IPMN (concomitant PDAC).

Design: The study cohort consisted of 23 IPMN lesions from 23 subjects consisting of 5 IPMN-I lesions (2 branch-duct and 3 mixed types; all intermediate-grade dysplasia) and 18 gastric-type IPMNs (IPMN-G; all branch-duct type; low- to intermediate-grade dysplasia). The latter included 4 with concomitant PDACs and 1 with invasive IPMN. *GNAS* and *KRAS* mutation assays using a SNaPshot platform were performed in 23 intraductal components and 5 invasive carcinomas.

Results: *GNAS* mutations were identified in 10 (56%) IPMN-G, and 4 (80%) IPMN-I, and *KRAS* mutations in 15 (83%) IPMN-G and 1 (20%) IPMN-I. Nine (50%) IPMN-G and 1 (20%) IPMN-I harbored concurrent *KRAS* and *GNAS* mutations. Interestingly, 2 concomitant PDACs harbored concurrent *KRAS* and *GNAS* mutations while the corresponding IPMN lesions only showed *KRAS* mutations. One concomitant PDAC harbored a *KRAS* mutation only while the corresponding IPMN showed both *KRAS* and *GNAS* mutations. The remaining concomitant PDAC and invasive IPMN and their corresponding intraductal components only showed *KRAS* mutations.

Conclusions: The results of this pilot study confirm that *GNAS* mutations are more prevalent in IPMN-I than in IPMN-G, and suggest that the genetic signature of concomitant PDAC may have overlap with that of IPMN and may be different from that of conventional PDAC (without any association with IPMN). A larger scale study is underway.

1766 Mesothelin Expression in Pancreatic Mucinous Cysts

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Background: Mesothelin (MSLN) is a differentiation antigen found to be overexpressed and a potential treatment target in pancreatic ductal adenocarcinomas. There is little to no MLSN expression in normal pancreas or chronic pancreatitis and MSLN is not detected in gastric or duodenal epithelium. The expression of MSLN in pancreatic neoplastic mucinous cysts is largely not investigated. The aim of this study was to determine the frequency of MSLN expression in neoplastic epithelium of mucinous pancreatic cysts.

Design: Fifty-four well-characterized cases of pancreatic neoplastic mucinous cysts were identified in our institutional pathology archive (2008-2011) including intraductal papillary mucinous neoplasm (IPMN) and mucinous cyst adenoma (MCN). Immunohistochemical analysis of MSLN was performed on a representative formalin-fixed paraffin embedded sections from each case using standard immunohistochemical protocols. MSLN expression was considered positive with either membranous or cytoplasmic staining and was recorded semiquantitatively in mucinous cysts, adjacent pancreatic ducts and bile duct, and duodenal and gastric foveolar epithelium as follows: 1+ (1- 25% positive cells), 2+ (26-75% positive cells) as and 3+ (>76% positive cells). A 2-tailed Fisher's exact test was used to determine statistical significance.

Results: MSLN expression was noted in the epithelial cytoplasm as well as on the cell surface. MSLN expression was seen more frequently in neoplastic epithelial cells from IPMN (33/41; $p< 0.0001$) and MCN (8/13; $p < 0.0005$) in comparison to expression in unremarkable adjacent pancreatic and bile ducts (8/58). When present, MSLN expression was only focally and weakly expressed in unremarkable pancreatic and bile ducts. There was no significant difference ($p = 0.26$) in MLSN expression between IPMN (81%) and MCN (62%). MSLN expression >2+ was significantly more frequent in IPMN (27/41) than in MCN (3/13), $p = 0.01$. No MSLN expression was seen in duodenal or gastric foveolar epithelium.

Conclusions: Our findings suggest that MLSN expression can be used as a marker of neoplastic transformation of epithelial cells in pancreatic mucinous cysts. While the biologic significance of MSLN in these neoplasms remains unclear, the findings could help distinguish neoplastic mucinous epithelium from gastrointestinal tract contamination on endosonography guided fine needle aspiration samples from pancreatic cysts.

1767 Neoplasms and Pseudotumors Associated with Low (Intrapancreatic) Union of Cystic Duct into Common Bile Duct: A Clinicopathologic Analysis of 15 Cases of a Hitherto Unrecognized Phenomenon

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Background: Anatomic variations and physical anomalies of biliary tract such as anomalous union of ducts or choledochal cysts have been established as risk factors for cancer, believed to be due to abnormal mucosal exposure to different mileu and subsequent carcinogenesis with different biologic/molecular properties. Over the years, we have encountered a previously uncharacterized anatomic variant, low (intrapancreatic) union of cystic duct into common bile duct (CBD), associated with various pathologic conditions.

Design: 15 cases with low union detected prospectively by the authors during routine practice were analyzed.

Results: The patients were 8 female, 7 male; 9 Caucasian, 4 African American, 2 unknown; Mean age= 62 (42-80). 5 cases had history of cholecystectomy, suggesting associated secondary problems in the gallbladder. 1 had diverticulum at the ampulla (suggestive of a conjoint anomaly). All patients underwent pancreatoduodenectomy with the diagnosis of a mass and the clinical impression of "cancer". 5 were classifiable clearly as CBD carcinomas (otherwise only 5% of Whipples in the authors' experience), 5 pancreatic ductal adenoca, 2 ampullary ca, 1 pancreatic neuroendocrine neoplasm and 2 proved to be pseudotumors (chronic pancreatitis forming pseudo-mass). In 6 cases, the pathology was localized immediately distal to the junction of these ducts. The median survival of the 13 cases with carcinoma was 7 mos. The median size of invasive carcinoma was 3.5 cm. The carcinomas were ordinary pancreatobiliary type. The two pseudotumors were also of focal type and largely localized to the groove region, suggesting a mechanistic relationship to the low union anomaly.

Conclusions: We hereby document a hitherto unrecognized anatomic variation in which the cystic and common hepatic ducts adjoin each other within the pancreas, which we propose to refer to as "low union". Low union cases can be associated with pancreatobiliary adenocarcinomas, as well as pseudotumors, often arising identifiably in this anomalous junction.

1768 Trypsin Expression in Pancreatic Neuroendocrine Carcinomas: Potential Diagnostic Pitfall and Prognostic Marker

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Background: Trypsin immunohistochemical stain is considered a specific marker for acinar cell carcinoma of pancreas. However, in our consultation practice, we have seen cases of metastatic pancreatic neuroendocrine carcinoma (PNECs) which label strongly and diffusely with chromogranin and synaptophysin showing focal trypsin expression. The significance of focal trypsin positivity in PNEC is unknown. In this study, we sought to determine the clinicopathologic characteristics of PNEC with focal trypsin expression and compared them to those without trypsin expression.

Design: We retrieved 132 PNECs from our database and obtained clinical and follow up data. Immunohistochemistry for trypsin was performed. For each trypsin positive case, a PAS-Diastase stain was done and confirmatory electron microscopy (EM) was performed on a single case. Trypsin–positive cases were compared to trypsin-negative

cases for differences in survival and other clinicopathologic features. Available metastases associated with trypsin-positive primary tumors were also evaluated with the trypsin stain.
Results: Of the 131 cases of PNEC, 9 (6.8%) showed focal trypsin expression in tumor cells. There were 8 men and 1 woman with a median age of 58 years. The trypsin-positive cases all had some cells with PAS-D positive cytoplasmic granules, and the single case assessed by EM revealed tumor cells with zymogen granules. Four of 5 metastases tested also showed focal trypsin positivity. The trypsin-positive tumors were functional in 6 of 7 cases, the median Ki-67 was 3.3% (range 1.4-7) and they were associated with liver metastases in 7 of 9 cases. By comparison, trypsin-negative tumors were functional in 56% of cases, had a median Ki-67 of 2.5% (range 0-25) and were complicated by metastases in 48%. Follow up data were available for 129 of 131 cases. At 5 and 10 years, tumor specific survival for patients with trypsin-positive tumors were 38% and 38% compared to 85% and 56% for those with trypsin-negative tumors (log-rank test p=0.035).
Conclusions: Rare PNEC may show focal trypsin positivity, which can be a potential diagnostic pitfall when evaluating small biopsies. We do not consider these tumors a type of mixed acinar-neuroendocrine carcinoma because of very focal trypsin expression in a background of nearly 100% cells positive for neuroendocrine cell markers. Still, when compared to trypsin-negative tumors, these tumors seem to have more aggressive clinical behavior. We hypothesize that trypsin expression in PNEC may represent an exocrine phenotype which portends a worse prognosis.

1769 EGFR Expression in Pancreatic Adenocarcinoma. Relationship to Tumor Biology and Cell Adhesion Proteins

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Background: In pancreatic ductal adenocarcinoma (PDAC), EGFR tumor expression has been reported to relate to an adverse outcome although with less relevance than VEGF, bcl2 bax or p16. The underlying mechanism is incompletely understood, possibly intricated since EGFR in PDAC relates to advanced tumor stage and metastases, to histological dedifferentiation and to cell proliferation proteins such as PCNA and cyclin D1.
Design: We aimed to study EGFR expression in surgically resected pancreatic ductal adenocarcinomas by immunohistochemistry and the relationship to clinicopathological features, cell proliferation and cell adhesion protein expression. A total of 99 PDAC were analysed on tissue microarrays for EGFR, E-cadherin, beta-catenin expression patterns in tumour cells. The percentage of cells expressing the three proteins (membrane, cytoplasmic, or nuclear pattern) and of Ki67 positive tumor cells was assessed. Tumour protein expression was studied with regard to clinicomorphological features, Ki67 index and for postsurgical survival.
Results: Membrane tumor EGFR correlated to histological dedifferentiation (poor differentiation), increased number of mitoses and severe tumor cell pleiomorphism (Fisher $p<0.01$, $p=0.05$, and $p=0.03$ respectively) as well as to aberrant adhesion protein expression such as nuclear beta catenin and cytoplasmic E-cadherin (Kendall $p<0.01$ tau 0.230 and $p<0.01$ tau 0.216). Cytoplasmic tumor E-cadherin correlated to a high Ki67 positive tumor cell component (Kendall $p<0.01$ tau 0.263) while nuclear E-cadherin to a shorter postsurgical overall survival (logrank $p<0.01$) as well as tumor necrosis and presence of an abundant clear cell component (logrank $p<0.01$ and $p=0.03$).
Conclusions: The results of our study suggest a complex role for EGFR in PDAC carcinogenesis, tumor expression of this protein being associated to tumor dedifferentiation, mitotic activity or pleiomorphism, as well as to aberrant tumor cell adhesion protein expression.

1770 Geographic Differences of Frequency and Associations with Invasive Carcinoma in the Two Distinct Carcinogenetic Pathways (Conventional Dysplasia Versus Tumoral Intraepithelial Neoplasia) in the Gallbladder

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Background: It is now well established that there are 2 distinct types of the intraepithelial neoplasm (IN) recognized in various organs including the gallbladder (GB): One, represented as conventional dysplasia (flat, non-tumoral IN; or FIN) and the other tumoral type, ("adenoma" or "intracystic papillary neoplasm and carcinoma" in WHO-2010, or as recently proposed intracholecystic papillary-tubular neoplasm-ICPN [in AJSP, 2012]). There was no data in the literature on the geographic differences (East vs West) of FIN vs ICPN in GB carcinogenesis.
Design: We searched total 30,249 cholecystectomy cases from Korea (10,028, Japan (2,198), USA (7,069) and Turkey (10,954) and collected those had the diagnostic terms of "dysplasia, adenoma, papillary tumor or neoplasm". We compared the frequency and relative proportion of the two precursor lesions (FIN vs. ICPN), as well as the frequency of associated invasive carcinoma between the East (Korea, Japan) and the West (USA, Turkey).
Results: I. Although the overall frequency of all IN (regardless of FIN or ICPN) was higher in the East (3.8%) than the West (0.7%), the INs in the East were predominantly of ICPN type (53%; 245/467), whereas the ICPNs constituted only 26% in the West ($p=0.00$), with the remaining 74% being FINs. II. Invasive carcinomas were more commonly accompanied by FIN than ICPN in both the East and the West: 73% of the invasive cases of the West, and 80% of those in the East had an FIN lesion in adjacent GB, as opposed to 27% and 20% had ICPN, respectively. Frequency of invasion was more commonly identified in cases with FIN in the East than the West (87 vs. 58 %; $p=0.00$). In contrast, ICPNs appeared to be more commonly associated with invasion in the West than the East (West vs. East: 42 vs. 29%); however this did not reach statistical significance ($p=0.13$).
Conclusions: There are significant geographic differences in the relative incidence of the two distinct types of IN (ICPN vs FIN) in the GB, and their rate of and association with invasive carcinoma. Both the overall incidence of ICPNs and its relative proportion in IN was higher in the East than the West, with the ICPNs constituting 53% of the GB INs in the East and only 26% in the West. In both geographic regions, invasive carcinomas are more closely associated with FINs than ICPNs. However, ICPNs, although lower in relative frequency, may be more prone to advance into invasive carcinoma in the West.

1771 SPARC Expression in Pancreatic Adenocarcinoma: Development of a Robust, Predictive Immunohistochemical Assay and Scoring Method

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Background: SPARC is a glycoprotein involved in the regulation of extracellular matrix deposition and remodeling. Our aim was to develop a robust SPARC IHC assay and scoring method that would be adaptable and reproducible in pathology laboratories worldwide. Overexpression of SPARC assessed by IHC has been associated with poorer prognosis and improved survival in patients with advanced pancreratic carcinoma (PC) treated with nab-paclitaxel plus gemcitabine, an albumin-containing formulation of paclitaxel (Celgene, Summit,NJ), in a single arm phase I/II trial (VonHoff et al., 2012). Another objective was to assess value for predicting response to this therapy using tissue of the phase I/II clinical trial.
Design: Three SPARC antibodies were evaluated on formalin-fixed paraffin-embedded tissues of 50 resected PC (Invitrogen clone ON-1; Novocastra clone 15G12; and Sygma-Aldrich polyclonal). Staining was assessed in tumor and stromal cells. Based on these results, a novel scoring method was developed and 20 samples from the nab-paclitaxel plus gemcitabine phase I/II trial stained using the Invitrogen antibody and a Ventana Benchmark Ultra autostainer by two laboratories (Baltimore and Madrid). Slides were scored using a published method (Infante *et al*., 2007) and a novel method similar to HER2 assessment in gastric carcinomas (Ruschoff J *et al*., 2010) All readers were blinded to each other and to the clinical data.
Results: The Invitrogen antibody had the most intense and specific staining in the carcinoma and stromal cells. Concordance was high (85-95%) for all scoring criteria between the different pathologists. Kaplan-Meier survival analysis of overall survival (OS) using the Infante method showed no significant difference between positive and negative tumors. In contrast, Kaplan-Meier survival analysis of OS according to stromal SPARC expression using the novel scoring method showed significant survival benfit (21.2 vs. 6.1 months) in tumors with SPARC positive stroma.
Conclusions: The Invitrogen antibody produced the most specific stain and was the easiest to interpret. These preliminary results also suggest that SPARC expression analysis using IHC and a novel scoring method is reproducible. This IHC methodology will be used to assess the correlation of clinical outcome with SPARC expression from a randomized Phase 3 trial of nab-paclitaxel followed by gemcitabine versus gemcitabine alone in metastatic PC.

1772 Carcinogenetic Progression of Pancreatic Mucinous Cystic Neoplasm Is of Aggressive Pancreatobiliary Lineage

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Background: Mucinous cystic neoplasm (MCN) is one of the precursors of invasive pancreatic carcinoma. The molecular alterations that take place during the progression of MCNs, however, have not been fully elucidated, with most of the literature focusing on the non-invasive examples.
Design: Immunolabeling of mucin core proteins (MUC1, MUC2, MUC 5AC, MUC6, CDX2), p53 and DPC4 were examined in 40 MCNs, consisting of 9 low-grade (LG), 8 high-grade (HG) and 23 with an associated invasive carcinoma. Mutational analysis for *BRAF* and *KRAS* was performed in 15 (4LG, 6HG, 5 invasive).
Results: Gastric lineage markers (MUC5AC and MUC6) were detected only in the cystic/LG areas and were virtually absent in papillary/HG components or invasion. Intestinal differentiation markers were not detected in carcinogenetic spectrum of MCNs, with the exception of metaplastic goblet cells highlighted by MUC2, and rare areas of cystic/LG components of the tumors showing weak CDX2 labeling. MUC1 (pancreatobiliary lineage marker) was expressed only in advanced lesions [50% (4/8) HG and 92% (20/23) of invasive]. Similarly, p53, if present, was detected only in advanced lesions; 25% (2/8) of HG and 39% (9/23) of invasive carcinoma. DPC4 stain was retained in most MCN cases except for 4 (17%) of invasive carcinoma. Immunophenotypic changes are summarized in Table 1. No *BRAF* mutations were identified, but *KRAS* was mutated in advanced lesions: 5/6 HG and 5/5 invasive (Table 2).

Table 1. Immunophenotypic changes of MCN

	MUC1	MUC2	CDX2	MUC5AC	MUC6	P53	DPC4
Low-grade (n=9)	0	0	0	4/9 (44%)	4/9 (44%)	0	8/9 (89%)
High-grade /CIS (n=8)	4/8 (50%)	1/8 (13%)	1/8 (13%)	0	2/8 (25%)	2/8 (25%)	7/8 (88%)
Invasive ca (n=23)	20/23 (92%)	0	0	2/23 (9%)	1/23 (4%)	9/23 (39%)	19/23 (83%)

Table 2. Mutational study of BRAF and KRAS in MCN

	Low-grade (n=4)	High-grade (n=6)	Invasive ca. (n=5)
BRAF	0	0	0
KRAS	0	5/6	5/5

Conclusions: At immunohistochemical and molecular levels, MCN pathway of carcinogenesis in the pancreas is of the pancreatobiliary lineage, showing similarities to the PanIN pathway and is dissimilar from the IPMN of intestinal or oncocytic types. Thus it is not surprising that the invasive carcinomas in MCNs are almost exclusively of tubular type (not colloid) and have aggressive behavior, as shown recently. *BRAF* mutation is not involved in MCN carcinogenesis.

1773 Deregulated Mucin Carbohydrate Antigens Tn and Sialosyl-Tn: Potential Utility in Fine Needle Aspiration Specimens of Pancreas

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Background: Abnormal levels of glycan antigen Tn and sialylated structure STn have been implicated in the initiation and progression of a variety of carcinomas. However, their impact on pancreatic cancers remains poorly understood. In addition, deregulated protein glycosylation has been well recognized as a common feature of malignancies, but the potential diagnostic utility of Tn and STn in pathologic diagnosis, especially in fine needle aspiration (FNA) cell blocks (CB), has not been clearly addressed. Hence, this study evaluated and compared the expression of Tn and STn in FNAs from different types of neoplasm and non-tumor tissues of pancreas, in an attempt to determine their potential diagnostic applicability.

Design: Tn and STn were evaluated immunohistochemically in 46 pancreatic ductal adenocarcinoma (PDAC), 5 neuroendocrine tumors, and 8 non-neoplastic pancreatic FNA CB. Membranous labeling and granular cytoplasmic staining for Tn or STn (3+ and >10% cells) was considered positive. The data were analyzed by statistical analysis.

Results: Forty-three and forty-one of 46 PDAC CB displayed positive staining for Tn (sensitivity 93.48%; specificity 87.50%) and STn (sensitivity 89.13%; specificity 87.50%), respectively; adjacent non-neoplastic tissue showed either negativity or minimal levels of Tn and STn (P<0.001). Only 12.5% (1/8) of non-tumor specimens showed minimal to moderate Tn or STn expression (focally 2+). Furthermore, no positivity was detected in pancreatic neuroendocrine tumors for either Tn or STn.

Pancreatic FNAs (number)	Tn +++		STn +++	
PDAC (46)	43 (93.48%)		41 (89.13%)	
Neuroendocrine tumors (5)	0 (0%)		0 (0%)	
Non-tumor (8)	1 (12.5%)		1 (12.5%)	
Total (59)	44	P<0.001	42	P<0.001

Conclusions: Tn and STn expression in PDAC is significantly increased, in sharp contrast to their absence in neuroendocrine tumors and non-tumor pancreatic tissue. These results indicate that the levels of Tn and STn are uniquely enhanced in pancreatic malignant epithelial, but not neuroendocrine, neoplasms, and raise the possibility of the future diagnostic and prognostic utility of Tn and STn in PDAC.

1774 Intestinal Markers Are Expressed in Pancreatic Well-Differentiated Neuroendocrine Tumors and Associated with Sclerosing Variant

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Background: Recent literature has shown that cadherin 17 (CDH17) and CDX2 can be used as markers of gastrointestinal adenocarcinoma. However, such markers may not be unique to gastrointestinal tract-derived tumors. A small group of pancreatic well-differentiated neuroendocrine tumors (PanNETs) have a distinctive histology, characterized by a trabecular or trabecular-glandular cellular pattern with interspersed fibrosis and frequent expression of serotonin (sclerosing variant)—features commonly seen in small intestinal neuroendocrine tumors (SINETs). This study utilized tissue microarrays (TMAs) for analysis of CDH17 and CDX2 expression in PanNETs, including the sclerosing variant.

Design: Institutional approval was obtained for this study. Two TMAs containing 51 PanNETs and one TMA containing 29 SINETs were included in the study. Immunohistochemical labeling for CDX2 and CDH17 were performed on the TMAs. Pathology slides were also reviewed. Fisher's exact test was used for comparison.

Results: As expected, the vast majority of SINETs expressed the intestinal markers. Of the 29 SINETs, 27 showed diffuse and strong membranous expression of CDH17 (93%), and 23 showed nuclear expression of CDX2 (79%). All CDX2-positive SINETs were also labeled diffusely with CDH17. Some PanNETs also expressed the intestinal markers: 5 cases (5/51, 10%) expressing both markers, 16 (16/51, 31%) with only CDH17 expression and 3 (3/51, 6%) with only CDX2 expression. Among the 21 CDH17-positive tumors, the stain was diffuse and strong in 9 cases (9/51, 18%). Five of the 51 (10%) pancreatic tumors were sclerosing PanNETs, all of which (100%) had strong and diffuse expression of CDH17 and two (2/5, 40%) expressing CDX2. CDX2 and CDH17 were more commonly expressed in the sclerosing variant than other PanNETs (p<0.05).

Conclusions: Intestinal markers, CDH17 and CDX2, are expressed in a subset of PanNETs. Sclerosing PanNETs are associated with strong and diffuse expression of CDH17. CDH17 and CDX2 may not be used as markers to differentiate PanNETs from SINETs in patients with unknown primary NETs.

1775 Validation Study for T Classification of Distal Bile Duct Carcinoma According to Depth of Invasion

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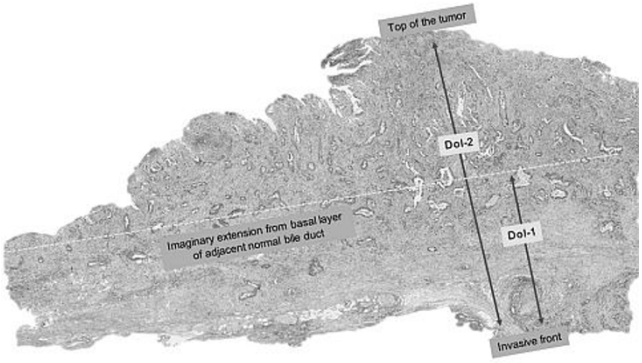
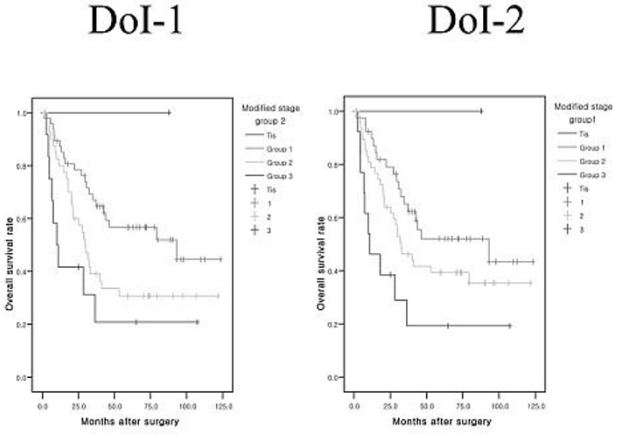
Background: In current AJCC staging system, T stage of distal bile duct carcinoma is classified according to tumor extent within or beyond the bile duct wall. However there are many cases those are difficult to clearly differentiate tumor extent within or beyond the bile wall because desmoplastic stromal reaction of invasive carcinoma obscures the boundary of bile duct wall. Alternative T stage by depth of invasion (DoI; T1: <5 mm, T2: 5 to 12 mm, and T3: >12 mm) has been proposed by Hong et al. In this study we validated new T stage by DoI in distal bile duct cancer.

Design: We evaluated the DoI in 101 cases of distal bile duct carcinoma with ruler tool of digital scan image. DoI was measured in two methods. First, DoI-1 was defined as distance between the basal lamina of the adjacent normal bile duct mucosa to the most deeply invasive tumor cells according to previously described by Hong el al. Second, DoI-2 was measured as distance from top of tumor to the most deeply invasive tumor cells except for intraductal papillary neoplasm.



Both data of DoI-1,2 were compared patient's survival. We also analyzed 101 cases of distal bile duct carcinoma by current T classification of 7th AJCC staging.

Results: T stage of current AJCC staging system showed poor correlation with patient's survival (p=0.157). However both new T classification by DoI-1,2 showed good correlation with patient survival with statistical significances (p=0.009 in DoI-1) (p=0.017 in DoI-2).



Conclusions: On the basis of the present data, we think that new T classification by DoI using cut-off value of 0.5 and 1.2 cm, which is measured from the basal lamina of the adjacent normal bile duct or top of tumor to the most deeply invasive tumor cells, is more appropriate T classification for distal bile duct carcinoma that showed good correlation with patient's survival. DoI could be more practical and reliable method in T classification of distal bile duct cancer staging.

1776 Characterization of Putative Precursor Lesions of Familial Pancreatic Cancer

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Background: The well characterized precursor lesions of pancreatic ductal adenocarcinoma (PDAC) such as PanIN, IPMN and MCN show a ductal phenotype. However, acinar cells might also be involved in PDAC origin, since genetically engineered mouse models revealed that pancreatic carcinomas may arise from atypical flat lesions (AFL) which derive from tubular cell complexes (TC) in areas of acinar-ductal metaplasia (ADM). Human AFL have also been detected in areas of ADM in pancreata from individuals with a history of familial pancreatic cancer (FPC). In this study, we analyzed pancreas resection specimens from FPC individuals in order to search for PDAC precursor lesions and analyze their phenotype. In particular, we looked for AFL as indication for a PanIN-independent carcinogenic pathway.

Design: We systematically screened pancreatic specimens, removed from six healthy individuals with a FPC history, for ductal and acinar lesions such as PanIN, IPMN, TC and AFL. Immunostaining for MUC1, MUC2, MUC5, MUC6, p53, smad4 and Her2neu and MIB1 as well as K-RAS mutation analysis was performed on all identified lesions.

Results: In addition to precursor lesions of ductal phenotype such as PanIN (many with lobulocentric fibrosis) and gastric type IPMN, all pancreata also showed AFLs in ADM areas that displayed a perifocal active stromal reaction. AFL showed predominantly a

MUC1+, MUC2- and MUC5+ expression pattern with a variable positivity for MUC6 and a focally elevated Ki-67 proliferation index. AFL from 2 cases also harbored mutations in the K-RAS-gene locus.
Conclusions: Pancreatic tissue from FPC individuals not only contain PanINs and IPMNs but also AFLs. The identification of the latter lesions suggests a potentially alternative pathway of carcinogenesis in FPC, that starts in ADM areas. Whether AFLs also play a role in the development of sporadic PDAC is not yet known.

1777 Epigenetic and Genetic Changes of *APC* Gene in Acinar Cell Carcinoma of the Pancreas

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Background: Genetic and epigenetic alterations involved in the pathogenesis of pancreatic acinar cell carcinoma (ACC) are largely unknown. ACCs generally lack mutations of KRAS, p53, DPC4 and p16, while mutations of the *APC* gene have been previously described in 17.6% of ACCs. However, it is not known whether loss of function of *APC* gene in ACC can occur through multiple alternative genetic mechanisms.
Design: We investigated promoter methylation and copy number of *APC* gene using MS-MLPA and FISH analysis in 14 ACCs. Droplet Digital PCR (ddPCR, Biorad Instrument) and immunohistochemistry were employed to evaluate APC mRNA and protein expression in the same tumors.
Results: *APC* methylation was found in 10/14 (71.4%) cases and FISH analysis revealed loss of *APC* in 7 ACCs. Interestingly 4 ACCs (4/14 28.6% %) revealed both methylation and loss of *APC*. Only one case did not show any loss and methylation. Absolute quantification of APC mRNA levels demonstrated a significant reduction of the transcript in all investigated ACCs compared with normal control pancreases (10.5 ± 3.2 RNA copies/µl in ACCs *versus* 56.77 ± 8.1 RNA copies/µl in normal controls; $p<0.0001$). APC protein expression was not found in any case investigated. *APC* gene methylation and loss did not correlate with stage, grade and prognosis.
Conclusions: *APC* gene inactivation is a frequent event in ACCs. Gene methylation and loss might be considered as a mechanism of APC haploinsufficiency. Moreover, our data suggest that *APC* gene alterations are an early event in ACC tumorigenesis.

1778 Comparison of PAX6 and PAX8 as Markers for Pancreatic Neuroendocrine Tumors (NETs)

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Background: Our previous study showed that PAX8 helps in distinguishing pancreatic NETs from ileal NETs. More recently it has been suggested that immunoreactivity of pancreatic NETs for PAX8 is due to cross reactivity of the PAX8 antibody with PAX6, a transcription factor and differentiation marker of neuroendocrine cells. The aim of this study was to compare the sensitivity and specificity of PAX6 and PAX8 for pancreatic NETs.
Design: PAX6 expression was investigated in 92 NETs, each of which had been previously stained for PAX8 in our prior study. Seventy-one primary NETs from different sites (22 pancreatic, 21 ileal, 12 pulmonary, 6 gastric and 10 rectal) and 21 metastatic pancreatic NETs were studied. Tumors showing moderate to strong nuclear staining of at least 5% of cells or tumors showing weak nuclear staining of at least 10% of cells were considered positive.
Results: Among primary NETs, PAX6 was positive in 68% (15/22) of pancreatic, 0% (0/21) of ileal, 0% (0/12) of pulmonary, 0% (0/6) of gastric and 90% (9/10) of rectal NETs. Forty-three percent (9/21) of metastatic pancreatic NETs in liver were positive for PAX6, all of which showed diffuse, moderate to strong staining. In comparison with surrounding islets (n =15), the staining intensity for PAX6 was found to be decreased in 64% of the primary pancreatic NETs. Moderate to strong expression of PAX6 was noted in 10 of 13 low grade primary pancreatic NETs but in only 2 of 9 intermediate grade primary pancreatic NETs ($p=0.027$). The comparison of staining of NETs for PAX6 and PAX8 is displayed in Table 1. The sensitivity and specificity for pancreatic NETs were 56% and 82% for PAX6 and 67% and 73% for PAX8, respectively.

Table 1. Comparison of PAX6 and PAX8 in pancreatic and non-pancreatic NETs

	Primary pancreatic	Metastatic pancreatic	Primary ileum	Primary lung	Primary stomach	Primary rectum
PAX6	15/22 (68%)	9/21 (43%)	0/21	0/12	0/6	9/10 (90%)
PAX8	18/22 (82%)	11/21 (52%)	0/21	3/12 (25%)	2/6 (33%)	8/10 (80%)

Conclusions: In comparison to PAX8, PAX6 is a more specific but less sensitive marker for pancreatic NETs. Considering the generally indolent behavior of rectal NETs, positivity of a metastatic NET of unknown primary origin for PAX6 positivity favors a pancreatic origin.

1779 DNA Mismatch Repair Deficiency in Acinar Cell Carcinoma of the Pancreas: Frequency and Significance

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Background: Acinar cell carcinoma (ACC) is a rare pancreatic exocrine malignancy, accounting for about 1%-2% of all pancreatic cancer diagnoses. The molecular features of ACC remain to be fully characterized. Two recent reports from our institution have indicated the occurrence of ACC in the context of Lynch syndrome (LS). The frequency and clinical implication of DNA mismatch repair (MMR) deficiency in ACC, however, have not been systematically analyzed. The aim of this study was to evaluate the frequency and biological implications of MMR deficiency in ACCs treated at our institution.
Design: Patients diagnosed of ACCs who were surgically treated at our institution and whose tumor blocks were available for study were analyzed. Tumors with a minor secondary component (ductal or neuroendocrine) were included. Pancreatoblastomas were excluded. MMR protein expression was evaluated by IHC using antibodies against MLH1, MSH2, MSH6, and PSM2; the results were correlated with clinicopathologic features.
Results: Twenty-two patients fulfilled the inclusion criteria, 4 females and 18 males, ranging in age from 47 to 80 years (median, 64 years). Tumor location was the head of pancreas in 50% of the cases, and tumor size ranged from 1.8 to 27cm (mean, 6.9cm). Most patients (13/18, 72%) presented with stage II disease. With a median follow up of 31.5 months, the median recurrence-free survival was 13.5 months. IHC detected loss of MMR protein in 4 cases (18%): 2 lost both MLH1 and PMS2, and 2 lost both MSH2 and MSH6. The 2 MLH1/PMS2 deficient cases occurred in males, age 55 and 75 years respectively with no known history suggestive of LS. Of the 2 MSH2/MSH6 deficient cases, one was a 49-yr-old male from a known LS family that carried a germline mutation in *MSH2*. The second was a 74-yr-old female whose paternal grandfather also had pancreas cancer; no LS work-up was done in this patient. All 4 MMR abnormal tumors occurred in the body/tail of the pancreas with a mean tumor size of 9.6 cm. All 4 patients presented with stage II disease; 2 patients recurred at 14 and 27 months after resection.
Conclusions: MMR abnormality is not uncommon in ACCs, occurring in 18% of this consecutive series. In addition to reaffirming previous observations that ACC can occur in LS individuals and manifest the patient's underlying genetic defect, our findings suggest that MMR deficiency may also occur as a sporadic event in ACCs similar to colorectal and other types of tumors, thus providing directions for further research efforts.

1780 Combining a Unique Biomarker Profile with Morphology To Differentiate Benign Reactive Epithelial Change from Adenomas and Adenocarcinomas in Ampullary Biopsies: A Large Cohort Study

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Background: Morphologic differentiation of benign reactive epithelial change, adenoma and adenocarcinoma in ampullary biopsy is challenging, particularly in the background of both duodenal and pancreaticobilliary epithelium, inflammation and fibrosis. Combining morphologic features with a panel of biomarkers would allow for straightforward and accurate diagnosis. We therefore identified 349 ampullary biopsies, analyzed their morphologic features and determined the usefulness of a biomarker panel including HMGA2, p53, β-catenin and Ki-67 in differentiating benign reactive epithelial change from adenoma and adenocarcinoma.
Design: Ampullary biopsies from 2003-2012 were identified in patients between 18 and 80 years old (n=349). All cases were separated into marked reactive change, adenoma and adenocarcinoma categories based on morphology. Immunohistochemistry (IHC) for HMGA2, p53, Ki-67 and β-catenin were performed on randomly selected slides from each category with appropriate controls. Nuclear positivity in epithelial cells from 5 high power fields was calculated for each marker and 5% positivity was considered a positive result in all cases.
Results: Morphologically, 22% of cases (n=76) demonstrated normal ampullary mucosa, 46% demonstrated benign reactive epithelial change (n=162), 19% were adenomas (n=68), 12% were adenocarcinomas (n=40) and 1% were others (n=3). Cases of benign reactive epithelial change demonstrated inflammation and/or fibrosis (n=105), pseudostratefication and hyperchromasia (n=39) similar to adenomas, or cytological atypia or mitosis (n=18) suspicious for adenocarcinoma. IHC revealed that HMGA2 was positive in 80% of the adenocarcinomas (16/20), 8% of those with benign reactive epithelial change (n=15) and none of the adenomas (n=15). p53 demonstrated 75% positivity in adenocarcinoma cases, but was negative in adenomas and reactive epithelial change. Ki-67 and β-catenin were diffusely positive in the proliferative zone, extending to the luminal surface in all adenoma cases (top-down pattern, n=15). In contrast, Ki-67 positive cells were limited to the epithelial proliferative zone in reactive epithelium with occasional cells positive for β-catenin.
Conclusions: The top-down growth pattern in ampullary adenomas is a unique morphologic feature that can differentiate adenomas from benign reactive epithelial change when combined with IHC for Ki-67 and β-catenin. Combining morphology with IHC for malignant biomarkers HMGA2 and p53 can also assist in the diagnosis of ampullary adenocarcinoma.

1781 Estrogen-Induced Genes Are Expressed in Pancreatic Neuroendocrine Tumors

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Background: High progesterone receptor (PR) expression, a well-known estrogen-induced gene (EIG), confers better prognosis in pancreatic neuroendocrine tumors (PanNETs). We, therefore, assessed mRNA expression of other EIGs and their prognostic significance in PanNETs. We also examined ERβ expression in a subset of PanNETs; we hypothesize that transcription of EIGs may be driven by ERβ, as ERα is not expressed in these tumors.
Design: 138 primary resected WHO G1-G2 PanNETs were analyzed for EIG [PR, insulin-like growth factor (IGF)-1, IGF-1R, EIG121, secreted frizzled-related protein (sFRP)-1 and sFRP-4] mRNA expression by RT-PCR and correlated with clinicopathologic parameters. Adjacent normal pancreas (42 cases) served as control. A

subset of tumors (40 cases) were evaluated for ERβ immunohistochemical expression and correlated with EIG mRNA. Allred scoring was used to categorize ERβ staining as follows: diffuse=7-8/8; weak=3-6/8; negative=0-2/8.

Results: Results are summarized in Table 1. PanNETs had significantly higher EIG mRNA levels compared to normal pancreas, regardless of gender. Diffuse ERβ positivity was seen in 24 of 40 (60%), while the remaining 40% showed weak positivity; none had negative staining. EIG121 mRNA was 10-fold higher compared to other EIGs and high mRNA expression correlated with diffuse ERβ positivity. High IGF-1R mRNA expression correlated wtih low WHO grade and AJCC stage and also showed diffuse ERβ positivity.

Correlation between Clinicopathologic Parameters and Estrogen-induced Genes in Pancreatic Neuroendocrine Tumors

	Estrogen-induced gene (mean mRNA/mean 18SrRNA) x10^4					
	PR	**IGF-1**	**IGF-1R**	**EIG121**	**sFRP-1**	**sFRP-4**
Tumor vs. Normal (p value)	<0.001	NS	0.04	<0.001	0.003	0.02
Normal (n=42)	4	1.7	22	110	2.0	21
Tumor (n=138)	63	2.3	35	470	15	62
Gender (p value)	NS	NS	NS	NS	NS	NS
Female (n=53)	57	2.3	36	530	12	63
Male (n=85)	66	2.4	33	430	17	61
WHO Grade (p value)	NS	NS	0.008	NS	0.003	NS
G1 (low, n=69)	71	2.3	43	440	7.3	57
G2 (intermediate, n=60)	55	2.4	25	480	21	73
Stage (p value)	0.05	NS	0.01	NS	NS	NS
1-2A (n=63)	71	1.9	41	500	14	56
2B (n=34)	81	2.5	41	510	14	79
4 (n=41)	35	2.9	19	400	17	57
Estrogen Receptor β	0.008	NS	0.03	0.007	NS	0.02
Diffuse (n=24)	115	1.6	64	630	13	27
Weak (n=16)	18	3.4	22	300	15	77

PR-progesterone receptor; IGF-insulin-like growth factor; EIG121-estrogen-induced gene 121; sFRP-secreted frizzled-related protein.

Conclusions: In a subset of PanNETs, high ERβ immunohistochemical expression correlated with high mRNA expression of EIGs predicting low grade and stage, suggesting a protective role in PanNETs. ERβ activation may serve as a new hormonal therapy for some patients with PanNETs.

1782 Evaluation of Immunohistochemical Study of Maspin for Pancreatic Tumors Should Be Made in Caution: Immunohistochemical Studies and Quantitative Analysis of mRNA Using Materials from Endoscopic Ultrasound Guided-Fine Needle Aspiration

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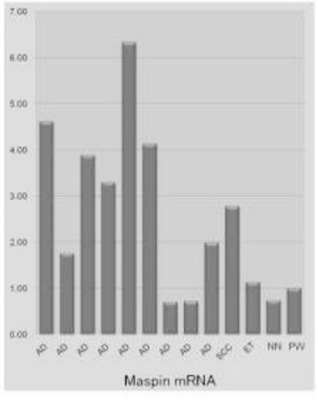
Background: Differentiating pancreatic adenocarcinoma (AC) from atypical glands of non-neoplastic lesions using materials from endoscopic ultrasound guided-fine needle aspiration (EUS-FNA) is always challenging for pathologists. Maspin is known to have tumor-suppressor function and is expressed in certain kinds of adenocarcinomas, however; immunohistochemical localization of maspin has not been studied well.

Design: Fifty-three specimens obtained from EUS-FNA (45 ACs, 5 autoimmune pancreatitis (type 1), 1 inflammatory peudotumor and 2 normal pancreas) were studied. After confirming the existence of targeted cells by Papanicolaou stain, cover glasses were removed, cell transfer technique was applied to immunohistochemical (IHC) studies for maspin. In addition, quantitative analysis of mRNA (Real-time PCR) of maspin in 12 cases (9 ACs, 1 squamous cell carcinoma, 1 neuroendocrine tumor with normal acini, and 1 intraoperative peritoneal washing) was performed.

Results: Maspin is positive in 44 of 45 ACs (either nuclear or nuclear plus cytoplasmic) and 2 of 8 non-neoplastic lesions (sensitivity 95.6%, specificity 85.7%) by IHC studies. Among 12 cases subjected to quantitative analysis of mRNA of maspin, 9 of 10 carcinomas showed nuclear plus cytoplasmic IHC stains, and 1 of 10 showed only nuclear stains. Increased mRNA of maspin was observed in 8 of 9 cases showing nuclear plus cytoplasmic IHC stains. One AC case showing only nuclear stain did not have increased mRNA of maspin.

Histology	IHC [maspin]
AD	Positive
AD	Positive
AD	Positive
AD	Positive
AD	Positive
AD	Positive
AD	Positive
AD	Positive (nuclear)
AD	Positive
SCC	Positive
ET	Negative
NN	Positive
PW	Negative

IHC: immunohistochemistry
AD: Adenocarcinoma
SCC: Squamous cell carcinoma
ET: Endocrine tumor
NN: Non-neoplastic
PW: Peritoneal washing



Conclusions: Sensitivity and specificity of maspin IHC using cell transfer technique were satisfactory. Although further studies are needed for the precise interpretation for IHC localization of maspin, only nuclear stain of maspin should be evaluated in caution for the diagnosis of malignancy according to the results of real-time PCR.

1783 MCT4 Expression in Pancreatic Cancer: Association with Prognosis, and Potential for Targeting Cancer Metabolism

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Background: Pancreatic ductal carcinoma (PDA) carries a poor prognosis, with a five-year survival rate of 6% despite treatment with surgery and chemotherapy. An emerging approach is to target the tumor's microenvironment and metabolic dependencies. PDA is known to have a glycolytic metabolism, producing large amounts of lactic acid. The monocarboxylate transporters (MCTs) are a group of membrane proteins that facilitate transport of lactic acid, preventing a fall in tumor cytosolic pH. We evaluated the expression, prognostic significance, and potential therapeutic relevance of MCT1 and MCT4 in cell cultures and clinical specimens of PDA.

Design: PDA tumor epithelial and stromal cell lines from human pancreatic adenocarcinoma were grown separately and in co-cultures. Cultures were treated with 20 nM gemcitabine for 48 hrs. Expression of MCT1 and MCT4 was assessed by immunofluorescence and Western blotting. Silencing of MCT4 expression was performed using siRNA. A tissue microarray containing 226 PDAs was immunohistochemically stained for MCT1 and MCT4 and scored using published criteria. Survival curves were computed by expression strata using the Kaplan-Meier method.

Results: MCT1 expression in pancreatic cancer cell lines was largely static, and there was no statistically significant association ($p>0.05$) of MCT1 expression with overall survival of PDA patients. In contrast, MCT4 expression varied across cell culture models and was induced by gemcitabine challenge or co-culture with cancer associated fibroblasts. Correspondingly, elevated MCT4 expression in both tumor epithelial cells ($p=0.0012$) and stromal cells ($p=0.0018$) was associated with a poor outcome in PDAs. This result remained significant in multivariate analyses. The attenuation of MCT4 expression by RNA-interference was associated with loss of pancreatic cancer cell viability both in mono and co-culture experiments.

Conclusions: MCT4 is highly expressed in pancreatic cancer cases and is associated with poor disease outcome. Functionally, MCT4 is induced by a variety of stress conditions, and could represent a viable target for therapeutic intervention.

1784 Differential Expression of Laminin ß1 and ß2 Isoforms in Pancreatic Adenocarcinoma

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Background: Laminins are a family of heterotrimeric glycoproteins that are integral components of vascular and parenchymal basement membranes and are involved in adhesive, signaling, and migratory functions. Vascular β chain isoforms are differentially expressed in gliomas and breast tumors (β1>β2), involved in tumor angiogenesis and invasion. Therapeutic targeting of laminin expression with antisense oligonucleotides resulted in tumor inhibition in a mouse model. This preliminary study has been performed to define the expression profile of laminin β1 (LB1) and laminin β2 (LB2) isoforms in capillaries and glands of pancreatic adenocarcinomas versus normal pancreas in order to determine if novel therapeutic laminin targeting drugs might be efficacious in treatment.

Design: 16 cases of formalin fixed paraffin embedded pancreatic adenocarcinoma (9 with adjacent normal pancreatic tissue) were immunohistochemically stained for LB1 and LB2. The predominant pattern of staining intensity was then evaluated in the capillaries and glands within the tumor and in adjacent pancreatic parenchyma in a semiquantitative fashion as follows: 0=no staining, 1=weak, 2=moderate, or 3=strong. The negative staining and positive staining was defined as 0-1 and 2-3, respectively.

Results: Extratumoral capillaries were positive for LB1 and LB2 (both; 9/9 = 100%), whereas intratumoral capillaries showed increased ($p = 0.0021$) staining for LB1 (15/16 = 88%) compared with LB2 (6/16 = 37.5%). The decreased LB2 in intratumoral capillaries was also significant ($p = 0.0028$) compared with extratumoral capillary staining. Extratumoral pancreatic ducts did not show any differential β isoform expression (β1 5/9 = 56%; β2 6/9 = 67%). Tumor basement membranes showed stable expression of LB1 (8/16 = 50%) and a loss of LB2 (3/16 = 19%) that was significant ($p=0.031$) when compared to normal. There was no significant relationship between the presence of lymph node metastases (11/16 = 69%) or tumor grade (high grade = 7; low grade = 8) and tumoral capillary or duct expression of LB1 and LB2.

Conclusions: Significantly less LB2 staining was observed in intratumoral vasculature when compared with LB1 staining as well as with extratumoral vessels. There was also significantly less LB2 staining in malignant ducts when compared with extratumoral ducts. This study provides novel data on tumor basement membrane remodeling in pancreatic adenocarcinoma, which could be useful for future diagnostic and/or therapeutic decision making.

1785 Comparison of Chromosomal Abnormalities by Fluorescence In Situ Hybridization between Intraductal Papillary Mucinous Neoplasm and Pancreatic Ductal Adenocarcinoma

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Background: Diagnosis of pancreatic malignancy on cytological specimen by fluorescence in situ hybridization (FISH) has been recently reported; however, few comparative studies of chromosomal abnormalities between intraductal papillary mucinous neoplasm (IPMN) and pancreatic ductal adenocarcinoma (PDAC) using FISH have been reported so far. The aim of this study is to examine the difference of chromosomal and locus abnormalities between IPMN and PDAC.

Design: Resected specimens of IPMN with low or intermediate-grade dysplasia (IPMN; n=15; gastric type: intestinal type = 12:3), PDAC (n=22; differentiation, well: moderate: poor = 9:11:2), and normal pancreatic ductal epithelial cells as controls (NM; n=18) were gathered. FISH analysis was performed to detect aneuploidies of chromosome (3, 6, 7, 8, 17, and 18) and loss of gene (p16 at 9p21 and p53 at 17p13). We evaluated chromosome (monosomy and polysomy) and locus (hemizygous deletion and homozygous deletion) abnormalities in IPMN and PDAC by comparison with NM. Furthermore, we compared these differences between IPMN and PDAC.

Results: Polysomies of chromosome 6, 7, 17, and 18 were significantly more frequent in PDAC than IPMN (chromosome 6, 12/22 vs 2/15, p=0.016; chromosome 7, 16/22 vs 3/15, p=0.003; chromosome 17, 13/22 vs 1/15, p=0.002; chromosome 18, 9/22 vs 1/15, p=0.028, respectively). Any kinds of monosomies did not differ significantly between IPMN and PDAC. The number of polysomy was significantly more in PDAC than IPMN (2.9±1.6 vs 0.8±1.1; P<0.0001). If specimen with polysomy 6 or 17 was considered positive for PDAC, the sensitivity and specificity were 100% and 80%, respectively. Loss of p16 and p53 was significantly more common in PDAC than in IPMN (p16, 12/22 vs 2/15, p=0.0164; p53, 13/22 vs 0/15, p=0.0002, respectively). Most of these deletions in PDAC were homozygous (p16, 11/12, 82%; p53, 12/13, 92%).

Conclusions: Our study reveals that chromosome and locus abnormalities correlate to pancreatic tumor malignancies and FISH is a useful modality to differentiate PDAC from IPMN.

1786 Pancreatic Ductal Adenocarcinomas with Multiple Large Cystic Structures: A Clinicopathologic and Immunohistochemical Study of Seven Cases

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Background: Pancreatic ductal adenocarcinoma (PDA) with cystic change may be confused with other cystic neoplasms of the pancreas, and must therefore be included in the differential diagnosis. Those tumors do not constitute a uniform group, and are classified into several types according to the features of the cysts.

Design: A total of 200 surgically resected PDAs were examined for the present analysis. Among them, 22 (11%) showed cystic lesions. Fifteen out of these 22 cases were classifiable as cystic PDAs by the previously reported criteria (ref. Kosmahl et al, 2005 and Adsay, et al, 2011). The remaining 7 cases were PDAs with a multiple large cystic (MLC) structure not classifiable by those criteria. Histological and immunohistochemical studies were performed using formalin-fixed, paraffin-embedded (FFPE) tissue. In the 7 PDAs with MLC structures, the *KRAS* oncogene mutation in codon 12 was studied. Follow-up information after surgical resection was available for all 22 patients. The mean follow-up period was 23 months (range, 4-60 months). Postoperative survival of patients with cystic PDA, with and without a MLC structure, were compared.

Results: PDAs with MLC were associated with more than 5 large cystic structures and numerous intratumoral microcysts, lined by epithelial cells with various degrees of atypia. The maximal cyst diameter (average 3.7 cm) was much larger than that of previously reported. Immunohistochemically, the tumor cells and cyst-lining epithelia were negative for mucin core protein (MUC) 1, MUC2, and MUC6, and showed only focal staining for MUC5AC. Maspin, CEA, and p53 were strongly positive, and the Ki-67 labeling index was high in both tumor cells and cyst-lining epithelia. *KRAS* oncogene mutation was identified in 6 of the 7 cases. Postoperative outcome in the MLC lesion group did not appear to differ significantly from cases without such lesions.

Conclusions: The MLC structures in PDA might be a mixture of ectatic neoplastic glands and retention cysts with ductal cancerization or pancreatic intraepithelial neoplasia; however, they might represent a new entity of cystic PDA because of the unusually large size of the dilated cysts. Diagnostic pathologists should bear in mind that MLC-type PDA can form cystic lesions similar to those of intraductal papillary neoplasms or mucinous cystic neoplasms.

1787 Pyrosequencing Identifies *GNAS* R201 "Hotspot" Mutations in Cytology Specimens Obtained from Intraductal Papillary Mucinous Neoplasms and Associated Adenocarcinomas: An Adjunct Tool for Molecular Diagnosis of Pancreatic Cystic Neoplasms

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Background: Intraductal papillary mucinous neoplasms are bona fide precursor lesions of pancreatic ductal adenocarcinomas (PDAC). Currently, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is used to obtain cyst fluid for evaluation. EUS-FNA cytology, however, can frequently be non-diagnostic or equivocal. Molecular analyses of IPMNs have identified activating mutations of *GNAS* at codon R201 in ~66% of lesions (including IPMN-associated PDACs), while this mutation is typically absent in other pancreatic cystic neoplasms and in non-IPMN associated PDACs. Assessment of *GNAS* mutations in EUS-FNA material could potentially be a valuable adjunct in the assessment of pancreatic cysts.

Design: We have previously reported *GNAS* mutational status in a series of ~100 IPMN-associated cyst fluid samples obtained at the time of surgical resection (Wu et al, *Sci Transl Med* 2011); in nine of these IPMNs, sufficient pre-operative EUS-FNA samples were available for DNA extraction and sequencing. Cytology specimens from entities not expected to harbor GNAS mutations, specifically, pancreatic neuroendocrine tumors (1), solid-pseudopapillary neoplasms (2), and PDACs not associated with an IPMN (3), were utilized as controls. From these preparations, DNA was extracted and pyrosequencing was used to detect *GNAS* mutations.

Results: Mutations in GNAS were detected in 7 out of 9 (78%) cytology samples of IPMNs, including non-invasive IPMNs (2/4, 50%) and in IPMN-associated PDACs (5/5, 100%). *GNAS* mutations were not detected in the remaining 6 samples from non-IPMN pancreatic lesions. Possibly as a manifestation of cyst multifocality, we found discordance in 5 of 9 cases when we compared the mutational status of *GNAS* in the cytology material with that of the cyst fluid obtained at the time of surgery. In the majority of these cases (4/5, 80%), *GNAS* mutations were detected in the cytology material but not in cyst aspirated at surgery. In one case, the cytology material contained only wild type sequence while *GNAS* mutant sequence was detected in the cyst fluid.

Conclusions: Pyrosequencing can detect *GNAS* mutations in routine cytology preparations from IPMNs and IPMN-associated PDACs. Cytology specimens might provide an added degree of sensitivity over acellular cyst fluid samples in the detection of mutant DNA sequence. This method could provide a valuable adjunct by allowing more accurate triage of cystic pancreatic neoplasms pre-operatively.

1788 Clinicopathologic Comparison of Ampullary Versus Pancreatic Carcinoma: Preinvasive Component, Size of Invasion, Stage, Resectability and Histologic Phenotype Are the Factors for the Significantly Favorable Outcome of Ampullary Carcinoma

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Background: The information on the clinical outcome differences of ampullary versus pancreatic carcinoma (AC vs PC) has been conflicting largely because of their poor definition and differential from each other, classified and analyzed together under the rubric of "periampullary cancers".

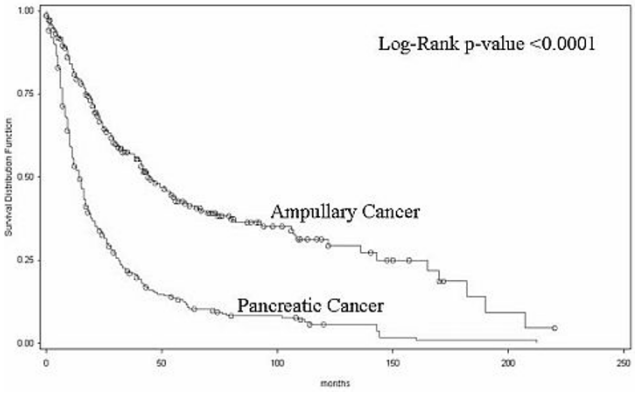
Design: 292 stringently-defined ACs identified based on the refined criteria set forth recently (Am J Surg Pathol, 2012) were compared with 297 invasive PCs.

Results: Compared to PCs, ACs often presented with a significant mass-forming preinvasive component (tumoral intraepithelial neoplasm), smaller invasive carcinomas, less advance stage (lower incidence of lymph node metastasis), higher resectability, and less aggressive histologic phenotype (higher incidence of non-pancreatobiliary cases and less perineural invasion).

Comparison of Prognostic Parameters of AC vs PC

	Ampullary Cancer (n=292)	Pancreas Cancer (n=297)	p-value
Overall size (cm)	2.6 (0.3-8.0)	3.3 (0.7-11.0)	<0.0001
Size of invasion (cm)	1.9 (0.2-6.0)	3.2 (0.7-11.0)	<0.0001
Frequency of invasion size < 1.0 cm	52 (17%)	1 (0.4%)	<0.0001
Frequency of LN mets	119 (42%)	177 (65%)	<0.0001
Margin positivity rate	13 (4%)	91(34%)	<0.0001
Histologic type as Pancreatobiliary	139 (47%)	286 (97%)	<0.0001
Perineural invasion	79 (27%)	158 (86%)	<0.0001
Vascular invasion	187 (63%)	84 (55%)	0.105

Fig.1 Overall Survival Between Ampullary Cancer vs. Pancreatic Cancer



Conclusions: Ampullary carcinoma, defined by the refined criteria, have an incomparably better clinical outcome than pancreatic. Relative abundance of preinvasive component (leading to early diagnosis), smaller invasion size at the time of diagnosis, higher resectability (higher R0 rate, due to central location in the Whipple), less aggressive histologic phenotypes, lower incidence of lymph node metastasis (due to early diagnosis and less aggressive phenotypes) are the important factors in this favorable outcome.

1789 Cell Signaling Pathways from Pancreatic Intraepithelial Neoplasia (Pan-IN) to Invasive Adenocarcinoma

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Background: Pancreatic adenocarcinoma (PDAC) still has a dismal prognosis. Multiple genetic alterations in pancreatic cancer progression have been identified, including tumor suppressor genes, oncogenes, and genome maintenance genes. Furthermore, the identification of non-invasive precursor lesions (PanIN) has led to the formulation of a multi-step progression model of pancreatic cancer and the identification of early and late genetic alterations. The aims of this study were to describe the expression of factors implicated in growth and proliferation cell signaling pathways, in order to analyze the role of these factors in pancreatic malignant transformation.

Design: We study the expression of pmTor, 4E-BP1, p4E-BP1, eIF4E, peIF4E, pS6 and pMAPK in 64 specimens from pancreatectomy, with PDCA and lesions of PanIN of all histological grades, adjacent to PDCA. Levels of expression were semiquantitatively

evaluated as percentage and intensity of stained tumor cells (H-score). Statistical evaluation used was Kruskal-Wallis tests.
Results: The expression of cell signaling factors implicated in mTor pathway increase with development of invasive adenocarcinoma, from low grade Pan-IN to DPCA (4E-BP1, p< 0'001; p eIF4E, p<0'001; pmTor, p<0'001; pS6, p: 0'013). pMAPK is expressed in Pan-IN 3 and DPCA, with higher levels in the invasive carcinoma areas (p:0'002)
Conclusions: In this study, we show that mTor pathway is activated in pancreatic malignant transformation since early stages, and that pMAPK is highly activated, mainly in advanced stages. With these results, we propose that: 1) the expression of factors such as pmTor, p4E-BP1 and pMAPK may provide a reliable way to identify high risk PanINs and the subsequent development of infiltrating carcinoma, 2) pmTor and pMAPK can be potential therapeutic targets in PDCA.

1790 Pancreatic Serous Cystadenomas Fine Needle Aspirations: Improving Diagnostic Yield with Cell Blocks and Inhibin

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Background: Serous cystadenomas (SAs) are rare benign neoplasms of the exocrine pancreas. Due to their generally benign prognosis, small and asymptomatic SAs can be followed conservatively. Greater numbers of pancreatic lesions are being detected with increasing and enhanced imaging modalities. Although characteristic imaging findings and chemical analysis of fluid have been described, they are not always recognized prospectively. Similarly, scant cellular yield on fine needle aspirations (FNAs) often leads to either a non-diagnostic or non-specific benign diagnosis. Alpha-inhibin (AI), a highly sensitive marker for SA and not endocrine tumors, is infrequently required for the histological diagnosis due to their characteristic appearance. The aim of this study was to determine if AI immunohistochemical staining can aid in improving the sensitivity and specificity of SA FNA diagnosis.
Design: We performed a retrospective database search for SAs diagnosed in surgical pathology (SP) and endoscopic ultrasound (EUS) FNA specimens. Cases with FNAs and corresponding SPs or follow-up FNAs were selected for the study. The FNAs were evaluated for cellularity, cellular arrangement, cytomorphology(sheets or 3D clusters;) and stroma. Al staining was performed both prospectively and retrospectively on cases with cell blocks (CBs). SP specimens were assessed for architecture (micro- vs macrocystic), cytology (clear vs oncocytic) and stromal predominance.
Results: We identified a total of 12 FNA cases (F:7, M:5, mean age 63.3 yrs), 75% of which had scant cellularity (9/12) and 3 were satisfactory. On smears, the cells were arranged mostly as flat sheets that corresponded to strips of cells on CB sections. The cells were typically small, round to cuboidal and had clear cytoplasm; occasional plasmacytoid cells and oncocytic cells were identified. Some cases had flattened cells, corresponding to attenuated epithelium lining macrocysts on the resection specimens. Stromal fragments were present in 5 FNAs and correlated with the hyalinized stroma in the corresponding SP specimens. AI stain was present in 86% (6/7) of cases and supported the diagnosis of SA.
Conclusions: A diagnosis of SA can significantly alter management of patients with pancreatic masses. Our results indicate that low cellularity and bland cytology is inherent to SAs and performing CBs and AI staining on EUS FNAs can improve the sensitivity and specificity of SAs.

1791 Is Investing Additional Time on Mitotic Counts in Neuroendocrine Neoplasm Clinically Relevant?

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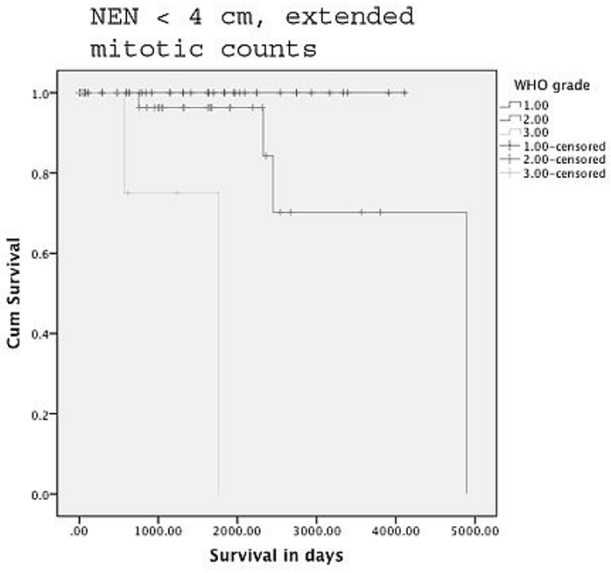
Background: Mitotic activity is a robust prognostic factor in pancreatic neuroendocrine neoplasms (PanNEN). Grading of NENs is based on mitotic counts and Ki67 labeling index. It is widely accepted that mitotic counts are performed on proliferation 'hotspots', however, the impact of the time expended on mitotic count has not been evaluated. We assess the impact of an extended evaluation of mitotic index on survival in PanNEN.
Design: The mitotic index in 130 PanNET was evaluated by two methods: (1) extended mitotic evaluation - identified all mitotic figures in one tumor bearing slide and used the 10 fields with the highest mitotic count, (2) abbreviated '3 minute' count to mirror routine clinical practice - the 10 fields with the highest counts were recorded. We compared mitotic counts from the two methodologies to overall survival. We used the 2010 WHO grading system with a modification - to critically assess the impact of mitotic counts we did not consider Ki67 labeling index.
Results: The 'extended' method of mitotic counts revealed significantly higher number of mitotic figures (6.7 vs 2.2, p=0.0001). For tumors less than 4 cm, the WHO grade on both the extended and 3 minute mitotic counts correlated with disease specific survival (p=0.0001 and 0.001). Most significantly, based on 'extended' counts the tumor deaths for grade 1, grade 2 and grade 3 tumors were 0/41, 4/30 and 2/5, respectively.



The tumor related deaths based on the '3 minute' counts: 3 deaths in grade 1 and 3 deaths in grade 2. There were no grade 3 tumors identified on the '3 minute' count. Interestingly, among tumors > 4 cm there was no correlation between the WHO grade based on either of the two methods and disease specific survival.
Conclusions: For tumors under 4 cm, an extended mitotic count provides a more robust separation of the 3 tumor grades and identifies an unrecognized group of grade 3 tumors whose behavior mirrors grade 3 tumors identified on 'conventional' mitotic counts. The lack of correlation between extended mitotic counts and outcome for tumors > 4 cm may be related to the greater heterogeneity in proliferation of larger tumors.

1792 Extracellular DNA Contributes to Pancreatic Cancer Invasion and Metastasis

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Background: Pancreatic cancer is the fourth leading cause of cancer death in US due to metastasis. Inflammation has been shown to facilitate metastasis. Extracellular DNA (exDNA) is a newly discovered component for inflammation and was reported on cancer cell surfaces. Analysis of exDNA as potential diagnostic, prognostic or predictive biomarkers for cancer indicated that their concentrations in the circulation are higher in cancer than in normal conditions. However, whether exDNA contributes to pancreatic cancer invasion and metastasis is not known.
Design: We used DNA fluorescent dye or antibody to detect exDNA. Using DNase I to digest exDNA, MTT, migration and invasion assays, we examined whether exDNA affected pancreatic cancer cell viability and contributed to invasion and migration in vitro. Finally human pancreatic cancer plugs were implanted into mouse pancreas to determine whether exDNA impacts metastasis in vivo. Two groups of mice received either introperitoneal saline or DNase I (1 U/mouse). Tumor growth and metastasis were monitored noninvasively every week by bioluminescence imaging. Organs were harvested for histology and bioluminescence analysis to evaluate the presence of exDNA and tumor metastasis.
Results: We detected exDNA on the surface and in vicinity of cultured pancreatic cancer cells and metastasized pancreatic cancer tissue, but not on normal human pancreatic ductal epithelial cells or in adjacent normal tissue. The presence of cell surface exDNA or DNase I treatment did not affect cell viability or growth, which is important for using DNase I to study the role of exDNA in metastasis. We showed that DNase I treatment significantly reduced pancreatic cancer cell migration and invasion. Normal pancreatic cells were not affected. Finally, the orthotopic human pancreatic cancer xenograft mouse model showed that tumor burden and metastasis were significantly inhibited by DNase I treatment.

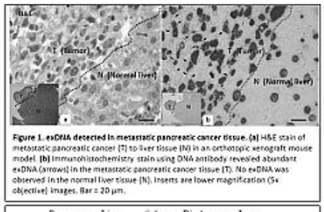

Figure 1. exDNA detected in metastatic pancreatic cancer tissue. (a) H&E stain of metastatic pancreatic cancer (T) to liver tissue (N) in an orthotopic xenograft mouse model. (b) Immunohistochemistry stain using DNA antibody revealed abundant exDNA (arrows) in the metastatic pancreatic cancer tissue (T). No exDNA was observed in the normal liver tissue (N). Inserts are lower magnification (5x objective) images. Bar = 20 μm.

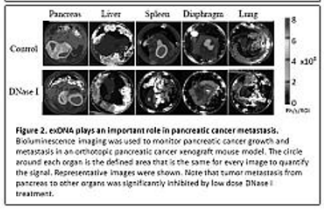

Figure 2. exDNA plays an important role in pancreatic cancer metastasis. Bioluminescence imaging was used to monitor pancreatic cancer growth and metastasis in an orthotopic pancreatic cancer xenograft mouse model. The circle around each organ is the defined area that is the same for every image to quantify the signal. Representative images were shown. Note that tumor metastasis from pancreas to other organs was significantly inhibited by low dose DNase I treatment.

Conclusions: Our results strongly support the hypothesis that exDNA contributes to the invasion and metastasis of pancreatic cancer. The increased presence of exDNA in pancreatic cancer and its role in metastasis support its potential as a useful diagnostic, prognostic or predictive biomarker as well as therapeutic target for pancreatic cancer.

1793 SOX9: A Novel Marker for Pancreatic Centroacinar Cell and Ductal Cell Differentiation

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Background: The function of the DNA binding protein SOX9 [SRY (Sex determining region Y)-box9] in chondrocyte, gonadal and neural crest differentiation has been well established. Recent studies from mouse models have implicated that SOX9 plays a role in pancreatic development. We therefore examined the expression of SOX9 in benign pancreatic tissue (BP) and different types of pancreatic neoplasms.

Design: We constructed tissue microarrays using archival tissue samples from 145 patients with pancreatic ductal adenocarcinoma (PDA), 39 patients with pancreatic neuroendocrine tumor (PEN), 23 patients with solid pseudopapillary neoplasm (SPN), 9 patients with acinar cell carcinoma (ACC) and their matched BP samples from 146 patients who underwent pancreatectomy at our institution. Benign pancreas and tumor were sampled in duplicate with a 1.0 mm punch from representative areas. Nuclear expression of SOX9 was evaluated by immunohistochemistry using a polyclonal antibody (1:2000, Millipore) and categorized as SOX9-negative (no staining in the tumor cells) or SOX9-positive (>20% staining in tumor cells). The nuclear expression of SOX9 was correlated with the clinicopathologic parameters.

Results: Nuclear expression of SOX9 was detected in the centroacinar cells and pancreatic ductal epithelial cells in 100% BP samples (146/146), 89.0% (129/145) PDAs, 2% (1/39) PENs, 11% (1/9) of ACCs and 0% (0/23) SPNs ($P<0.05$). No correlation between the nuclear expression of SOX9 and other clinicopathologic parameters were identified ($P>0.05$).

Conclusions: The expression of SOX9 in centroacinar and pancreatic ductal epithelial cells but not in acinar or endocrine cells of the pancreas suggests that SOX9 may have an important role in ductal differentiation. In addition, nuclear expression of SOX9 in PDA's may be a useful tool to distinguish it from challenging cases of SPT, PEN and ACC.

1794 A Comparison of the Morphology of Familial and Sporadic Pancreatic Cancers

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Background: Nearly 10% of patients with pancreatic cancer report a family history of the disease. Genetic syndromes, including Peutz-Jeghers and familial breast cancer (BRCA-2) increase pancreatic cancer risk. Certain subtypes of pancreatic cancer (such as medullary carcinoma) can occur more frequently in given genetic syndromes (such as Hereditary Nonpolyposis Colorectal Cancer Syndrome). The purpose of this study was to compare the morphology of familial (FC) and sporadic (SC) pancreatic cancer, with FC defined as a pancreatic cancer that occurred in an individual with a first-degree relative who also had a diagnosis of pancreatic cancer.

Design: The National Familial Pancreas Tumor Registry (NFPTR) is a research study enrolling patients with a family history of pancreatic cancer. The NFPTR includes 1201 patients with pancreatic resections whose pathology was assessed at our center. These included 529 patients with familial pancreatic cancer and 672 apparently sporadic pancreatic cancer patients. We retrospectively reviewed all available pathology slides in a blinded fashion.

Results: Primary tumor types were classified as moderately-differentiated ductal carcinoma (FC=307, SC=357); Poorly-differentiated ductal carcinoma (FC=156, SC=238), Well-differentiated ductal carcinoma (FC=23, SC=45) mixed ductal/neuroendocrine carcinoma (FC=2); well-differentiated neuroendocrine neoplasm (FC=2, SC=2); medullary (FC=1), adenosquamous (FC=6, SC=8), colloid (FC=4, SC=11), acinar (SC=1), large duct adenocarcinoma (FC=1, SC=4), undifferentiated (FC=5, SC=4), undifferentiated with osteoclast-like giant cells (FC=1, SC=1), solid-pseudopapillary neoplasm (SC=1) and neuroendocrine (FC=2, SC=2). No statistically significant differences were noted in the primary tumor types between FC's and SC's ($p=0.53$, overall or only non-adenocarcinomas).

Conclusions: We report the pathology of 1201 familial pancreatic cancers. Although certain subtypes of pancreatic cancer have historically been linked with genetic syndromes, the current study illustrates no statistically significant differences between the morphologies of familial vs. sporadic pancreatic cancers among the patients in our cohort.

1795 Analysis of KRAS Mutations in 518 Aspirated Pancreatic Cyst Fluid Samples: KRAS Is Highly Specific for Intraductal Papillary Mucinous Neoplasms but Fails To Detect Mucinous Cystic Neoplasms

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Background: With improvements in abdominal imaging, incidental pancreatic cysts are becoming increasingly common. Analysis of pancreatic cyst fluid from fine needle aspiration (FNA) is particularly important in identifying intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN), which have significant implications in clinical intervention and follow-up. Previous controlled studies have shown that KRAS mutations in pancreatic cyst FNAs are highly specific for both IPMNs and MCNs; however, this has not been examined prospectively in the clinical setting.

Design: A total of 518 pancreatic cyst FNAs were submitted for KRAS codons 12 and 13 mutational analyses between 2007 and 2012. In all cases, samples were submitted by the endoscopist due to uncertainty as to whether a pancreatic cyst represented either an IPMN or MCN. Of the 518 specimens, 505 (97%) were satisfactory for molecular analysis. Cytopathology slides as well as subsequent surgical resection material were also reviewed.

Results: KRAS mutational analysis was performed on 505 pancreatic cyst FNAs from 448 patients. Patients ranged in age from 17 to 90 years (mean, 63.7 years) and were predominantly female (77%). Pancreatic cysts were relatively evenly distributed throughout the pancreas and ranged in size from 0.6 to 10.1 cm (mean, 2.2 cm). Mutations in KRAS were detected in 197 of 505 (39%) FNAs. Although sufficient for molecular studies, 268 of 505 (53%) specimens were either less than optimal (37.8%) or unsatisfactory (15.2%) for cytopathologic diagnosis. Surgical follow-up information was available for 98 of 448 (22%) patients and corresponded to 41 KRAS-mutated and 57 KRAS wild-type cysts. Of 57 resected IPMNs, KRAS mutations had an overall specificity of 95% and overall sensitivity of 68%. In contrast, KRAS mutations were neither specific nor sensitive for MCNs with 1 of 14 cases harboring a KRAS mutation.

Conclusions: Although the sensitivity was low, KRAS mutations in pancreatic cyst FNAs was highly specific for IPMNs. However, contrary to published reports, KRAS mutations were inadequate in identifying MCNs. Future molecular studies including other fluid markers are required to improve the detection and classification of pancreatic mucinous neoplasms.

1796 Protein Expression of the SWI/SNF Chromatin Remolding Subunits in Intraductal Papillary Mucinous Neoplasm (IPMN) and Pancreatic Ductal Adenocarcinoma (PDAC)

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Background: Pancreatic adenocarcinoma remains one of the most intractable cancers to date, with an extremely high mortality rate, and IPMN is one of the precursor lesions to PDAC. An understanding of their molecular alternations may lead to therapeutic strategies. A recent study reported mutations of the genes encoding SWI/SNF complex subunits, namely ARID1A, BRG1 and PBRM1, in a minority of PDACs. Another study has found decreased protein expression of BRG1 in about half of 60 IPMN lesions, most frequently in high-grade dysplasia (HGD). However, little is known about protein expression of the other subunits in PDAC and IPMN.

Design: Our study cohort consisted of 185 IPMN lesions from 109 patients and 90 PDACs. Of the IPMNs, 86 were classified as gastric, 79 intestinal, 14 oncocytic, and 6 pancreatobiliary (PB) epithelial subtypes; 32 low-grade dysplasia, 64 intermediate-grade dysplasia, 63 HGD and 26 invasive IPMN (15 tubular, 10 colloid and 1 oncocytic carcinomas). Protein expression of BRG1, ARID1A and PBRM1 were evaluated using tissue microarrays of IPMN and PDAC by immunohistochemistry. The nuclear expression of each protein was scored using H-score (0-3 intensity and % of the positive cells) in each lesion. The cut-off for positive expression was set at the H-score of 100 or greater.

Results: Of 166 IPMN lesions available for BRG1 evaluation, 58 (35%) showed negative expression (14 with complete loss of expression). The negative BRG1 expression was more prevalent in the PB type (71%) than the gastric (33%), intestinal (33%) or oncocytic (36%) types (PB vs. others, $p = 0.051$). There was no significant difference in the BRG1 expression between different histological grades, including invasive IPMN. Of 90 PDACs, 30 (33%) showed negative expression of BRG1. As for PBRM1, loss of expression was seen in 11 IPMN lesions, including 4 HGD and 3 tubular carcinomas. PBRM1 expression was preserved in the PDAC cohort, and ARID1A expression was also preserved in both IPMN and PDAC lesions.

Conclusions: Decreased BRG1 expression is seen in both PDACs and IPMNs in our study, but to a lesser degree than what was reported in the previous study (~33% vs. ~50%). There is a significant association of negative BRG1 expression in PB-type IPMNs, which are essentially classified as HGD. Although not frequent, reduced expression of PBRM1 is also seen in IPMN, particularly in higher-grade lesions. Our results indicate that alterations of the SWI/SNF chromatin remolding complex may play a role in the progression of pancreatic ductal neoplasia.

1797 SMAD4 Expression in Mucin-Producing Cystic Neoplasms of the Pancreas and Associated Carcinomas

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Background: SMAD4, a tumor suppressor mediating TGF-β superfamily signaling, is inactivated in 55% of ductal adenocarcinomas (DA) by homozygous deletion or inactivating mutation/allelic loss. These mechanisms lead to complete absence of SMAD4 expression (ubiquitously expressed in the nucleus/cytoplasm of non-neoplastic tissue). Loss of expression is well-characterized in DA, and SMAD4 immunohistochemistry (IHC) is often used in the distinction of DA from reactive lesions in small tissue samples. Data regarding expression in DA precursors is limited and, particularly as regards intraductal papillary mucinous neoplasm (IPMN), conflicting. For example, 1 group found intact expression in all non-invasive IPMN's and 97% of invasive carcinomas (inv) ex IPMN, while another found SMAD4 to be absent in 75% of these cancers.

Design: Tissue microarrays (TMA) were made from 13 mucinous cystic neoplasms (MCN), 34 IPMN, and 43 normal pancreata. Effort was made to capture varying dysplasia grades (low [lg], moderate-high [hg]) and inv from a single tumor; different areas were each arrayed in duplicate. Samples included 9 inv (7 DA, 2 colloid), all ex IPMN. SMAD4 IHC was graded as intact, weak, and absent (lost). Cores without intact internal control were excluded. For discrepant cores from the same component of a tumor, grade was based on the "more intact" staining. Given preliminary findings, we also stained whole sections from 10 separate MCN.

Results: No MCN (or normal pancreas) showed absent staining. 5/9 (56%) inv ex IPMN displayed absent SMAD4; all were DA. Loss was also seen in the corresponding hg non-invasive component in all 4 tumors in which it was sampled (overall loss in hg: 4/27; 15%). The rate of loss in IPMN-lg was 13% (2/15), although in 1 of these a corresponding hg area was intact. In fact, on initial blinded review, we were surprised by frequent weak or absent expression in individual lg-appearing cores (e.g., MCN-lg: 29% weak, 8% lost). This prompted evaluation of whole sections, which showed heterogeneous staining (modulating between all 3 scores in a single section) in 8 and uniform, intact staining in 2.

Conclusions: We found a similar rate of SMAD4 loss in carcinoma ex IPMN as has been reported for all DA. Interestingly, this was confined to the ductal subtype. Frequent heterogeneity of staining in our TMA cores, especially in lg lesions, highlights a potential pitfall in similarly sized small biopsies/cytology specimens and underscores the importance of verifying TMA findings in whole sections.

1798 Metastasis Suppressors KISS-1, RKIP and GPR54 Are Expressed in Pancreatic Neuroendocrine Tumors and Their Expression Is Altered in Metastases

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Background: Pancreatic neuroendocrine tumors (PNET) are a group of neoplasms whose metastatic potential and lethality can be difficult to predict. Metastasis suppressors have biologic effects on metastasis but do not impact tumorigenicity. Kisspeptin-1 (KISS-1) and Raf kinase inhibitory protein (RKIP) are metastasis suppressors known to play biological roles in pancreatic islet cells. GPR54 is a putative receptor for KISS1. These proteins have not been studied in PNET, thus we sought to determine their expression and biological impact in PNET.

Design: Tissue microarrays (TMAs) of PNET (n=107) were constructed from archived samples. These TMAs consisted of both primary (n=85) as well as paired and unpaired metastatic samples (n=22). TMAs were immunohistochemically stained for KISS-1, RKIP and GPR54 and scored as strongly positive (2+), weakly positive (1+), or negative (0).

Results: The expression of KISS-1, RKIP and GPR54 are shown in Table 1.

Table 1. Expression of KISS-1, RKIP, and GPR54 in Primary and Metastatic PNET

		Score		
Protein	**Site**	**2+**	**1+**	**0**
KISS-1	Primary	15% (13/85)	38% (32/85)	47% (40/85)
KISS-1	Metastasis	5% (1/22)	59% (13/22)	36% (8/22)
RKIP	Primary	46% (38/82)*	44% (36/82)	10% (8/82)
RKIP	Metastasis	33% (7/21)*	38% (8/21)	29% (6/21)
GPR54	Primary	24% (20/82)	42% (34/82)	34% (28/82)
GPR54	Metastasis	14% (3/21)	38% (8/21)	47% (10/21)

*-Statistically Significant

RKIP was statistically down-regulated (p= 0.027) in metastatic tumors compared to primary tumors, while there were no significant differences in KISS-1 and GPR54. Furthermore, the expression of KISS-1 strongly correlated with the expression of GPR-54 (p<0.001). Correlation between expression levels of KISS-1, RKIP, GPR54 with tumor and clinical parameters are presented in Table 2.

Table 2. Correlation between KISS-1, RKIP and GPR54 and Pathological and Clinical Parameters

	KISS-1	**RKIP**	**GPR54**
	p	p	p
Mitotic Count	0.935	0.09	0.951
Ki-67	0.785	0.026**	0.982
Tumor Size	0.124	0.374	0.983
Presence of Metastasis	0.033**	0.184	0.727
Length of Survival	0.320	0.136	0.936

**-Statistically Significant

Overexpression of KISS-1 was associated with the presence of metastasis while absence of RKIP expression was associated with a higher Ki-67 proliferation rate.

Conclusions: These results suggest that KISS-1, RKIP and GPR54 are expressed in PNET and that RKIP may act as a metastasis suppressor due to its loss in metastatic lesions. Finally, overexpression of KISS-1 may promote metastasis.

1799 Multicenter Study of Pancreatic Juice Cytology Scoring System for Differential Diagnosis in Pancreatic Neoplasm

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Background: We perform multicenter study for diagnostic precision improvement of the pancreatic juice cytology and examined the scoring system for the differential diagnoses in pancreatic neoplasm.

Design: The pancreatic juice cytology specimens were obtained from the patients pathologically diagnosed with intraductal papillary mucinous adenoma/carcinoma: IPMA/C and invasive ductal carcinoma: IDC. Using the virtual slide microscopy, twenty-eight cytotechnologists of many institutes participated in this examination. Several multivariate analyses were carried out to sort the diagnostic clue of cell findings to be important. Furthermore, ROC analysis was performed whether it was diagnostic using the selected factors.

Results: As a result of multivariate analysis, the important findings to distinguish adenocarcinoma from adenoma were the mucinous background, the intracytoplasmic mucin, the cellular stratification, the peripheral irregularity of cell cluster, the irregular nuclear arrangement, the irregular nuclear appearance and the abnormal chromatin distribution. By the ROC analysis, the degree of precision for the differential diagnosis showed the 96 % of sensitivity and the 90% of specificity between the IPMAs and the IPMCs, and showed the 86% of specificity and the 96% of sensitivity between the IPMAs and the IPMCs+IDCs.

Conclusions: By using pancreatic juice cytology in this scoring system, the possibility to determine with high accuracy IPMA and IPMC was suggested. This diagnostic system might be expected as an examination to contribute to the patient stratification.

1800 Activation of Src and STAT3 in Intraductal Papillary Mucinous Neoplasm of the Pancreas (IPMN)

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Background: The STAT3 transcription factor is an important regulator of stem cell self-renewal, cancer cell survival, and inflammation, and the majority of pancreatic ductal adenocarcinomas (PDACs) show constitutive activation of STAT3, classically mediated by the JAK family of tyrosine kinases. Intraductal papillary mucinous neoplasm (IPMN) is a precursor to PDAC and is being increasingly diagnosed due to advances in imaging modalities. Recent studies have shown that somatic *GNAS* activating mutations are present in two thirds of IPMNs, and a fraction of benign and malignant hepatocellular tumors with an inflammatory phenotype. In the latter, the weak activation of STAT3 via Src has been reported. Thus, the aims of this study are: 1) to evaluate Src activation in IPMNs; 2) to correlate it with STAT3 activation and histomorphologic features.

Design: The study cohort consists of 86 IPMN lesions from 53 pancreatectomies. Of those, 42 were of the gastric type (IPMN-G), 33 the intestinal type (IPMN-I), 3 the pancreatobiliary type (IPMN-PB), and 8 the oncocytic type (IPMN-O). The grade of dysplasia was low (LGD) in 17, intermediate (IGD) in 31, and high (HGD) in 29, and 9 were invasive carcinoma (INV, 4 tubular and 5 colloid). The protein expressions of phosphorylated Src (pSrc) and STAT3 (pSTAT3) were evaluated using the H-scores (maximum score 300). The cut-offs for positive expression were set at 50.

Results: pSrc expression was seen in 49% of IPMN lesions and was correlated with that of pSTAT3 (Pearson r = 0.3648, P=0.0006). pSrc expression was significantly more prevalent in IPMN-G (67%) than IPMN-I (33%), IPMN-PB (33%) and IPMN-O (25%) (IPMN-G vs. others, P=0.0023). Similarly, Src was more frequently activated in LGD (76%) and IGD (55%) compared to HGD (34%) and INV (22%) (LGD + IGD vs. HGD + INV, P=0.0052). pSTAT3 expression was positive in 28% of IPMNs and there was no difference in the expression between epithelial types or grade.

Conclusions: Src is frequently activated in IPMNs and Src activation is related to STAT3 activation. Given the more prevalent Src activation seen in LGD and IGD, it may be involved in the initiation and/or development of IPMN.

1801 Tumor-Infiltrating Lymphocytes Do Not Affect Ki-67 Labeling Index in a Series of Pancreatic Neuroendocrine Tumors (PanNETs)

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Background: Pancreatic neuroendocrine tumors (PanNETs) show poor clinical outcomes, with 10-year overall survival of 45%. The World Health Organization (WHO) 2010 grading system for PanNETs permits the use of either mitotic rate or Ki-67/MIB-1 proliferative index (Grade 1: < 2 mitoses/10HPF or < 3% Ki-67 index; Grade 2: 2-20 mitoses/HPF or 3-20% Ki-67 index), which may lead to discordant grading by method used. We postulated that the presence of tumor-infiltrating lymphocytes, which may label for Ki-67, falsely elevates the Ki-67 labeling index in PanNETs with discordant grading.

Design: A dual immunohistochemical stain protocol was employed using the Ki-67 anti-CD45 antibodies (mouse monoclonal, anti-human CD45 Leucocyte Common Antigen (clone 2B11+PD7/27) and rabbit monoclonal anti-Ki67 (clone 30-9)) on 17 PanNETs with discordant grading resected at a single institution (1989-2009) with available histologic material. Ki-67+, CD45+, and Ki-67+/CD45+ labeling indices were obtained by averaging 10 high-powered fields per tumor and expressed as a percentage of total tumoral cells. Statistical analysis was performed using a paired T-test at 95% certainty.

Results: The average patient age was 54.2 years at diagnosis (58.8% female, 41.2% male), all with unifocal PanNETs (average size 4.3 cm) arising in the pancreatic head (47%, 8/17), uncinate (5.9%, 1/17), body (11.8%, 2/17), and tail (35.3%, 6/17). Using the Ki-67 labeling index cutoff of 3%, 35.3% (6/17) were Grade 1 and 64.7% (11/17)

were Grade 2. The CD45 labeling indices for Grade 1 and 2 tumors were 7.4 ± 19.7% and 4.7 ± 11.1% (p=0.47). The Ki-67+/CD45+ labeling indices for Grade 1 and 2 tumors were 0.0029 ± 0.10% and 0.60 ± 3.6% (p=0.45).
Conclusions: These results suggest that tumor-infiltrating lymphocytes did not affect the Ki-67 labeling index in this series of PanNETs, and will require validation with a larger patient population.

1802 MicroRNAs as Diagnostic Markers for Pancreatic Ductal Adenocarcinoma and Pancreatic Intraepithelial Neoplasm

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Background: Since the discovery of small non-coding RNAs, the analysis of microRNA (miRNA) expression patterns in human cancer have provided new insights into cancer biology. Evidence suggests that deregulated miRNA expression is associated with pancreatic cancer development. In this study, we analyzed the expression of several miRNAs in different types of pancreatic disease to determine if miRNAs expression could aid the diagnosis of pancreatic ductal adenocarcinoma (PDAC) and its precursor – pancreatic intraepithelial neoplasm (PanIN).
Design: Resection specimens containing PDAC (n=16), paired pancreatic tissue with negative margin in each case (n=16), chronic pancreatitis (n= 4), normal pancreatic parenchyma (n=5), PanIN (n=5) with different grade of dysplasia (I to III) were selected from our department archive between 2004 and 2011. These formalin-fixed paraffin embedded tissue blocks were evaluated for miR-148a, miR-196 and miR-217 expression by quantitative reverse transcription polymerase chain reaction.
Results: Our data show that miR-148a and miR-217 expression levels were significantly down-regulated in PanIN and PDAC compared to the normal pancreatic parenchyma. Comparison of these miRNA levels between the neoplastic lesions and chronic pancreatitis showed that miR-148a ($P < 0.05$) and miR-217 levels ($P < 0.0001$) were much lower in PDAC. Dramatic reduction of expression levels in PDAC was further confirmed by comparing their levels in PDAC and paired controls with negative margin which predominantly consists of chronic pancreatitis. In addition, we observed that miR-148a levels were much lower in PanIN than in chronic pancreatitis ($P < 0.05$). MiR-217 expression level also decreased, although to a lesser extent, in PanIN compared to in chronic pancreatitis ($P = 0.09$). In contrast, the level of miR-196 expression was significantly overexpressed in PanIN ($P<0.0001$) and PDAC ($P<0.0001$) compared to the non-neoplastic pancreatic parenchyma. Interestingly, the degree of deregulation of all three miRNA markers is much higher in PanIN II-III compared to that in PanIN I.
Conclusions: Our study demonstrates that miR-148a, miR-217 and miR-196a are significantly deregulated in pancreatic ductal adenocarcinoma, including in the early stage of the pancreatic ductal adenocarcinoma. Thus, these markers can be potentially used as diagnostic markers to distinguish pancreatic ductal adenocarcinoma and its precursor from benign lesions.

1803 Molecular Comparison between Intraductal Tubulopapillary Neoplasms and Intraductal Tubular Adenomas of the Pancreas Indicates Their Distinctive Nature

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Background: Intraductal tubulopapillary neoplasm (ITPN) is composed of tubulopapillary masses with high-grade atypia in the pancreatic duct. Intraductal tubular adenoma (ITA) is composed of tubular glands mimicking pyloric glands with low-grade atypia. Some may consider that ITA could be a benign counter part of ITPN, however, whether ITPN and ITA are distinctive or not is not fully assessed. In this study, we compared molecular features between ITPNs and ITAs to know their distinct or similar nature.
Design: Formalin-fixed, paraffin-embedded tissues of 14 ITPNs and 15 ITAs were investigated in this study. Foci of tumor and normal tissues were dissected separately from serial sections under microscopic guidance. Genomic DNA was extracted and somatic mutations in exons 10 and 21 of *PIK3CA*, exons 8 and 9 of *GNAS*, exons 2 and 3 of *KRAS,* and exon 15 of *BRAF* were analyzed.
Results: Somatic mutations in *PIK3CA* were found in 3 of 14 ITPNs (21.4%) but not in any of ITAs ($P = 0.0996$; Fisher exact test). In contrast, mutations in *GNAS* were found in none of the ITPNs but were found in 9 of 15 ITAs (60.0%) ($P < 0.001$; Fisher exact test). Mutations in *KRAS* were detected in 1 of 14 ITPNs (7.1%) and 12 of 15 ITAs (80.0%) ($P<0.001$; Fisher exact test). *BRAF* mutation was found in 1 ITPNs but none of ITAs.
Conclusions: These results clearly indicate that ITPNs and ITAs are molecularly distinctive from each other, which suggests that ITPNs do not evolve from ITAs. Furthermore, the molecular features of ITAs were similar to those reported in IPMNs, i.e., prevailed mutations in *GNAS* and *KRAS*, which is consistent with the renewed (2010) World Health Organization classification system for intraductal neoplasms of the pancreas that describes that intraductal neoplasms are classified into IPMNs and ITPNs, and ITA is a variant of IPMNs.

1804 Inconclusive Cytology and Non-Contributory Cyst Fluid Tumor Marker Analysis of Pancreatic Cystic Lesion: Is It the End of the Story?

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Background: Pancreatic cystic lesions constitute a broad spectrum of entities ranging from non-mucinous to mucinous and benign to malignant cysts. Endoscopic ultrasound -guided fine needle aspiration (EUS-FNA) cytology is routinely utilized for pre-operative diagnosis. Cyst fluid obtained from EUS-FNA is commonly submitted for chemical analysis of tumor markers to aid in the diagnosis. However, the value of cytologic interpretation is frequently limited by low cellularity of aspirated fluid and the reliability of cyst fluid tumor marker is variable. Recently, molecular tests of cyst fluid have been applied and show high specificity in the differentiation of the pancreatic cystic lesions. In this study, we investigated the usefulness of molecular analysis of pancreatic cyst fluid in the setting of inconclusive cytology and non-contributory tumor markers test.
Design: We retrospectively reviewed pancreatic cystic lesions with EUS-FNA cytology, cyst fluid tumor markers (CEA and amylase) and molecular tests (K-ras mutation and loss of heterozygocity) from 2009-2011. The inconclusive cytology diagnosis was defined as "non-diagnostic" or "atypical cytology". Non-contributory cyst fluid chemical analysis includes 2 situations: 1) amylase<250U/L and CEA <192 ng/ml or 2) amylase >250 U/L and CEA >192ng/ml. The molecular tests and the histopathologic results of the resection specimens were analyzed.
Results: The cohort consists of 32 pancreatic cyst EUS-FNA cytology cases, which shows 20 cases (62.5%) with non-diagnostic (34.4%) or atypical (28.1%) cytology. Among the 20 cases, non-contributory tumor marker analysis was found in 11 cases (55%). The molecular analysis shows K-ras mutation or loss of heterozygocity in 5 out of 11 cases (45%), which also indicates a mucinous cystic neoplasm with potential for neoplastic progression. Interestingly, 3 out of 4 pancreatic cyst resection specimens showed pancreatic intraepithelial neoplasias (PanIN)-1A or 1B with benign molecular features; while elevated cystic fluid CEA and amylase were identified in all the cases with PanIN-1A and 1B.
Conclusions: Inconclusive pancreatic cyst cytology and non-contributory cystic fluid tumor markers were frequently encountered, making accurate diagnosis challenging. Our study indicates that cyst fluid molecular tests for K-ras mutation and allelic imbalance provide extra information and may help with the diagnosis and clinical management. Our novel finding is that PanIN-1A and 1B might be related to both elevated cyst fluid CEA and amylase without K-ras mutation.

Pan-genomic/Pan-proteomic approaches to Cancer

1805 Characterization of Cell Type Specific miRNA Profiles and Application to miRNA Profiles Derived from Tissues

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Background: MicroRNAs (miRNAs) are highly conserved RNAs that serve as master regulators of gene expression. They are exciting biomarkers and therapeutic targets. Studies have found altered expression of miRNAs in tissue disease states; however, the interpretation of these studies may be fundamentally flawed because the changing cellular composition of tissues in disease states is not adequately accounted for. Techniques to control for this source of variability should increase the relevance of changes in miRNA species identified in profiling studies of diseased tissue.
Design: Our approach characterizes both factors that can affect miRNA expression levels: altered cellular composition of tissue and disease specific expression changes. We have utilized miRNA expression profiles from a panel of cell types to deconvolute observed miRNA profiles generated from various tissues. We have then explored the impact of this two factor analysis via a modeling study of ulcerative colitis.
Results: We utilized publicly available data from 16 cell types including inflammatory, endothelial, stromal, and epithelial cells to predict the cellular composition and model the observed expression of the miRNA expression profile from 4 tissues (Fig. 1A). The predicted cellular compositions are consistent with the expected composition of these tissues and the modeled miRNA levels are highly correlated to the observed signals. We next performed a modeling experiment of ulcerative colitis using image analysis to predict cellular ratio changes (Fig. 1B). We generated hypothetical miRNA profiling data of normal colon and ulcerative colitis in which we manually altered only 3 miRNAs at the cell level – potential disease specific changes. We then analyzed the data via routine univariate hypothesis testing with and without correction for changes in tissue composition. Without tissue composition correction, most miRNAs identified are false positives (Fig. 1C).