1765 Hemangioblastoma: A Targeted Next Generation Sequencing Study

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Background: Hemangioblastomas (HB) are rare CNS tumors and up to 25 % are associated with von Hippel-Lindau syndrome and biallelic inactivation of VHL genes is an important mechanism for pathogenesis. Same mutation is also seen in clear cell renal cell carcinoma (ccRCC) and part explains the overlap of histomorphological and immunohistochemical characteristics. However, molecular difference between the ccRCC and HB are not yet extensively studied. In this study we looked at common gene mutations by targeted NGS in HB and compared to those in ccRCC.

Design: Our study composed of two sections: 1) analysis of targeted genetic sequence and 2) study of expression of CAIX in hemangioblastoma by IHC and correlate with VHL mutation status. Five cases of hemangioblastom from elderly patients (mean = 53) with no known family history of VHL disease were selected. Macrodissection of formalin-fixed paraffin-embedded (FFPE) tissue corresponding to Hematoxylin and Eosin (HE)-stained slides was used to ensure that at least 20% of the retrieved cells were neoplastic. Genomic DNA was extracted from FFPE tissues by Qiagen AllPrep DNA/RNA Kit. The isolated genomic DNA was subject to targeted sequencing by using Illumina TruSeq Amplicon - Cancer Panel which includes highly cancer-associated 48 genes.

Results: The most common mutation was APC (3/5 cases), ATM (3/5 cases), ErbB family mutations (3/5 cases). The angiogenesis pathway including the genes EGFR and FGFR are seen in 2/5 cases. PTPN11 gene was also found in 2/5 cases. However VHL mutation was not found in any of the cases. However, CAIX was expressed diffuse and strongly in all 5 cases of hemangioblastoma. Therefore, expression of CAIX in absence of VHL mutation raises possibility of alternate interactions of downstream proteins, HIFa and CAIX, EGFR and FGFR.

Conclusions: In this study, we demonstrated multiple genes that are mutated in hemangioblastoma, especially, APC, ATM and ErbB family mutations are highly associated with this tumor. Notably, VHL mutation was not detected among the cases, may indicate VHL structure variant and/or epigenetic changes. Our data demonstrate clearly difference between clear cell renal cell carcinoma and hemangioblastomas at molecular level.

1766 Detection of IDH1/2 and TP53 Mutations and MGMT Methylation in High Grade Gliomas of the Central Nervous System by the Next Generation Sequencing and Pyrosequencing

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Background: Genetic alternations in gliomas, including IHD1/2 and MGMT promoter methylation, are important markers for treatment plan and prognosis. The next generation sequencing (NGS) offers a convenient means of surveying mutations in common cancer associated genes, including TP53 and IDH1/2 genes. In addition, pyrosequencing provides a sensitive means to detect MGMT promoter methylation.

Design: We retrospectively reviewed twenty-nine patients diagnosed with the CNS gliomas. We subgrouped the patients based on the diagnosis and correlated with the IDH1/2 and P53 mutations as well as MGMT methylation status.

Results: Among 29 patients, 20 were diagnosed with high grade gliomas, including 3 anaplastic oligodendrogliomas (grade III), 8 anaplastic astrocytomas (grade III) and 9 glioblastomas (GBM). Nine patients had low grade gliomas, including 6 astrocytomas (grade I and II) and 3 gangliongliomas (grade I). IDH 1/2 mutations were detected in high grade gliomas, including 4 anaplastic astrocytoma and 3 anaplastic oligodendroglioma (grade III). Only one of 9 GBMs had IDH1 mutation. IDH1/2 mutations detected by the NGS were also compared with IDH immunohistochemistry (IHC) results. IDH mutations were found in all IHC-positive tumors, in contract, IHC was positive in only 50% tumors with NGS-confirmed IDH mutations. Moreover, MGMT methylation detected in 11 (55%) high grade gliomas. As expected 90% of TP53 mutations were also found in high grade gliomas.

	No. of patients	Average age in year (range)	IDH1 R132H	MGMT methylation	TP53 mutation
Ganglionglioma (Grade I)	3	46 (26-73)	0	0	0
Astrocytoma (Grade I)	2	17 (7-27)	0	0	0
Astrocytoma (Grade II)	4	53 (21-84)	0	0	1
Anaplastic oligodendroglioma (Grade III)	3	38.3 (34-40)	3* with an IDH2 R172K	1	0
Anaplastic astrocytoma (Grade III)	8	36.5 (26-67)	4	5	6
Glioblastoma (Grade IV)	9	53 (29-74)	1	6	3
Total	29		8	12	10

Conclusions: Our results showed that NGS is more sensitive and specific to detect IDH1/2 mutations than IHC and should be the method of choice. Moreover, our data demonstrated that IDH1/2 and TP53 mutations and MGMT methylation occur concomitantly in high grade glioma, especially anaplastic oligodendroglioma and astrocytoma, suggesting their interactive roles in the development of high grade glioma.

1767 Prognostic Significance and Characterization of a Small Cell Population in Uveal Melanoma

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Background: Uveal melanoma is the most frequent primary intraocular tumor. Despite the use of different treatment modalities, 50% of the patients will develop metastasis, after which the mortality rate is at 90%. Uveal melanoma is composed of two main types of cells: spindle and epithelioid, with the latter associated with a poor prognosis. Nevertheless, there is a subset of small cells located predominately in the infiltrative margins of the tumor and surrounding blood vessels. The aim of the present study is to characterize and evaluate the correlation between the presence of these small cells and clinical outcome.

Design: The clinical and pathological features of 70 patients with uveal melanoma were evaluated. The presence of small cells was quantified as a percentage of the tumor volume (SC). The small cells were characterized using melanocytic markers (HMB-45, Melan A, SOX-10) and stem cell markers (CD133, CD24 and CD38). Univariate and multivariate analysis were conducted to evaluate survival. Clinical details of follow-up visits were available for all patients (mean 148.6 months; SEM 15.1).

Results: For all tumors, SC ranged from 0 to 30% (median: 1%). Thirty-nine tumors (55.7%) had SC areas. Univariate analysis showed that the classic cell type (p=0.006), lymphocytic infiltration (p=0.04), macrophage infiltration (p=0.02), ciliary body involvement (p=0.02) and SC greater than 5% (SC>5%, p<0.0001) had a significant impact on metastasis-free survival. SC was >5% in 24 cases; of these, 3 were spindle-type and 21 were mixed tumors. Multivariate analysis revealed that a SC>5% was the most significant adverse prognostic factor (p<0.001, HR=4.98). Moreover, the small cells were negative for HMB45, focally and weakly positive for MELAN A and SOX10 and were negative for stem cell markers.

Conclusions: A high SC is a strong prognostic indicator in patients with uveal melanoma. Based on the loss of melanocytic markers, these cells are less differentiated than the spindle and epithelioid cells and may represent amoeboid transition. Further characterization may lead to new therapeutical targets and a better understanding of the metastatic process in uveal melanoma.

1768 Programmed Cell Death Ligand 1 Expression in Uveal Melanoma Is Associated with Better Patient Outcome and Decreased Tumor Infiltrating Lymphocytes

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Background: Programmed cell death-1/ligand (PD-1/PD-L1) interaction negatively regulates T-cell activity. PD-L1 expression in tumor cells, antigen-presenting cells and lymphocytes of the tumor microenviroment is associated with response to treatment with PD1/PD-L1 inhibitors, but is still in debate the cutoff value correlated with responders. In uveal melanoma (UM), 40% of patients will develop liver metastases and 90% will succumb to their disease. The aim of this study was to analyze PD-L1 as a prognostic marker and as a possible therapeutic target for UM.

Design: Sixty seven enucleated eyes from UM patients with relevant clinical information were analyzed. Immunostaining was performed with the anti-PD-L1 E1L3N clone and with the retinal pigment epithelium as an internal positive control. PD-L1 knockdown in the 92.1 cell line was used as a negative control. Two types of qualifying PD-L1 expression were analyzed: the conventional method that described >5% membranous staining in tumor cells, and an alternative that defined positivity as >5% in either tumor cell or non-tumor cells (TNT). Cases were evaluated by two ocular pathologists and by using an image analyzer after a 2-hour bleaching protocol. Univariate and multivariate analysis were used to evaluate association of PDL1 with survival, and the kappa score was calculated to compare the agreement between manual and automated evaluation of PD-L1 expression.

Results: PD-L1 expression was positive according to tumor cell and TNT counts in 46.3% and 55.2% of the cases, respectively. With univariate analysis, tumor cell and TNT PD-L1 expression were associated with a longer metastasis free survival (MFS) (*P*=0.04, P=0.007). However, on multivariate analysis, only TNT positivity was associated with longer MFS (P=0.01). Furthermore, tumoral and TNT PD-L1 expression was associated with decreased tumor infiltrating lymphocytes (TIL, P=0.02). Automated evaluation of TNT showed good agreement with the pathologists' evaluation (κ=0.764) and was correlated with longer MFS after both univariate (P=0.01) and multivariate analysis (P=0.02).

Conclusions: PD-L1 is expressed in uveal melanomas and is associated with better outcome and decreased TIL. These results support the evaluation of anti-PD1/PD-L1 therapy in UM. Automated analysis showed good correlation with conventional methods. Due to its higher objectivity and reproducibility, it is a promising tool that can be utilized in designating a cutoff value for future clinical trials.

Pancreas and Biliary Tree

1769 Surgical Resection of the Pancreas for Benign Conditions: A Ten-Year Single Center Retrospective Review

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Background: Surgical resection of the pancreas (SR) is usually performed when there is clinical suspicion of malignancy. SR is sometimes performed for the management of benign conditions, including chronic pancreatitis, however, cases arise when SR is performed for suspected malignancy and the resulting pathologic diagnosis is benign. The purpose of this study is to investigate SR with benign diagnoses that are suspected to be malignant, with a focus on pancreatitis diagnoses.

Design: 726 pancreas SR cases were retrospectively reviewed from 2006-2016. Medical records for select benign cases reviewed in detail to assess the clinical indication for the procedure. Slides from select cases were reviewed by the investigators for accuracy of diagnosis.
Results: The cases included 357 (49.2%) females and 369 (50.8%) with a mean age of 61.6. There were 450 pancreatoduodenectomies (61.8%), 201 distal pancreatectomies (27.5%), 38 partial pancreatectomies (5.2%), 29 total pancreatectomies (4.0%) and 9 complex procedures (1.4%). There were 610 (84.0%) malignant diagnoses and 116 (16.0%) benign diagnoses. Benign diagnoses are summarized in the table below:

	# of cases	%
Pancreatitis	51	44.0
Benign neoplastic	46	39.7
No residual tumor after neoajuvant therapy	7	6.0
Cyst, non-neoplastic	5	4.3
Complex resection, pancreas not involved	4	3.4
Intrapancreatic accesory spleen	3	2.6
Total Benign	116	100.0%

Pancreatitis diagnoses included 40 (78.4%) chronic pancreatitis, 5 (9.8%) groove pancreatitis, 3 (5.9%) autoimmune pancreatitis, and 3 (5.9%) cholangitis. 16 of the 51 (31.4) pancreatitis cases were suspected to be malignant, including 10 of 40 (25.0%) chronic pancreatitis, 1 of 5 groove pancreatitis, 3 of 3 (100%) autoimmune pancreatitis, and 2 of 3 (66.7%) cholangitis. Additionally, there were 3 distal pancreatectomies with intrapancreatic accessory spleens suspected to be malignant.
Conclusions: SR specimens were overall most frequently performed for malignant diagnoses. SR specimens diagnosed as pancreatitis were more frequently indicated for pancreatitis than suspected malignancy.

1770 SMAD4 Expression in Pre-Treatment Fine Needle Aspiration Specimens *Versus* Post-Treatment Surgical Specimens in Patients with Pancreatic Ductal Adenocarcinoma

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Background: SMAD4 is a tumor suppressor gene found on chromosome 18q21.1 and is a key mediator of the transformation growth factor beta signaling pathway. Loss of SMAD4 immunohistochemical staining is observed in more than 60% of the pancreatic ductal adenocarcinoma (PDAC). It has been proposed that SMAD4 loss in PDACs is associated with worse prognosis. As such, stratifying patients based on their pattern of SMAD4 expression may provide useful prognostic information. Preoperative endoscopic-ultrasound fine-needle aspiration (EUS-FNA) offers the first opportunity to classify patients with PDACs into SMAD4-positive and SMAD4-negative PDACs patients. The former may benefit from local chemoradiotherapy, while systemic chemotherapy might be a better therapeutic choice for the latter. We evaluate the validity of (EUS-FNA) as a tool to categorize those patients.
Design: 196 cases of PDAC were retrieved from our surgical department. These cases included 92 treatment naïve EUS-FNA (no prior therapy) and 104 surgical pancreatectomies. 36 of the cases were paired EUS-FNA/ pancreatectomy specimens. 45 of the pancreatectomy specimens were treatment naive, and 59 received prior neoadjuvant therapy. All cases were evaluated for SMAD4 (B8, Santa Cruz, USA) expression by immunohistochemistry. Greater than 10% PDAC nuclear staining was the cutoff for positive staining. Correlation of SMAD4 IHC on paired EUS-FNA/ resection specimens was also determined.
Results: SMAD4 expression was negative in 58/92 (63%) and 53/104 (51%) cytology specimens and pancreatectomy specimens, respectively. Of the 36 patients with paired FNA and surgical resections, SMAD4 expression was concordant in 24 (67%) cases. Where discordant SMAD4 expression was observed, all cases showed SMAD 4 expression on the pancreatectomy specimen.
Conclusions: Loss of SMAD4 expression on FNA cytology specimens is a potential source of false negative results. This may be due to tumor sampling, technical issues related to FNA specimen handling or processing, or inaccurate pathological interpretation. Caution should be taken when interpreting the results of SMAD4 expression on FNA samples in order to avoid incorrectly categorizing those patients.

1771 Altered Vitamin D Receptor Expression in Pancreatic Ductal Adenocarcinoma: Insights into Vitamin D Signaling Pathway in Endocrine and Exocrine Pancreas

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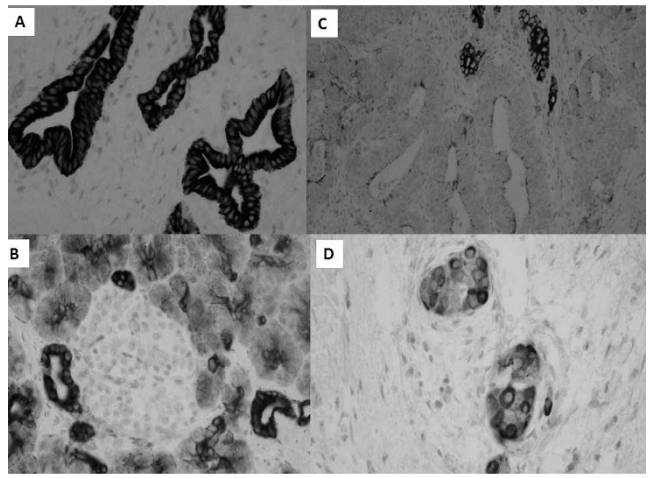
Background: Vitamin D is a pluripotent fat-soluble prohormone that regulates various cellular pathways that affect proliferation, differentiation, and apoptosis. Vitamin D status has been correlated with cancer incidence, prognosis, and mortality. Studies have demonstrated inhibitory effects of vitamin D on the proliferation of pancreatic cancer cell lines. Vitamin D receptor (VDR) has also been identified as a transcriptional regulator in pancreatic stellate cells and studies suggest that vitamin D signals exert antiproliferative and pro-differentiating effects on cancer cells. VDR IHC expression is evaluated to gain insights into the Vitamin D signaling pathway of PDAC.
Design: The pathology database was searched for pancreatic specimens resected between 2008-2015. IHC for VDR was performed and 3 fields were evaluated at 4x. Epithelial cells, endocrine cells of benign cases, and endocrine cells within 1 mm of tumor were assessed for cytoplasmic staining. VDR expression was classified as lost if <50% of tumor epithelial cells showed strong cytoplasmic expression. In endocrine cells, VDR expression was labeled positive if ≥ 5% of cells showed cytoplasmic staining.
Results: We identified benign (n=9) and PDAC (n=29) cases. All benign cases (9/9) showed strong and diffuse VDR expression in epithelial cells (Fig.1A). Most of the endocrine cells of benign cases were negative (Fig.1B) but 2 cases had cytoplasmic VDR expression in endocrine cells (22.2%). In malignant cases, there was loss of epithelial VDR expression (Fig.1C) in 72.4% of cases (21/29) and endocrine expression of VDR (Fig.1D) present in 59.2% (16/27) of cases.



Conclusions: We evaluated the expression of VDR to examine the possible role of vitamin D-related mechanisms in PDAC. Our results suggest that during the development of PDAC, most tumors exhibit alterations of VDR in both in the endocrine and exocrine pancreas. The altered VDR staining in PDAC implies a deregulation of VDR in epithelial cells and increased expression in endocrine cells during pancreatic tumorigenesis. These findings suggest a cross-talk between these two cell types and emphasize the need to study the role of the vitamin D in pancreatic malignancies.

1772 Alternative Lengthening of Telomeres and Loss of ATRX/ DAXX Can Be Reliably Detected in Fine Needle Aspirates of Pancreatic Neuroendocrine Tumors

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Background: The Alternative Lengthening of Telomeres (ALT) pathway for telomere maintenance is often activated in pancreatic neuroendocrine tumors (PanNETs), and ALT is strongly associated with ATRX and DAXX alterations. Recent studies have demonstrated that loss of ATRX/DAXX and activation of ALT is a prognostic marker. Because fine needle aspiration (FNA) is a non-invasive way of sampling tumors, we evaluated whether we could accurately detect ALT and loss of ATRX/DAXX in FNAs of a primary PanNETs. For a subset of cases from patients undergoing tumor resection, we also evaluated the resected case to determine the concordance of the alterations detected in the FNA with those identified in the surgical specimen.
Design: After approval from the Institutional Review Board of a waived consent retrospective data review, the pathology database at the Johns Hopkins Medical Institutions was searched for all pre-operative PanNETs FNA cytology cases between 2005 and 2016. All cases were acquired either under trans-abdominal or endoscopic ultrasound (EUS) FNA with on-site evaluation of adequacy. Cases in which the cell block H&E or subsequent levels contained less than 100 cells were not included due to insufficient material. ATRX and DAXX expression was evaluated by immunohistochemistry and ALT was determined by telomere-specific FISH.
Results: Loss of nuclear expression of DAXX was identified in 5 PanNETs (8%), and loss of nuclear expression of ATRX was identified in 6 PanNETs (10%). All cases of ATRX loss were associated with intact DAXX and vice versa; thus, 11 total cases displayed loss of DAXX or ATRX (18%). ALT was detected in 15 cases (23%). While all ATRX-negative and DAXX-negative tumors were ALT-positive, 3 of 14 (21%) ALT-positive cases did not show alterations in ATRX or DAXX. ALT-positive tumors were associated with larger radiographic tumor size (4.9 v. 2.4 cm, on average, $p<0.05$) and higher grade ($p<0.05$). Overall, there was 100% concordance for ALT status between the FNA and surgical specimen. Additionally, we observed 100% concordance between ATRX/DAXX IHC between the FNA and surgical specimen.
Conclusions: Both ATRX/DAXX IHC and telomere-specific FISH for ALT can be accurately performed on FNA specimens with adequate material. This finding is especially important for patients undergoing surveillance or for patients with unresectable tumors, as ALT status may in the future be used for clinical management.

1773 Intraductal Tubulopapillary Neoplasm of the Pancreas: A Clinicopathologic and Immunohistochemical Analysis of 33 Cases

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Background: Intraductal tubulopapillary neoplasm (ITPN) is a relatively recently described member of the pancreatic intraductal neoplasm family. Thus, the literature on its histologic/immunohistochemical features, clinical behavior, and its similarities/ differences from other pancreatic neoplasms is limited.
Design: Thirty-three cases of ITPN, the largest series to date, were identified. Immunohistochemical labeling for cytokeratins, glycoproteins, pancreatic enzymes,

markers for intestinal and neuroendocrine differentiation, and antibodies associated with genetic alterations previously described in pancreatic neoplasms were performed. Clinicopathologic features/survival was assessed.
Results: Female:male ratio was 1.2. Mean age was 55 yrs (range, 25-79). Median overall tumor size was 4.5 cm (range, 0.5-15). Forty-five percent of the tumors occurred in the head, 32% in the body/tail, and 23% showed diffuse involvement. The tumors were characterized by intraductal nodules composed of tightly packed small tubular glands lined by cuboidal cells lacking apparent mucin. Although it was often challenging to determine its extent, invasion -usually in the form of thin strands of cells/individual glands extending away from the nodules- was present in 71%. Almost all tumors labeled for CAM5.2/CK7/CK19; most expressed CA19.9/mCEA/MUC1/MUC6. CDX2/MUC2/trypsin/chymotrypsin/chromogranin/synaptophysin were not expressed. SMAD4 expression was retained in 100%, p16 expression and nuclear p53 acumulation was seen in 33% and 27%. Follow-up information was available for 22 patients (median, 45 mos; range, 11-173). Two with invasive carcinoma died of disease at 23 and 41 mos. One died of unrelated causes at 49 mos. Twelve were alive with disease (4 had histologically proven local recurrence, 3 had liver metastasis). Seven were alive with no evidence of disease. The overall 1-, 3- and 5-yr survival rates were 100% in patients without an invasive component and 100%, 91% and 71% in patients with an invasive component (p=0.7).
Conclusions: ITPN is a distinct clinicopathologic entity in the pancreas. Despite the difficulties of determining the extent of invasive carcinoma in many cases, the overall outcome appears relatively favorable and substantially better than that of conventional ductal adenocarcinoma, even when only the cases with invasive carcinoma are considered.

1774 Pathologic Findings from Totally Sampled and Mapped Gallbladders in a High-Risk Population

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Background: To better understand the distribution of pathologic abnormalities in the gallbladder, we fully sampled and mapped gallbladders in a high-risk region in Chile, focusing on women given their higher risk of gallstones and gallbladder cancer (GBC).
Design: We recruited 140 female cholecystectomy patients from two hospitals in Temuco and Pucon, Chile. The gallbladders were submitted totally and mapped using a standardized protocol.
Results: The mean age was 49 (range=37-70), with 8 blocks on average. On initial review, 3 patients (2%) had GBC, 16 had dysplasia (13 low-grade, 3 high-grade), 65 had intestinal metaplasia, and 88 had pseudopyloric metaplasia (not mutually exclusive). Comparing the presence of dysplasia with metaplastic changes, 94% (15/16) had intestinal metaplasia, compared to 68% (50/74) of patients without dysplasia ($\chi 2$ $p<0.01$). All GBCs (100%) and 8 dysplasias (50%) were confirmed on independent review. Patients with and without dysplasia were similar in terms of age (47 vs 49 years), length of the gallbladder (9 cm), and chronic inflammation score (1.2 vs 1.5). Similarly, age, gallbladder length, and chronic inflammation score did not vary by the presence or absence of epithelial atypia, which occurred in 52 (38%). Only 3 (2%) had adenomyoma and 7 (5%) had polyps (3 adenoma tubular, 2 cholesterol, 2 metaplastic), but 49 (35%) had cholesterolosis, 31 (22%) had PMNs in the epithelium, and 35 (25%) had PMNs in the stroma.
Conclusions: In this group of 140 female cholecystectomy patients from a high-risk area in Chile, a high proportion had histologic abnormalities. Agreement was good for the diagnosis of cancer, but poor on low-grade dysplasia. Special attention is needed to fine tune differential diagnosis of intestinal metaplasia with or without dysplasia. Chronic inflammation score did not discriminate between patients with and without dysplasia or atypia. Atypia was common, suggesting that it should not be used to trigger full mapping. Additional consensus efforts are needed to establish reliable diagnosis of dysplasia.

1775 MUC13 Mucoprotein Is a Reliable Diagnostic and Prognostic Marker for Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer related death in the United States, with a 5-year survival rate of less than 5%. PDAC is lethal because of late presentation/diagnosis, and ineffective treatment options. Identification of newer biomarkers for early diagnosis and effective therapeutic targeting of PDAC is therefore highly desirable. MUC13, a recently identified high molecular weight glycoprotein, offers such a marker as it is aberrantly expressed in PDAC and controls PDAC progression by influencing multiple signaling pathways.
Design: MUC13 expression was assessed in tissue microarrays of PDAC, prepared from formalin-fixed, paraffin-embedded specimens, using our newly generated anti-MUC13 mouse monoclonal antibody and analyzed by immunohistochemistry, immunoblotting, RT-PCR and computational analysis.
Results: MUC13 is not present in normal pancreas but can be detected as early as in Pancreatic Intraepithelial Neoplasia (PanIN)- 100% (n=29). High level MUC13 expression was detected in PDAC samples- 94.6% (n=213 of 225, Mean composite score: MCS=9.7), as compared to low to moderate expression in tumor-adjacent normal pancreas (MCS=4), or very faint to no expression in distant normal pancreatic tissues (MCS=0.8) from PDAC cases. Subcellular localization of MUC13 varied with PDAC staging and behavior: with substantially enhanced nuclear staining positively correlating with nodal metastasis, invasion and poor patient survival. Interestingly, MUC13 expression also positively correlated with history of smoking and drinking in PDAC patients.
Conclusions: Our results confirm a very high specificity for MUC13 expression in PDAC and its subcellular localization significantly correlating with higher stage PDAC. Higher MUC13 expression also significantly correlates with smoking and drinking in our cohort of patients. Thus MUC13 offers a very attractive and novel diagnostic and prognostic marker for PDAC.
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1776 Pancreatic Neuroendocrine Tumors Expressing Proinsulin: A Clinicopathologic Analysis

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Background: Proinsulin (PI) is an insulin (IN) precursor, also produced in the ß cells of the islets. Rare cases of pancreatic neuroendocrine tumors (PNETs) produce PI-only and manifest with hypoglycemia, respond to tumor resection and are called proinsulinomas. Since clinical hypoglycemic symptoms are milder and PI levels are not routinely checked, we suspect proinsulinomas may be underdiagnosed. Expression of PI in PNETs has not been studied. Our goal was to study the expression of PI in a large number of PNETs and evaluate their clinicopathologic features.
Design: 69 cases of resected PNETs identified from our files (1998-2011) were included. Tissue microarrays created from these cases were stained using IHC against IN and PI. Immunoreactivity was evaluated for percentage of positive cells and intensity of staining (0-3+) by two independent pathologists blinded to the final diagnosis. Tumors demonstrating >1+_staining in >10% of cells were considered positive and analyzed.
Results: Of 69 cases, 13 (19%) stained positive for PI (7 male, mean age 54), and 10 co-expressed IN. Two of 7 cases (29%) tested also expressed somatostatin by IHC. The mean size of all cases was 2.4 cm, and 12 (92%) arose in the body/tail. None of the cases showed necrosis, lymphovascular invasion or any metastases. All cases were well-differentiated and 12 (92%) had low Ki-67 (<2%). No patient died of their disease, and 11 (85%) are alive over a mean follow-up of 8 years. The mean size of 3 PI positive-only cases was 3.8 cm; all arose in the body/tail and showed low KI-67. Blood glucose levels were available in 7 cases, all hypoglycemic. Serum IN was elevated in 3 cases, suggesting a diagnosis of insulinoma. One of the patients with hypoglycemia and normal IN levels was a 15 year old male with a 2 cm low-grade PNET, suggesting a possible proinsulinoma. His tumor was positive for PI and IN.
Conclusions: Our results show that 14% of PNETs coexpress PI along with IN, and 4% express PI only. All except 1 case of PNETs with co-expression of PI and IN had elevated serum IN and represent insulinomas. Those expressing only PI are likely proinsulinomas; but could not be confirmed in our retrospective analysis due to missing information. Our study suggests that in patients with hypoglycemia and normal IN levels, a possibility of proinsulinoma should be considered. Proinsulinoma represents an underdiagnosed entity and larger prospective studies are needed to further understand their clinicopathologic features.

1777 Prognostic Significance of New AJCC Tumor (T) Stage in Patients with Pancreatic Ductal Adenocarcinoma Treated with Neoadjuvant Therapy

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Background: The newly proposed tumor (T) stage by American Joint Committee for Cancer (AJCC, 8th edition) for pancreatic ductal adenocarcinoma (PDAC) is a size-based T stage system as follows: pT1, ≤2 cm (pT1a, ≤0.5 cm; pT1b, >0.5 cm and <1 cm; pT1c, 1-2 cm), pT2, >2 cm and ≤4 cm, and pT3, >4 cm. However the prognosis of this new T stage system has not been validated in patients who received neoadjuvant therapy and pancreaticoduodenectomy (PD).
Design: We retrospectively analyzed 398 cases (176 females and 222 males; median age: 64.1 years) who received neoadjuvant therapy and underwent pancreaticoduodenectomy (PD) for PDAC at our institution from 1999 – 2012. All PD specimens were processed using a standardized pathologic evaluation system. The tumor size was measured grossly or microscopically when there was no grossly identifiable mass lesion. The results were correlated with clinicopathologic parameters and survival using SPSS Statistics.
Results: Based on the new T stage system, there were 9 ypT0 (pathologic complete response with no residual carcinoma, 2.3%), 152 ypT1 (38.2%: 16 ypT1a [4%], 14 ypT1b [3.5%], and 122 ypT1c [30.7%]), 203 ypT2 (51%), and 34 ypT3 (8.5%) patients. The new T stage correlated with lymph node metastasis ($p<0.001$), tumor regression grade (TRG, $p<0.001$), disease-free survival (DFS, $p<0.001$) and overall survival (OS, $p<0.001$) among all study patients. Applied to patients who were ypT3 based on AJCC 7th edition (358 patients, 89.9%), the new T stage also showed significant correlations with lymph node metastasis, TRG, DFS and OS ($p<0.05$). Among the patients with ypT1 disease, patients with ypT1a and ypT1b had better DFS ($p=0.046$) and OS ($p=0.03$) than those with ypT1c. However there was no significant difference in either DFS or OS between ypT1c and ypT2 or between ypT2 and ypT3 groups ($p>0.05$). In multivariate analysis, new T stage system was not a significant predictor for either DFS ($p=0.18$) or OS ($p=0.11$).
Conclusions: Our study shows that the size-based new T stage system correlates with lymph node metastasis, TRG, DFS and OS in patients who received neoadjuvant therapy and PD. The new T stage system performs better than the T stage system of the AJCC 7th edition in this group of patients. Our study suggests that tumor size cutoff for T2 should be 1.0 cm for patients with PDAC who received neoadjuvant therapy.

1778 Prognostic Validation of T2-Substaging of Gallbladder Carcinomas: Survival Analysis of 127 Korean Cases with T2 Substaging and Survival Correlation

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Background: Majority of Gallbladder carcinoma (GBC) correspond to pT2 stage however it is regarded as one holistic prognostic group, while there are 3 sub-stages for a much smaller region of muscle confined tumor (Tis, T1a, T1b) that is much less common. Recently a sub-staging protocol for pT2-GBCs into 4 categories was proposed and found to have a good prognostic correlation in a study of 326 cases from Chile and U.S. Here we performed additional validation study of pT2 sub-staging with 127 GBCs from Korea.

Design: Seven international experts reviewed 127 pT2 GBCs from Korea and sub-staged T2 into 4 levels (L1-L4) according to the recent proposal (briefly, L1: slightly beyond muscle layer, L2: fills the fibrous-rich layer, L3: slightly into adipose-rich layer, L4: deep into loose-adipose layer).

Results: Among 127 GBCs, there were 11 pT1 (3 pT1a, 8 pT1b), 106 pT2, and 10 pT3. In pT2 group, there were 14 L1, 20 L2, 46 L3, and 26 L4. When we analyzed survival by pT2 sub-staging of 4 categories, it showed statistically significant correlation (p=0.0059). There was a good agreement (kappa, 0.72) with ≥ 6 of the reviewers agreeing in 79% of the cases. Taking majority opinion as "consensus", the survival of minimally perimuscular invasive group (L1 + L2) was incomparably better than that of advanced group (L3 + L4)(p=0.00059), although separation between L1 vs. L2, and L3 vs. L4 was didn't reach significance in this cohort, presumably due to lower case numbers.

Conclusions: This study validates that pT2-substaging protocol is reproducible and applicable in other populations, and it also confirms that minimally muscle penetrating GBCs have incomparably better prognosis than those with more advanced invasion of peri-muscular tissues. Considering that the former group has close to 90% 5-yr survival, an attempt for cure would be more justifiable in this group, and this group may be more eligible for conservative treatments in patients with co-morbidities.

1779 Loss of Hes1 Is a Common Finding in Human Pancreatic Ductal Carcinoma and Is Associated with Loss of SMAD4

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Background: Hes1, the main downstream transcriptional factor of Notch signaling pathway, has been shown to be required for appropriate development and differentiation of pancreas in knockout mice studies. In pancreatic ductal adenocarcinoma (PDAC), Hes1 was reported to have higher frequency of positivity in epithelial cells of infiltrating ductal adenocarcinoma compared to normal interlobular ductal epithelium in a study using human specimen (n=33). Hes1 has also been studied as potential therapy target of in cell culture studies. Our study is focused on characterizing the expression characteristics of Hes1 in a variety of human PDACs.

Design: A tissue microarray (TMA) comprised of 198 cases of PDAC, were created using 2 punches of 1 mm tumor with cases from a single tertiary medical center. Immunohistochemistry of Hes1, SMAD4 and CDX-2 were performed. Intensity and distribution of the staining of Hes1 was evaluated.

Results: Hes1 shows characteristic expression pattern in different components of benign adult pancreatic tissue. A robust nuclear expression pattern is present in the pancreatic islet cells (+++). A moderately positive nuclear expression pattern is observed in pancreatic ductal cells (++), and mixed expression pattern is found in pancreatic acinar cells (+/-). Using these as the internal grading standard, expression pattern and intensity of Hes1 in PDAC were evaluated. We found that 51% and 10% of PDAC showed complete loss of Hes-1 expression, 10% show mixed expression pattern and 29% showed positive Hes-1 expression (++ or +++). We also found a statistically significant association of loss of Hes1 and loss of SMAD4 (p=0.04) in PDACs. No significant association was found between loss of Hes1 and presence/absence of CDX2.

Conclusions: Although Notch signaling pathway had been studied as potential therapeutic target for PDAC, our study suggests that Hes1 expression is lost or decreased in more than half of the PDAC. Our study provides valuable insight of Hes1 expression as an indicator of Notch signaling dysregulation in PDAC, and suggests that Notch signaling pathway is not a suitable therapeutic target for PDACs without proper testing in the era of precision medicine. Also the loss of Hes1 in association with loss of SMAD4 suggests that Hes1 may play an important role in pancreatic carcinogenesis which needs further investigation.

1780 Diagnostic Feasibility and Accuracy of Endoscopic Ultrasound-Guided Fine Needle Core Biopsy (EUS-FNB): A Single-Center Large Cohort Study

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Background: The aim of this study was to evaluate the diagnostic accuracy of Endoscopic Ultrasound-guided Fine Needle Core Biopsy EUS-FNB in patients with solid and cystic pancreatic lesions in a single center large cohort.

Design: All consecutive patients who underwent EUS-FNB using 19G and/or 22G needles between 2008 and 2015 were enrolled in the study. The EUS-FNB technique was standardized among the endoscopists and diagnoses were made by dedicated pathologists.

Results: The study included 531 patients, 289 (54.4%) women and 242 (45.6%) men, mean age 66 years. The lesions were located in the head (67.7%), in the isthmus (4.7%) and in the body-tail (27.6%). Sample quality was adequate and highly diagnostic for histological and immunohystochemical (IHC) assessment in 465 cases (87.6 %): 364 (78.3%) malignant, 8 (1.7%) suspicious for malignancy, 93 (20%) benign. The diagnoses were: pancreatic adenocarcinoma (56.3%), neuroendocrine tumors-NET (14.8%), intraductal papillary mucinous neoplasms (IPMN) (8.6%), chronic (5.4%) or autoimmune (3%) pancreatitis, metastasis (2.6%), serous cystoadenoma (1.7%), lymphoma (0.9%), acinar carcinoma (0.4%), solid pseudopapillary tumor (0.2%), mucinous cystic tumor (0.2%) and rare malignant or benign lesions (3.4% and 2.4%, respectively). The accuracy was calculated by comparing the results of FNB with the final pathological examination in 124 consecutive operated cases. Excluding the non-diagnostic samples (13 cases, especially cystic lesions), the sensitivity, specificity, likelihood ratio +, likelihood ratio - of EUS-FNB were 98.1%, 100%, 50.038, and 0.019, respectively; while in all cases the sensitivity, specificity, likelihood ratio +, likelihood ratio - were 87.4%, 100%, 44.571, and 0.129, respectively.

Conclusions: Our data confirms that EUS-FNB is a safe, feasible and highly accurate technique for obtaining histological samples for diagnosis both in solid and cystic pancreatic diseases. It improves the quality of pathological analysis by providing well preserved tissue, crucial to better discriminate between benign inflammatory/regenerative lesions and neoplasm, to define neoplastic lesions in type and grade, and to perform IHC when necessary. Furthermore, in the personalized medicine era, it is essential to obtain tissue for molecular analysis which allows the characterization of genomic alterations and predictors of optimal therapy.

1781 p16 Immunohistochemistry Reveals Clinicopathologically Distinct Subsets of Pancreatic Cancer

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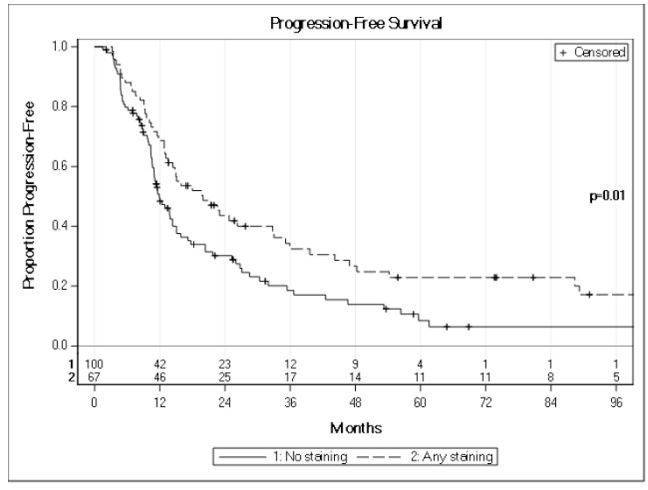
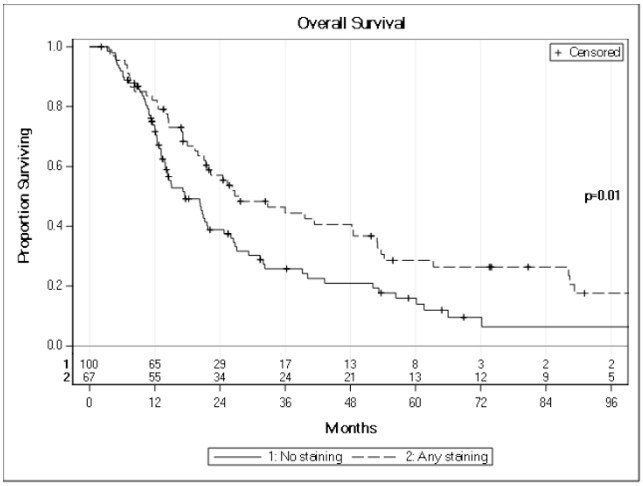
Background: p16 expression is altered in diverse tumor types. In high-risk human papillomavirus (HR-HPV)-driven cancers, it is markedly upregulated due to Rb inactivation; expression should be abolished in most pancreas cancers (PDAs), which typically demonstrate biallelic p16 inactivation. We recently encountered a patient with carcinomatosis and uterine cervical and pancreatic masses. Based on p16 overexpression, we favored an endocervical origin. Given the genetics of PDA, previous immunohistochemistry (IHC) studies have focused on absent staining. We performed p16 IHC on a large PDA cohort to determine if it ever demonstrates diffuse, strong p16 staining, also correlating expression with outcome.

Design: Tissue microarrays were constructed from 248 primary and 97 metastatic PDAs. p16 IHC (clone E6H4) was scored for extent (%) and intensity (0-3+), and an H-score (extent*intensity) was calculated. Detailed clinical annotation was available for 172 primaries. For these, survival probabilities were estimated using the Kaplan-Meier method; Cox proportional hazards regression was used to assess the effects of clinicopathologic variables on progression-free (PFS) and overall survival (OS).

Results: 4.8% primary and 3.9% metastatic PDAs demonstrated an H-score between 200-300 (highly expressing pattern); 63% of primary and 70% of metastatic tumors demonstated no staining (null pattern); the remaining demonstated an H-score >0 and <200 (wild-type pattern). Null-pattern staining was prognostically adverse, while any staining conferred improved PFS and OS, with hazard ratios of 0.53 (95% confidence interval 0.35-0.80) remaining significant on multivariate analysis (p<0.01). PFS and OS curves for no vs. any p16 staining are depicted.





Conclusions: Up to 5% of PDAs demonstrate the p16 highly expressing pattern, similar to HR-HPV-driven tumors, possibly related to Rb inactivation. Null pattern slightly predominates over wild-type pattern in the remainder and is powerfully prognostically adverse.

1782 A Novel *BRAF* FISH Probe Identifies Potentially Targetable *BRAF* Rearrangement in Pancreatic Carcinomas with Acinar Differentiation

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Background: Acinar cell carcinoma (ACC) of the pancreas is characterized by trypsin expression, and approximately 25% of cases harbor *BRAF* fusion genes by comprehensive genomic profiling in a single study. This finding has not been validated in another series, and genomic profiling is not always feasible or available in routine clinical care. Furthermore, a clinicopathologic comparison of carcinomas with acinar differentiation with *BRAF* fusion genes and those without has not been performed.

Design: We retrieved 30 cases of pure ACC and 5 cases of mixed acinar-neuroendocrine carcinoma (ACC-NEC). The histologic sections were reviewed and a tissue block selected for ancillary immunohistochemistry and FISH. A break-apart FISH probe was designed to assess for *BRAF* rearrangements, including all of those previously described. Clinicopathologic features of *BRAF*-rearranged cases were compared with those without *BRAF* rearrangement.

Results: The tumors affected 26 men and 9 women (mean age 65 +/- 12.3 years), and the maximum tumor dimension ranged from 2.8 cm to 8.0 cm. All cases were positive for trypsin. In the 5 mixed cases, neuroendocrine markers were positive in >30% of the tumor cells, whereas the pure ACC were negative for neuroendocrine markers (n=23; 77%) or showed <30% cells positive for those markers (n=7; 23%). CK7 was positive in 19 of 28 cases (32%), only 1 of which was a mixed ACC-NEC. *BRAF* rearrangements were found in 6 cases (17%), 5 of which were pure ACC and 1 was a mixed ACC-NEC. There was no difference in the tumor size and pT stage of patients whose tumors harbored *BRAF* rearrangements compared to those whose tumors did not have *BRAF* rearrangements. Follow-up was available in 69% of cases; the 2-year overall survival rates were 40% for cases with *BRAF* rearrangement and 32% for cases without *BRAF* rearrangement.

Conclusions: In this series of pancreatic carcinomas with acinar differentiation, which is the largest to date, *BRAF* rearrangements were present in ~20% of cases. *BRAF*-rearranged carcinomas with acinar differentiation displayed similar clinicopathologic features to carcinomas without *BRAF* rearrangement. Importantly, *BRAF* rearrangement may be amenable to targeted therapy, and the novel *BRAF* probe described herein can be used on routinely processed tissue to allow for selection of patients who may benefit from *BRAF* inhibition.

1783 Is Routine Histologic Examination of Gallbladders Necessary?

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Background: Gallbladders (GBs) are routinely sent for pathologic examination after cholecystectomy to rule out the presence of malignancy. Primary GB neoplasms, while rare, include aggressive adenocarcinomas (ACA) with poor prognosis and a timely diagnosis can have a significant effect on a patient's clinical course. Recent studies have tried to determine the necessity of routine histopathologic examination of GBs, especially looking at the cost-effectiveness of this approach. Our study evaluates the spectrum of pathologic changes in cholecystectomy specimens with a focus on malignant lesions and examines the validity of routine pathologic examination of cholecystectomy specimens.

Design: Pathology laboratory data of all GB specimens over a period of 5 years (Sept. 2011 to Sept. 2016) were analyzed retrospectively. The case notes were retrieved in all cases of malignancies.

Results: The total number of specimens was 5494. There were 25 (0.45%) malignancies detected, including 17 (0.3%) primary ACAs and 8 (0.15%) metastatic carcinomas (CA). 7/17 (41%) of the primary GB ACAs and 1/8 (12.5%) of the metastatic CAs were incidental findings in cholecystectomies performed for clinical symptoms of cholecystitis. For the remaining cases, malignancy was suspected based on pre-op imaging studies or on intra-operative (IO) findings. All of the incidental cases of GB ACAs however, did have grossly abnormal findings (e.g. mass, polyp, firm areas) that were detected by the prosectors. In addition, 7 (0.12%) cases of incidental high-grade dysplasia were also identified.

Histologic Findings	No. of cases	Percentage
Primary gallbladder adenocarcinoma	17	0.31%
Metastatic carcinoma to gallbladder	8	0.15%
Dysplasia	14	0.25%
Adenomyomatosis/adenomyoma	35	0.64%
Pyloric gland polyp/adenoma	4	0.07%
Hyperplastic/fundic polyp	4	0.07%
Tubular/villous adenoma	2	0.04%
Porcelain gallbladder	5	0.09%
Chronic cholecystitis	4576	83.29%
Acute and chronic cholecystitis	1031	18.77%
Xanthogranulomatous cholecystitis	16	0.29%
Gangrenous/necrotic cholecystitis	147	2.68%
Hemorrhagic cholecystitis	86	1.57%
Cholelithiasis	3950	71.90%
Cholesterolosis	812	14.78%
Cholesterol polyps	44	0.80%
Atrophy	9	0.16%
No pathologic changes	208	3.79%

Conclusions: Malignancies involving GB are rare. However, our study shows pre and IO evaluation of the GB is insufficient to rule out all malignancies. Approximately 41% of primary GB CAs were missed until the specimens were examined in the gross room. Interestingly, all of the incidentally detected CAs did have macroscopic abnormalities which raised the prosector's suspicion for a possible malignancy, highlighting the importance of gross examinations by experienced and reliable prosectors. In view of these results, careful pathologic examinations of GBs should continue and tissue should be selectively submitted for histologic examination on grossly suspicious cases. Relying exclusively on pre- and IO evaluations however, could lead to a compromise on patient safety.

1784 Effect of Sampling on the Prevalence of In Situ/ Invasive Gallbladder Carcinoma

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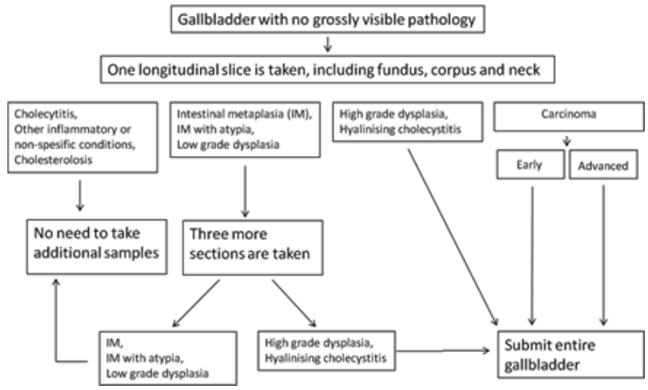
Background: Gallbladder (GB) carcinomas and preneoplastic lesions are mostly not recognizable clinically and macroscopically. Many of the carcinoma diagnoses are rendered incidentally in cholecystectomies indicated for cholelithiasis. This makes sampling more important in GB than other organs, where the lesion is readily distinguishable. To our knowledge, there isn't a standard sampling method for GB in countries with low incidence of GB carcinoma; usually, random sections from fundus, corpus and neck are submitted. Furthermore, in low-risk populations there is also no standard protocol for additional sampling in GBs with preneoplastic/neoplastic lesions seen in initial sections. Our aim in this study is to find out GB dysplasia/carcinoma prevalence in low-risk populations by using the modified sampling protocol established in high-risk areas.

Design: 5254 GBs in 5 years were sampled as one longitudinal full-thickness slice, including fundus-corpus-neck. The cystic duct margin was also included. Gross and microscopic examination was done by 2 pathologists. In the presence of intestinal metaplasia (IM), IM with atypia and low grade dysplasia, three more sections were taken. In case of high grade dyplasia and hyalinising cholecystitis, entire GB was submitted. Sampling algorithm for GBs with no grossly visible pathology is summarised in Figure 1.



Results: There were 79 gallbladder carcinomas diagnosed out of 5254 cholecystectomies, 54 early (TIS, T1a and T1b) and 25 advanced (T2 and T3). 19 were male and 60 were female. GB carcinoma prevalence was 1.5%. Low grade dysplasia was detected in 162 GBs. IM was found in 339 cases.

Conclusions: The prevalence of GB carcinoma is less than 0.5% in western countries. In our institute GB carcinoma prevalence was 0.44% prior to 2011. This study indicates

the importance of sampling in the detection of GB carcinoma (prevelance was 1.5% in this study). Since the neoplastic lesions of GB are usually very subtle, submitting one longitudinal slice for examination provides us a better conception of the GB and by this way enables the diagnosis of early and advanced carcinoma more accurately.

1785 Significance of T1a and T1b Carcinoma Arising in Mucinous Cystic Neoplasm of Pancreas

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Background: Mucinous cystic neoplasm of pancreas (MCN) is one of the precursor lesions of pancreatic adenocarcinoma. The 5-year disease-specific survival for non-invasive MCNs was 100% and 57% for those with invasive carcinoma. However the significance of T1a (≤5.0 mm) and T1b (>0.5 mm and <10 mm) carcinoma arising in MCN as defined by the upcoming AJCC 8th edition is not clear.

Design: From our institutional pathology databases, we identified 71 cases of MCN (63 females and 8 males, median age: 55.5 years). All cases were reviewed and classified as MCN with low-grade dysplasia (LGD), MCN with high-grade dysplasia (HGD), MCN with T1a or T1b carcinoma, and MCN with grossly invasive carcinoma (PDAC). In all cases, tumor was entirely submitted for histologic examination when carcinoma was not grossly identified. The results were correlated clinicopathologic parameters and survival.

Results: Sixty-one patients underwent a distal pancreatectomy and 10 patients had a pancreaticoduodenectomy. There were 51 MCN with LGD (72%), 7 MCN with HGD (10%), 3 MCN with T1a or T1b carcinoma (4%), and 10 MCN with PDAC (14%). The resection margins were negative for all cases. Tumor was located in the head, body or tail in 10, 23 and 38 cases respectively. The median tumor size is 4.5 cm, ranging from 1.3 cm to 16 cm. For patients who had MCN with T1a or T1b carcinoma, one had a 10 cm MCN in the body with multifocal invasive adenocarcinoma measuring up to 6.0 mm in size and 43 negative lymph nodes (0/43); one had a 16 cm MCN in the tail with 3 foci of invasive adenocarcinoma measuring up to 0.9 mm in size and 28 negative lymph nodes (0/28); and one had a 13 cm MCN in the tail with two foci invasive adenocarcinoma measuring 3.0 mm and 7.0 mm in size and one focus of osteoclast-like giant cell tumor (3.0 mm) and 51 negative lymph nodes (0/51). All three tumors were entirely submitted in 133, 296, and 200 blocks respectively. All three patients were alive with no evidence of recurrence during the follow up of 6.9, 100.3, and 119.3 months, respectively. Similarly, none of the patients who had MCN with either LGD or HGD had recurrence or died of disease. In contrast, 7 of 10 patients who had MCN with PDAC had recurrence and late died of disease with a median survival of 22.9 months ($p<0.001$).

Conclusions: MCN with T1a and T1b carcinoma had excellent prognosis similar to MCNs with LGD or HGD after complete sampling for histologic examination.

1786 Rhabdoid Gallbladder Carcinomas Show Loss of SMARCA4 or SMARCB1

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Background: The SWI/SNF protein complex including SMARCB1 and SMARCA4 is responsible for chromatin remodeling. Mutations in these tumor suppressor proteins characterize clinically aggressive and histologically rhabdoid tumors of various types including epithelioid sarcoma, small cell carcinoma of the ovary, hypercalcemic type, and undifferentiated gastrointestinal rhabdoid carcinomas. We hypothesize that rhabdoid gallbladder carcinomas may show SMARCB1 or SMARCA4 loss.

Design: Study cases included resected gallbladder carcinomas categorized as poorly differentiated or undifferentiated (1995-2015). The histology was reviewed and immunohistochemistry was performed for SMARCB1, SMARCA4 (clone EPNCIR111A), CK7, CK20, CDX2 and P53. Clinical records were reviewed.

Results: Gallbladder carcinoma cases (n=103) were reviewed from patients with a mean age of 66.1 +/- 11.1 years. Five cases (5%) showed loss of either SMARCA4 expression (n=4) or SMARCB1 (n=1). Loss of SMARCB1 and SMARCA4 were mutually exclusive. SMARCA4 or SMARCB1-deficient cases showed monotonous rhabdoid cells with marked nuclear atypia, were pT2 (n=2) or pT3 tumors (n=3), and had a median maximum dimension of 2.7cm [range 0.8 – 6 cm]. All SMARCA4 and SMARCB1-deficient carcinomas were positive for CK7 and CDX2 (focal), 2 were CK20 positive, and 4 were P53 wild type. Ongoing analysis for mutations in *SMARCA4* and *SMARCB1* is pending. The cases with intact SMARCA4 and SMARCB1 expression (n=98) did not show significant differences in age, maximum tumor dimension, and pT stage from cases with deficient SMARCA4 or SMARCB1 expression. Survival data analysis is pending.

Conclusions: This is the first report of gallbladder carcinomas with loss of SMARCA4 and SMARCB1 protein expression. These tumors are a morphologically distinctive subset of gallbladder carcinomas composed of monotonous, severely atypical rhabdoid cells, and are histologically similar to rhabdoid tumors at other sites. Awareness of this histologic type of gallbladder carcinoma may prevent misclassification. Ongoing survival analysis may help to determine the prognostic significance of this group of gallbladder carcinomas.

1787 Programmed Cell Death Ligand 1 Cut-Point Is Associated with Disease Specific Survival in Resected Pancreatic Ductal Adenocarcinoma

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Background: Programmed Cell Death Ligand 1 (PD-L1) has been postulated as a predictive biomarker to immune checkpoint inhibitors which have recently shown promising anti-cancer effects in a number of solid tumor types through inhibition of the programmed cell death 1 (PD1) signaling axis. The conventional cut-point for the determining PD-L1 positivity is an H-Score >=1. In this study, we explore the biological significance of differing cut-points on resected pancreatic ductal adenocarcinoma (PDAC).

Design: A tissue microarray (TMA) from 258 resected PDAC patients acquired between 1987 - 2013 in the Vancouver Coastal Health Region, Canada was stained with the SP142 antibody for PD-L1. Disease specific survival (DSS) was modeled with parametric survival methods using PD-L1 H-Score as the regressor. Individual proposed cut-points were assessed with the Kaplan-Meier method. Multivariable DSS was performed with the Cox Proportional Hazards Method.

Results: Parametric survival analysis indicated that there was a gradient dependent relationship between increased PD-L1 expression and reduced DSS ($p = 0.0123$). Three H-Score cut-points were assessed: >=1, >=10, and >=20. For the >=1 cut-point, 32 (12.4%) of the cases were positive and no significant association with DSS was found ($p = 0.38$). For the >=10 cut-point, 17 (6.6%) of the cases were positive and a statistically significant association with DSS was observed ($p = 0.0079$). For the >=20 cut-point, 11 (4.3%) of cases were positive and a further increase in statistical significance was observed ($p <0.0001$). In multivariable DSS, both the >=10 and >=20 cut-points were independently significant when considered with the application of adjuvant chemotherapy ($p <= 0.0066$).

Conclusions: This data suggests that there is an inverse relationship between PD-L1 expression and disease specific survival times in resected PDAC. This would suggest that if the application of anti-PD1 therapy is to be biologically based, a cut-point greater than H-Score >=1 should be considered for this disease.

1788 Evaluating Tumor Budding in Pancreatic Cancer: Simple and Reliable

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Background: Pancreatic ductal adenocarcinoma (PDAC) is still a highly lethal malignancy with increasing incidence and suboptimal management. Tumor budding (TB) is a strong and independent prognostic factor in PDAC, however information on TB is currently not included in most histopathology reports due to lack of a standardized scoring system. Our aim is to assess the reliability and reproducibility of a new method for scoring TB in a well characterized PDAC-cohort, based on the scoring system proposed by the International Tumor Budding Concensus Conference (ITBCC) 2016.

Design: TB (presence of small groups up to 5 cells or single cells detached from main tumor) was scored independently by two pathologists on H&E-stained sections of 110 PDACs with complete clinico-pathological and follow-up information, by assessing the densest budding area at 20x-magnification. Findings were correlated to the patient and tumor characteristics and interobserver agreement was assessed.

Results: Inter-correlation coefficient (ICC) was 0.77 suggesting strong agreement. Increasing density of TB was associated with higher grade ($p=0.003$) and significantly shorter disease-free (DFS, $p=0.0008$; HR(95%CI)=1.032(1.013-1,051)) and overall survival (OS, $p<0.0001$; HR(95%CI)=1.039(1.024-1.054)). Moreover, TB was an independent prognostic factor for OS ($p=0.0001$; HR(95%CI)=1.032(1.05-1.049) and DFS ($p=0.0255$; HR(95%CI)=1.024(1.003-1.045)), after adjusting for T-stage, N-stage, M-stage and therapy.

Conclusions: The ITBCC scoring system is a simple, reliable and reproducible method for evaluating TB in PDAC and facilitates therefore the inclusion of tumor budding in histopathology reports of PDAC.

1789 Prognostic Significance of Cleaved Caspase-3 in Biliary Tract Carcinomas

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Background: Biliary tract carcinomas (BTCs) are rare neoplasms associated with poor prognosis, high mortality rates and resistance to conventional therapies. Programmed cell death(apoptosis) has a crucial role in the regulation of the tumor cells and we evaluated the expression of cleaved caspase-3 (CC3) protein (a cysteine protease causing apoptosis) in BTCs.

Design: We investigated CC3 expression by immunohistochemistry in a well-characterized tissue microarray cohort of 161 BTCs (gallbladder carcinomas, extrahepatic and intrahepatic cholangiocarcinomas). Subcellular CC3 expression was determined and cytoplasmic and/or nuclear expression noted in the tumor cells. CC-3 expression was further grouped into 8 classes based on number of tumor cell staining: Group 0 (absent staining), 1 (<6); 2 (<16); 3 (<31); 4 (<51); 5 (<76); 6 (<106); 7 (<141) and 8 (>140) cells. High CC-3 expression, defined as BTCs with group 0- 3, was correlated with clinicopathologic features including outcome such as overall and recurrence free survival.

Results: Incidence of BTCs with high CC3 expression was 20.9%(32/153).High CC3 expression levels were correlated with early AJCC stage ($p=0.0331$), lower lymph node

metastasis (p=0.0272), lower perineural invasion (p=0.0629; trend), lower number of deaths (p=0.0062) and reduced recurrences (p=0.0149). The Kaplan-Meier method with the log-rank test revealed that patients with high CC3 tumors expression had significantly greater survival than those with low CC3 expression among all stages, (p = 0.0192), and also the subgroup that did not receive adjuvant chemotherapy (p=0.0179). After adjustment for known clinical prognostic factors, such as age, AJCC stage and site the hazard ratio for high CC3 was 0.49 with 95% confidence interval between 0.27 and 0.82 (P = 0.0072). Within the subgroup that did not receive any adjuvant chemotherapy, the hazard ratio was 0.46 with 95% confidence interval between 0.22 and 0.87 (P = 0.0155).
Conclusions: CC3 expression was an independent prognostic marker and could be useful not only to prognosticate BTCs but also potentially identify BTCs with low CC3 expression that may need aggressive therapy.

1790 Validation of Ampulla of Vater Cancer Staging form the 8th Edition of the American Joint Committee on Cancer Staging System

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Background: The American Joint Committee on Cancer (AJCC) proposed 8th edition of cancer staging system and validation study is required for evaluation of prognostic stratification of ampulla of Vater cancer patients.
Design: Three hundred sixty nine patients with surgically resected patients with ampulla of Vater cancer at Asan Medical Center from 1997 to 2009 were selected. H&E slides of selected cases were reviewed and cases were grouped based on the newly proposed 8th T (T1a, limited to sphincter of Oddi; T1b, invasion to duodenal submucosa; T2, invasion to duodenal proper muscle; T3a, invasion to pancreas upto 0.5 cm; T3b, invasion to pancreas >0.5 cm; and T4, involvement of celiac axis or superior mesenteric artery) and N (N0, no nodal metastasis; N1, 1-3 nodal metastasis; and N2, >4 nodal metastasis) classifications of ampulla of Vater cancer staging.
Results: Overall 5-year survival rate for T and N classifications were as followed: T1a, n=65, 83.0%; T1b, n=42, 70.5%; T2, n=83, 45.8%; T3a, n=56, 47.5%; T3b, n=109, 28.5%, T4, n=14, 7.1%; p value<0.001; N0, n=250, 44.8%; N1, n=95, 20.0%; N2, n=24, 4.2%; p<0.001. Group-wise comparisons demonstrated statistically significant difference between T1a vs T1b (p=0.005), T3a vs T3b (p=0.03), N0 vs N1 (p<0.001), and N1 vs. N2 (p=0.007), but not between T1b vs T2 (p=0.20), T2 vs T3a (p=0.84), and T3b and T4 (p=0.17)
Conclusions: T classification of ampulla of Vater cancer of the 8th AJCC cancer staging system did not stratify prognosis in patients with ampulla of Vater, therefore T classification is needed to further improvements.

1791 Well Differentiated Neuroendocrine Neoplasms of the Pancreas with a Ki67 Index Above 20% Differ from Their Poorly Differentiated Counterparts: Results from Massive Parallel Sequencing Applying a 409 Gene Panel

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Background: Pancreatic neuroendocrine neoplasms (PanNENs) with a Ki67-index of more than 20% represent a heterogeneous group comprising well differentiated PanNENs, provisionally termed NET G3, and poorly differentiated small or large cell PanNENs, called NEC G3. Recent studies have shown that pancreatic NECs frequently show *TP53* and *RB1* gene alterations, while NETs G1/2 mainly harbor *MEN1* and *ATRX / DAXX* mutations. A detailed genetic profile of NETs G3 is still lacking. The aim of the study was therefore to analyze the genetic alterations in pancreatic NETs G3 in order to find out how much this tumor group differs either from NETs G1/2 or NECs.
Design: Twenty PanNENs with a Ki-67 index above 20%, including 9 NETs G3 and 11 NECs, were analyzed by massive parallel sequencing applying a 409 gene panel on an Ion Torrent system. Copy number variation analysis in order to detect gene amplifications and deletions was also performed.
Results: In the two cohorts we found 84 mutations in 62 of the 409 genes examined. NETs G3 harbored 30 mutations in 25 different genes. *MEN1* gene alterations (two nonsense and two frameshift mutations) were found in four of nine NETs G3 (44%), whereas *ATRX / DAXX, TP53* or *RB1* mutations were absent. NECs harbored 54 mutations in 40 different genes. Seven and four of the eleven NECs were *TP53* and *KRAS* mutated, respectively (64%, 36%). Histology revealed that three of the four *KRAS* mutated NECs showed in addition an exocrine component. *CSMD3, KDM5C, EP300* and *KMT2D* were each mutated in two NECs (18%). The only gene alterations that were shared by NETs G3 and NECs were found in the genes *LRP1B* and *ARID1A* with two mutations in each of the genes. One NET G3 and two NECs did not show any mutations in the investigated genes.
Conclusions: NETs G3 and NECs of the pancreas show substantial genetic differences. NETs G3 are closely related to NETs G1/2 regarding *MEN1* alterations and the lack of *TP53* mutations, while pancreatic NECs are associated with *TP53* alterations. The presence of *KRAS* mutations in the poorly differentiated PanNENs seems to indicate a glandular component in the tumors.

1792 Combination Immunohistochemistry for SMAD4 and Runt-Related Transcription Factor 3 (RUNX3) May Identify a Favorable Prognostic Subgroup of Pancreatic Ductal Adenocarcinomas

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Background: SMAD4/DPC4 mutations have been associated with aggressive behavior in pancreatic ductal adenocarcinomas (PDAC), and it has been suggested using animal models that RUNX3 expression combined with SMAD4 status may predict the metastatic potential of PDACs. We evaluated the prognostic utility of SMAD4/RUNX3 status in human PDACs by immunohistochemistry.
Design: A retrospective analysis was performed on 210 cases of consecutively resected PDACs. Immunohistochemical stains were performed for SMAD4 and RUNX3 on tissue microarray slides and whole sections of 14 paired primary tumors and metastases, and the results were correlated with the clinicopathological features.
Results: Loss of SMAD4 expression was detected in 145 cases (69.0%) and associated with poor overall survival (OS) (p=0.082) and recurrence-free survivals (RFS) (p=0.051), although not statistically significant. Nuclear RUNX3 expression was detected in 121 cases (57.6%) and associated with decreased OS (p=0.004) and RFS (p=0.027), and was more frequent in poorly differentiated PDACs (p=0.028). On combining RUNX3/SMAD4 status, RUNX3-/SMAD4+ PDACs demonstrated longer OS (p=0.008) and RFS (p=0.003) compared to RUNX3+/SMAD4+ and SMAD4- groups; RUNX3-/SMAD4+ was a significant independent predictive factor for both OS [p=0.039, HR 1.753 (95% CI 1.029-2.985)] and RFS [p=0.0.041, HR 1.862 CI 1.027-3.375)]. Of the paired primary and metastatic PDACs, none of the tumors demonstrated RUNX3-/SMAD4+ status.
Conclusions: SMAD4-positivity with RUNX3-negativity was a significant independent predictive factor for better OS and RFS in PDAC. Combination immunohistochemistry for SMAD4 and RUNX3 may help identify a favorable prognostic subgroup of PDAC and have implications for treatment decisions; RUNX3-/SMAD4+ PDACs may benefit from more aggressive locoregional treatment due to the lower metastatic potential.

1793 Gene Expression Analysis of Low and High Grade Pancreatic Intraepithelial Neoplasia (PanIN)

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death where the majority of patients present with locally advanced or metastatic disease. Current low survival rates are partly due to the lack of an effective early detection methodology. With the goal of discovering biomarkers useful in early detection of PDAC, we performed gene expression analysis of PanIN, precursor lesions of PDAC. Since extracting high quality RNA from archival pancreatic tissue is difficult due to the abundance of ribonucleases and fixation related RNA degradation, we have chosen to perform the gene expression analysis using a novel extraction free approach.
Design: Selected formalin-fixed paraffin embedded (FFPE) tissue blocks from 7 PDAC resections were sectioned at 10µm. Low and high grade PanIN (15 total), adenocarcinoma, normal exocrine pancreas and normal pancreatic duct epithelium were micro-dissected, with dissected areas ranging 1 - 20mm^2. Without RNA extraction, libraries were prepared directly from the micro-dissected tissue on the HTG platform (HTG Molecular Diagnostics) using the HTG EdgeSeq Oncology Biomarker Panel (2,560 biomarkers).
Results: Low and high grade PanIN were successfully profiled by the HTG EdgeSeq and compared to carcinoma, benign pancreas and benign ductal epithelium. Among many differentially expressed genes, we found genes previously reported to be upregulated in PanIN : trefoil factor 1 (*TFF1*), Kruppel-like factor-4 (*KLF4*), and pepsinogen C(*PGC*). Correlation heatmaps and principle component analysis show that gene expression profiles of high grade and low grade PanIN can be distinguished and that distinct subgroups may exist within the group of high grade PanIN.
Conclusions: Robust and reproducible gene expression analysis of PanIN can be performed on archived FFPE tissue using a nucleic acid extraction-free approach. We speculate that this analysis will contribute to our understanding of the biology of the early events in pancreatic carcinogenesis.

1794 Cholecystopathy of PancreatoBiliary Maljunction (PBM): Analysis of 75 Gallbladders from Patients with PBM Elucidates a Diagnostic Pattern of Mucosal Hyperplasia

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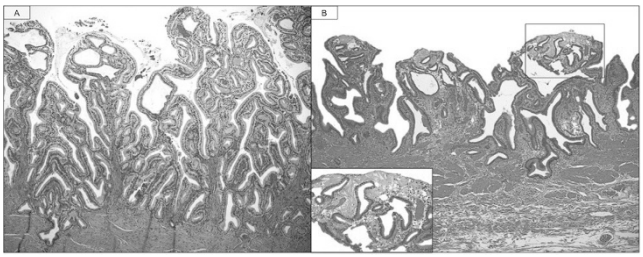
Background: Pancreaticobiliary maljunction (PBM) is the anomalous union of the main pancreatic duct and CBD outside the duodenal wall, which allows pancreatic juice to by-pass the Oddi and reflux into the gallbladder (GB). While it is well recognized in Asia, there is virtually no data from the West.
Design: 75 GBs from patients with PBM (63 Japan; 12 USA) were analyzed and contrasted with 66 from non-PBM patients.
Results: GBs with PBM were F/M= 2, mean age, 53 (14-81). Microscopically, there was diffuse mucosal hyperplasia (83%, vs 43% in non-PBM group) without any significant inflammation, and frequently with markedly taller folds (mean 1.12mm, vs 0.68mm). This hyperplasia had distinctive characteristics, with broad-based pushing into the muscle and prominent Rokitansky-Aschoff sinuses (2.0/cm, vs 1.0) at the

base. At the tips, this villiform hyperplasia displayed frequent horizontal bridging of the folds (69%, vs 45%, p=0.0063), bulbous dilatation (52%, vs 21%, p=0.0003) of the tip often showing lymphangectesia, mild hypercellularity and common amyloid-like hyaline material deposition in the stroma of the tip (59%, vs 15%, p<0.0001). Also noted significantly more common in PBM group were ICPN (15%, vs 0%, p=0.0011), pyloric gland metapl (71%, vs 47%, p=0.0059), cholesterolosis (48%, vs 27%, p=0.0169) and intestinal metapl (24%, vs 6%, p=0.0039). 67% (8/12) of US cohort was resected for biliary carcinoma, and their mucosa was 0.3mm thicker than Japanese cohort, but no other significant differences were detected between the two populations.



Conclusions: Gallbladders with PBM display a fairly specific pattern of mucosal hyperplasia with distinctive features, which we propose to refer to as PBM-cholecystopathy. Since most PBM are clinically undetected, it is important for pathologists to recognize and remark these findings so that the patient can be investigated for this anomaly, and its complications (biliary tract cancers and pancreatitis) can be prevented.

1795 Pathologic Findings in Gallbladders: An Analysis of the True Frequency and Distribution in 203 Totally Sampled and Mapped Gallbladders from a North American Population

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Background: Previous studies have not established the true frequency of various pathologic findings encountered in gallbladders due to the inadequate information generated from random gallbladder sampling, as well as ever-evolving criteria in the classification of lesion types.

Design: 203 consecutive isolated cholecystectomy specimens from one hospital in a southeastern US institution were submitted totally and mapped according to the Chilean/NCI protocol. Epithelial alterations were classified based on the Santiago consensus meeting criteria (Mod Pathol, 9(2S):438A, 2016).

Results: Female/Male (F/M)=135/68=2. Mean age=51 (range=19-93). Mean blocks sampled/examined=22. Adenomyomatous nodule (9%): all fundic, mean=1.2 cm. Cholelithiasis (80%): F/M=2, age=50, mean=13 stones. Cholesterolosis: 36%. Acute cholecystitis (CCitis): 20% (1/3rd each mild/moderate/severe). Chronic CCitis: 57% (55 % mild, 43% moderate, 2% severe); activity (intraepithelial PMNs or acute/subacute changes) in 16%; lymphoplasmacytic-mucosal-predominant (obstruction-associated) findings: 11%. Follicular CCitis (9%) in elderly (mean age=68; F/M=1). Rokitansky-Aschoff (RAS): 70% (15% substantial, 7% diffuse/extensive). "Pyloric" gland metaplasia: 54%. Intestinal metaplasia (goblet cells): 7% (65% very focal), with striking predilection for fundus (65%). Some degree of epithelial atypia was seen in 24%, but most were focal epithelial atypia (favor reactive/indeterminate for low-grade dysplasia in the Santiago consensus). Convincing low-grade dysplasia was seen in 4 (2%) and overt high-grade dysplasia/CIS ("early GBC") in 2 (1%), both were EGBC class-3 (complex intramucosal adenocarcinoma without overt invasion). No conventional invasive adenocarcinoma was seen.

Conclusions: In this cholecystectomy cohort from the southeastern US characterized by frequent gallstone disease, as well as chronic mucosal changes (including prominent RAS formation and pyloric metaplasia) and cholesterolosis but relatively low amount of inflammatory findings, the frequency of epithelial atypia was not uncommon (24%) but was mostly reactive, whereas intestinal metaplasia was uncommon (7%), as was convincing low-grade dysplasia (2%) or high-grade dysplasia /CIS (1%). Comparison of these findings with those from other populations, including high-risk regions such as Chile is an important next step.

1796 Prognostic Values and Clinicopathological Significances of MUC13 and AGR2 Expressions in Intraductal Papillary Mucinous Neoplasms (IPMNs) of the Pancreas

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Background: Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a primary pancreatic ductal epithelial neoplasm and has a potential to develop to invasive adenocarcinoma. The aim of this study was to investigate the associations between several possible biomarkers and prognosis/progression in IPMN and IPMN with an associated invasive carcinoma.

Design: 104 patients with IPMN, who underwent surgical resection at the Jichi Medical University Hospital from January 1st 2000 to May 31st 2016, were analyzed clinicopathologically and immunohistochemically. IPMNs (110 lesions in total) were histologically classified into IPMN, low grade (n=68) and high grade (n=16), and IPMN with an invasive carcinoma (n=26). We evaluated the immunohistochemical expression of MUC13, AGR2, FUT8, and FXYD3, which are previously reported their overexpression in pancreatic ductal adenocarcinoma.

Results: MUC13 expression was more frequently observed in cases of IPMN with an invasive carcinoma compared with IPMN without invasive carcinoma (p<0.001), and MUC13 expression was associated with poor prognosis of the patients (p=0.004). MUC13 expression was not associated with patient's age, gender, tumor location, histological subtype, lymphatic /vascular invasion, or neural invasion. AGR2 expressed in most of IPMNs, and loss of AGR2 expression tended to be associated with tumor recurrence and poorly differentiated histology of invasive carcinoma although they were not statistically significant (p>0.05). FUT8 and FXYD3 were not observed any association with clinicopathological features of IPMNs.

Conclusions: An overexpression of MUC13 and loss of expression of AGR2 may predict the progression of IPMN and an unfavorable prognosis of the patients with IPMN. Especially MUC13 may represent a useful biomarker for the treatment strategies of IPMNs.

1797 Can Pathologic Subclassification of Ampullary Carcinoma Identify Clinically Meaningful Groups?

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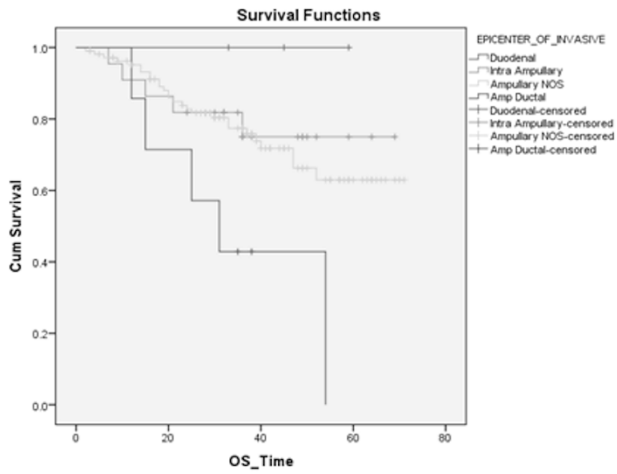
Background: Ampullary (AMP) carcinoma is a rare and poorly understood malignant tumor marked by ambiguity in definition and need for robust prognostic stratification. Recently proposed classification by Adsay et al. identified 4 prognostically distinctive site-specific types of Ampullary carcinoma (AC). Validation of the new classification for this uncommon malignancy is urgently needed. We aimed to reclassify our cohort of AC cases to determine the feasibility and prognostic significance of the proposed classification.

Design: A retrospective review of clinical and pathologic findings of pancreaticoduodenectomy resections operated at a single tertiary-care oncology centre, from 2010-2014, was undertaken and cases were re-classified in accordance with Adsay et al. classification [AJSP 2012;36:1592–1608].

Results: 150 ACs were re-classified into 4 types: 1) **Intra-AMP**(n=24; 16%): displayed large preinvasive intrampullary component, small invasive T(iT) size, intestinal histology, low T-stage, margin negativity(R0) and most favorable 4-yr survival of 75%. An iT-size < 5mm was significantly associated with a progression-free outcome (p=0.01). 2) **Peri-AMP-duodenal**(n=3; 2%): had large exophytic duodenum-centred tumors, small iT size, intestinal type, R0 resection and 3-yr survival of 100% (4-yr data unavailable). 3) **AC, Not Otherwise Specified**(n=116; 77.3%): lacked specific traits, displayed heterogenous histology, T stage, R1 rate (5%) and showed intermediate prognosis with a 66% 4-yr survival. 4) **AMP-ductal**(n=7; 4.7%): showed circumferential schirrous growth along duct walls, lack of preinvasive component, smallest tumors, pancreatobiliary type, high T and N stage, margin positivity (R1; 14%) and worst 4-yr survival of 43%. iT-size(p=0.0005), tumor budding(p=0.001), histology(p=0.012) varied significantly with the 4 types.



There was a significant difference in survival of the 4 subtypes(log rank, p=0.02); type 1 and 2 displayed favorable, type 3 intermediate and type 4 worst prognosis.

Conclusions: The present study provides data on a large cohort of AC cases evaluated at a single centre. Pathologic subclassification of AC, in accordance with the proposed new classification, is feasible and segregates heterogenous AC cases into pathologically distinct and clinically relevant prognostic subtypes.

1798 Prognostic Significance of the Number of Involved Lymphatic Vessels in Patients with Resectable Pancreatic Head Cancer

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Background: Little is known concerning the prognostic significance of the degree of lymphatic vessel invasion in patients with resectable pancreatic cancer. Thus, we investigated the relationship between the number of involved lymphatic vessels and prognosis in pancreatic cancer patients.

Design: We retrospectively studied 64 patients (median year of age 70, range 36-83; 31 men and 33 women) with pancreatic head cancer who underwent pancreatoduodenectomy at Jichi Medical University hospital between 2000 and 2015. All cases were histopathologically diagnosed as primary pancreatic ductal adenocarcinoma and classified according to the UICC TNM classification system [T1 (n=2), T2 (n=1), T3 (n=61), T4 (n=0); N0 (n=28), N1 (n=36); and M0 (n=64)]. Of these, 10 cases received neoadjuvant chemotherapy, and 49 cases received adjuvant chemotherapy. The number of involved lymphatic vessels were counted using D2-40 immunohistochemistry on the whole mount tissue sections that covered the greatest dimension of the tumor at the horizontal sectional view. We investigated the association between post-operative disease free survival, overall survival and clinicopathological factors (sex, age, neoadjuvant chemotherapy, adjuvant chemotherapy, tumor size, lymphatic vessel invasion, bile duct invasion, duodenal invasion, pancreatic anterior serosal invasion, pancreatic posterior invasion, portal invasion, bile duct cut end margin, dissected peripancreatic tissue margin, and lymph node metastasis) by the Kaplan-Meier method, log rank test and Cox proportional hazards analysis.

Results: Univariate analysis demonstrated that among clinicopathological factors investigated, only 5 or more numbers of invaded lymphatic vessels was significantly associated with shorter recurrence-free survival time (median 9 vs 41 months, P<0.05). Meanwhile, overall survival time was found to be shorter in patients without adjuvant therapy (median 24 vs 54 months, P<0.05), with duodenal invasion (mean 7 vs 14 months, P<0.05), and with pN1 (mean 32 vs 112 months, P<0.05) by univariate analysis. However, multivariate analysis demonstrated that factors influencing overall survival time were adjuvant therapy (hazard ratio 0.27, 95% CI 0.10-0.67), pN1 (hazard ratio 6.72, 95% CI 1.93-23.3) and 6 or more numbers of invaded lymphatic vessels (hazard ratio 3.08, 95% CI 1.21-7.83).

Conclusions: Our study strongly suggests that the degree of lymphatic vessel invasion is associated with prognosis in patients with resectable pancreatic head cancer.

1799 Paraduodenal Pancreatitis (PDP) Shows Histomorphologic Mimicry of Autoimmune Pancreatitis (AIP): An Analysis of 62 PDP Reveals Common Occurrence of Periductal Lymphoplasmacystic Infiltrates with IgG4 Positive Cells and Granulocytic Epithelial Lesions

Takashi Muraki, Michelle D Reid, Grace E Kim, Gabriella Bedolla, Yue Xue, Bahar Memis, Hyejeong Choi, Ipek Erbarut, Alyssa Krasinskas, David Kooby, Shishir Maithel, Juan M Sarmiento, Volkan Adsay. Emory Uni., Atlanta, GA; Uni. of California, San Francisco, San Francisco, CA; Ulsan Uni., Seoul, Korea.

Background: The diagnosis of autoimmune pancreatitis (AIP) have far reaching implications for the patient (and the family), from exhaustive steroid-therapy, to relapse potential, to life-long screening for multi-organ involvement. Paraduodenal pancreatitis (PDP) and AIP both present as pancreatic pseudotumors, and accurate distinction relies heavily on histological examination.

Design: Histopathologic examination and immunohistochemical staining for IgG4 were performed in 62 resected PDP.

Results: All cases showed characteristic histologic features as well as lesional distribution and imaging findings of PDP. The patients were predominantly male (77%); mean age, 51 (30-80); H/O alcohol 88%, smoking 93%, hypertension 56%. There was no extra-pancreatic IgG4-related lesion or inflammatory bowel disease in the patients. Serum IgG4 was performed in 5 cases and were all within normal limits. Histomorphologic findings that are considered characteristic of AIP were frequent in PDP including: periductal condensation of lymphoplasmacytic infiltrate 66% (41/62); ≥10 IgG4-positive cells/HPF in 40% (25/62); ≥50 IgG4-positive cells/HPF in 18% (11/62); luminal or intraepithelial neutrophilic infiltrates in pancreatic ducts in 63% (39/62); "granulocytic epithelial lesions" (GELs) in 42% (26/62). However, none of the cases had the distinctive sclerotic pattern of IgG4-disease including storiform fibrosis, dispersion of plasma cells within lacunae and periductal expansion or peri-phlebitis. Increased IgG4-positive cells had a strong association with GELs (mean, 22 cells/HPF, vs 11 in no GELs, p=0.0263) and a tendency for h/o alcohol use (mean, 18 cells/HPF, vs 6 in no alcohol use, p=0.0992).

Conclusions: Findings previously considered "pathognomonic" of AIP (including periductal IgG4-positive plasma cell-rich lymphoplasmacytic infiltrates and granulocytic epithelial lesions) are frequently observed in PDP (40-66%), and may cause misdiagnosis if other histopathologic findings (such as storiform fibrosis, lacunal plasma cells, periphlebitis) and clinical/serologic criteria of AIP are not carefully sought and excluded.

1800 The Nature of Cystic Lesions > 1 cm Associated with Pancreatic Ductal Adenocarcinoma

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Background: The vast majority of pancreatic ductal adenocarcinomas (PDACs) are solid tumors. PDACs associated with cystic changes present as a diagnostic challenge and some indiscriminately classified as "IPMN-associated".

Design: Cysts >1 cm detected in juxtaposition with ordinary PDAC were analyzed.

Results: In the institutional files, the frequency of cyst size > 1 cm documented in the gross report in the 501 consecutive ordinary PDACs was 4.0 % (20/501). Analyzing all cases together (including consultation), there were 88 resected cases primarily as a cystic mass, with the PDAC representing a smaller/incidental component (58 IPMN-associated, 30 MCN-associated). Whereas, 41 were resected with a primary diagnosis of PDAC and the cysts identified incidentally, and this group could be classified as: **I. Epithelial/carcinoma lined. 1) Secondary duct ectasia ("retention cysts")** (n=15); often small, chain of relatively round, evenly-shaped, smooth-contoured ducts, partially lined by epithelium but all also colonized by carcinoma cells. **2) Large duct type PDAC** (n=6); massively dilated invasive ductal units some > 1 cm. **3) Simple mucinous cyst** (SMC, n=4); large cysts with hyalinized band-like wall (AJSP, 2016). **4) MCN-associated** (n=2); subtle ovarian stroma discovered in re-analysis. **5) Congenital/choledochal cyst** (n=2); large, band of muscle coat, other epithelia (respiratory), and peri-biliary mucous glands. **II. Necrosis/degeneration related. 1) Fat necrosis associated (true pseudocyst)** (n=5). **2) Centrally necrotic/hyalinized** (n=3); clinically presenting as "pseudocyst". **3) Paraduodenal wall cyst of paraduodenal pancreatitis** (n=3);partially lined by epithelium and partially by hypercellular reactive tissue and stromal deposition of acinar secretions associated with inflammatory/fibroblastic reaction.**4) Hypercellular/inflammatory necrosis with cyst formation** (n=2).

Conclusions: A variety of mechanisms lead to cyst > 1 cm in PDACs. PDACs associated with precursor lesions (IPMN/MCN) are often diagnosed as such pre-operatively (98%). Cases that present as PDAC with an associated cystic component are typically due to secondary effects of the tumor such as retention cysts (37%) or necrosis (32%). Examination for muscular coat, ovarian stroma or other epithelia is warranted. Colonization of the cyst lining by carcinoma is frequent (63%) and should not be regarded as evidence of IPMN. Some PDACs are found in association with SMCs, raising the concern that SMCs may have precursor nature.

1801 Characterization and Diagnosis of Small Pancreatic Serous Cystadenomas

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Background: Serous cystadenomas (SCAs) are benign pancreatic cystic neoplasms that can be managed conservatively unless they become symptomatic. While large SCAs can usually be recognized radiographically by a pathognomonic central stellate scar, smaller SCAs may be difficult to distinguish from other neoplastic lesions.

We studied a large series of SCAs, with special emphasis on the radiologic-pathologic correlation of small SCAs.

Design: All SCAs were diagnosed or resected from a single institution. SCAs were divided into two groups based on size: "small" (≤4 cm) or "large" (>4 cm). Corresponding clinico-radiologic information and pathology reports were reviewed.

Results: 74 SCAs were collected (56 females, 18 males; median age 62 years, range: 27-95). Median tumor size was 2.5 cm (range: 0.5-11). 18 SCAs were large (24.32%) and 56 were small (75.7%). SCAs were located in the pancreatic head (n=23, 31%), body and/or tail (n=45, 61%), or unknown (n=6, 8%). Sixty-eight patients (91.9%) underwent surgical resection. SCAs in the head of pancreas had significantly higher chance of being >4cm (p<0.01). Fine needle aspiration (FNA) was available in 27 (36.4%) cases with cytological diagnoses as follows: cyst content (n=11), neuroendocrine tumor (n=4), mucinous neoplasm (n=4), SCA (n=2), and inadequate (n=6). Six of 74 (8.1%) had a core biopsy, all confirmed the diagnosis of SCA.

Abdominal CT scans were available in 52 patients (table 1). 78.8% (41/52) of SCA cases were initially misdiagnosed on CT, of which 24.4% (10/41) were >4cm while 75.6% (31/41) were ≤4cm. Chi Square test revealed that >4cm and ≤4cm groups were associated with different entities. The >4 cm group was significantly more associated with inflammatory conditions (33.3% vs 2.5%, p=0.008), while the ≤4 cm group tended to have more neoplastic conditions (75.0% vs 50.0%, p=0.15).

Radiology	>4 cm (n=12)	≤4 cm (n=40)
Neoplastic	6 (50%)	30 (75%)
Inflammatory and nonspecific	4 (33.3%)	1 (2.5%)
SCA and probable SCA	2 (16.7%)	9 (22.5%)

Note: Neoplastic diagnoses included intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, neuroendocrine tumor, suspicious for carcinoma.

Conclusions: Small SCAs are frequently misinterpreted as other pancreatic neoplasms radiologically. Core biopsy is a more accurate modality for the diagnosis of SCA compared to FNA cytology (p<0.0001).

1802 Investigation of the Genomic Landscape of Gall Bladder Carcinoma for Predictive and Prognostic Markers

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Background: Gall bladder carcinoma (GBC) "rare in most of the world, is common in south-east Asia. Cases present in advanced stages with dismal prognosis and limited therapeutic options. The current study investigated the genomic landscape of GBC for predictive targets and driver mutations.

Design: Study group comprised 268 cases of GBC diagnosed in radical cholecystectomy(24.3%), simple cholecystectomy(14.9%), biopsy(42.5%) and metastatic sites including omentum(3.0%), liver(4.5%), lymph node(3.7%), scar site(5.2%) and distant metastasis(1.9%). Immunohistochemistry for EGFR, VEGF, Her2 and p53 was performed and positive expression interpreted as in Table 1. EGFR and Her2 amplification was validated in FISH using TMA and broader spectrum of gene variation was screened in NGS in representative FFPE tissue using the Ion amplisec cancer hotspot panel V2 (Thermofischer Scientific).

Results: Mean age was 49.5 years with female preponderance (76.6%). Histological types included 221 cases of adenocarcinoma, 23 papillary adenocarcinoma, 11 mucinous, 8 adenosquamous, 1 signet ring, 3 neuroendocrine and 1 undifferentiated carcinoma. Well differentiated adenocarcinoma comprised 48.5% of 262 cases graded by WHO criteria. Majority (76%) presented with stage 3 or 4 disease. Marker expression was not significantly different with stage, grade, type and metastasis in GBC except VEGF with tumour grade (p=0.032). Overall positive expression of p53, VEGF, Her2 and EGFR is shown in Table 1 and Fig1. Intratumoral heterogeneity was evident as shown in fig2.

NGS revealed consistent p53 mutations along with gene variations in the RAS activation pathway including EGFR, Her2, Her3, FGFR, PDGFRA, KRAS and RET. Additionally some cases showed APC gene, CDKN2A, SMARCB1, NOTCH1 alterations.

Markers	Criteria:%/intensity	Number of cases	Positive(%)
p53	≥50%,2-3+	230	127 (55.2)
EGFR	≥10%, 3+	214	74 (34.6)
VEGF	≥30%, 2-3+	165	131 (79.4)
Her2	≥10%, 3+	245	67 (27.3)

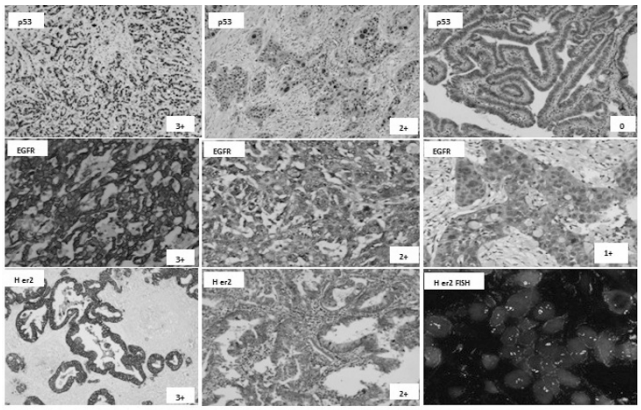

Fig1: Variable percent and intensity of p53, EGFR and Her2 IHC expression in GBC, 2+ Her2 IHC showing amplification in FISH.

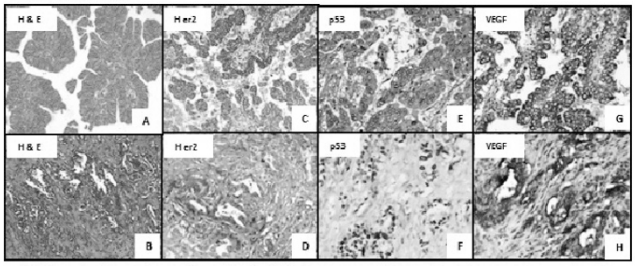

Fig2: Case of papillary adenocarcinoma(A) with infiltrative component(B), showing heterogenous expression of Her2(C,D), p53(E,F) and VEGF(G,H) in both tumor component.

Conclusions: Targetable markers like Her2, VEGF and EGFR are expressed in high proportion of GBC. *RAS* pathway molecules including Her2, EGFR and VEGF showed significant co-expression.

1803 Molecular Correlates of Major Morphologic Subtypes of Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) can exhibit a variety of morphologic patterns. However, the clinical significance and molecular underpinnings of these distinct patterns are largely unknown. The aim of this study was to evaluate the spectrum of molecular alterations across PDAC morphologic subtypes, focusing on pancreaticobiliary and intestinal-type PDAC, the two most common patterns.

Design: Resected PDAC specimens from patients without prior neoadjuvant therapy were scored for extent of different morphologic patterns and then assigned an overall morphologic subtype. Paired tumor and normal sequencing was performed by an NGS panel designed to interrogate 429 genes specifically implicated in PDAC pathogenesis. Dynamic adjustment of sequencing depth and a customized bioinformatics pipeline were employed to reliably detect mutations in samples with low tumor cellularity. Fisher's exact test was used to evaluate differences in molecular alteration frequency.

Results: Morphologic review of 200 cases yielded 120 pancreaticobiliary-type PDACs, 32 intestinal-type PDACs, and 48 cases with less common morphologic patterns. Integrated SNV/CNV analysis of key PDAC genes revealed a similar frequency of *KRAS* mutation in both major subtypes and similar rates of *SMAD4* inactivation. However, the frequency of *TP53* inactivation was significantly lower in intestinal-type PDAC than in the pancreaticobiliary type (48% versus 72%, p=0.017). While the overall rate of *CDKN2A* inactivation was not statistically different between subtypes, the method of inactivation differed, with SNVs accounting for a larger percentage of *CDKN2A* inactivation in pancreaticobiliary-type PDAC (21% versus 3%, p=0.017). Multiple genes, including *COL14A1*, *ARID1A*, *PSPH* and *LRP1B* showed a significantly higher rate of SNV alteration in intestinal-type versus pancreaticobiliary-type PDAC. Copy number gains of *IKBKE*, *CHD7* and *PTGS2* were significantly more common in intestinal-type PDAC, while *MED29* gain was more common in pancreaticobiliary-type PDAC. Although the overall frequency of *GNAS* mutations in the cohort was low, they were more common in intestinal-type PDAC (13%) than in pancreaticobiliary-type PDAC (3%).

Conclusions: Our findings suggest that while pancreaticobiliary and intestinal-type PDAC are molecularly related, they harbor partially distinct molecular profiles that can help elucidate their underlying pathogenesis and that may play a role in guiding therapy.

1804 Histologic Definition and Prognosis of "T3" Gallbladder Adenocarcinoma

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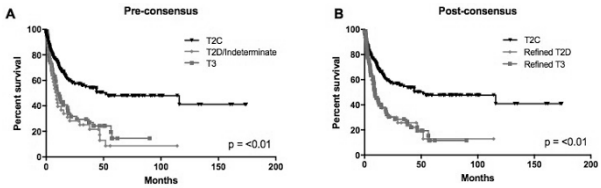
Background: The literature on the prognosis of T3 gallbladder carcinoma (GBC) has highly conflicting data, partially attributable to the challenges in the histologic criteria for "perforates the serosa" or "directly invades the liver".

Design: Among 626 GBCs, 102 were found to display close proximity of the cancer cells to the external-surfaces/liver, and were analyzed and compared to T2c cases, (i.e., those showing substantial invasion into adipose-rich layer of the GB but > 1 mm away from surfaces; abstract in *Modern Pathology* 29(2S):445A, 2016).

Results: The 102 cases could be classified in 2 groups: 1) Definite T3 (n=62), with clearly demonstrable carcinoma cells on the serosal surfaces or in the liver; 2) T2D/indeterminate (n=40), extending close to(within 1 mm) but not showing carcinoma cells literally on the surfaces or in the liver. Both groups were found to have equally aggressive behavior, significantly worse than the T2C cases, and in fact, the T2D/indeterminate group seemed to do worse. Subsequently, an attempt was made to classify the indeterminate cases by extrapolating from the criteria proposed for T4a colon cancers: 1) Refined T3 (n=71) also including cases with surrogate evidences of external surface involvement such as carcinoma cells forming a nodule/retraction pushing into and associated with fibrin/granulation-tissue/ulceration or mesothelial reaction on the immediately adjace; versus refined T2D (n=31) delimited from the surface by a band of non-inflamed fibrous tissue or accompanied on the surface by intact/continuous/flattened mesothelial cells without reactive changes; or maintaining the integrity of the imaginary line (at low power) between the liver and GB wall. However, even with these refined criteria, no survival benefit was observed.



Conclusions: Similar to the recent observations in colon (PMID:23774176), the prognosis for gallbladder cancers in which the tumor closely approximates (within 1 mm of) the serosal surface or liver ("T2D or indeterminate") is virtually identical to those with overt serosal or liver involvement documentable on the histologic samples. Therefore these T2D/indeterminate cases also ought to be managed as T3.

1805 Combined FISH, IHC and NGS Identifies New Therapeutic Targets in Cholangiocarcinomas

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Background: Cholangiocarcinoma (CCA) is the most common biliary tract malignancy. It can affect any point of the biliary tree and based on its location, may be classified as intrahepatic, perihilar or extrahepatic. Unfortunately, CCA is often asymptomatic until it has metastasized thereby restricting therapeutic options, with poor 5 year overall survival. Although rare, CCA incidence has increased significantly in the last decades.

Design: Increasing our knowledge of CCA (identifying novel therapeutic targets and better classifications) will offer new targets for therapy and allow for more effective treatments. A cohort of 54 clinically well characterised CCA cases was included. Formalin-fixed-Paraffin-embedded blocks were used to perform immunohistochemistry (mismatch-repair proteins), and FISH (amplification of FGFR1, FGFR2 and HER2 as well as FGFR2 break-apart). DNA was extracted from the FFPE blocks and a number of genes were targeted for using NGS.

Results: The majority of tumours were MMRp (mismatch repair proficient) (92.6%). There was no evidence of FGFR1 or FGFR2 amplification whilst HER2 amplification was seen in six samples (11.3%). NGS revealed mutations in KRAS (24.5%), NRAS (15.1%), BRCA1 (13.2%), BRCA2 (7.5%) and EGFR (13.2%). No mutations were seen in BRAF. Half of the HER2 amplified cases had mutations in the genes mentioned above. FGFR2 translocations were seen in 12.96% of samples, interestingly we also saw amplification of parts of the C-terminal and N-terminal of the receptor.

Conclusions: Our results add further evidence that the EGFR pathway is a promising molecular target for CCA. We have also shown that FGFR2 translocations are present in CCA which adds to the evidence of using FGFR inhibitors as part of a treatment course. HER2 blockade is also promising treatment strategy for patients with gene amplification. Correlating this data with the clinical information of the patients may help in identifying higher risk individuals and may potentially provide early screening opportunities should novel biomarkers be identified.

1806 Immunohistochemical Detection of Loss Expression of ATRX/DAXX in Neuroendocrine Tumors from Various Organs

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Background: Nearly all liver neuroendocrine tumors (NETs) are metastatic well- or moderately differentiated neuroendocrine tumors, a significant percentage of which are from the pancreas. Identification of the tumor origin can be challenging because of the similar histological features and overlapping immunostaining profiles, regardless of origin. Limited studies suggested that loss of expression of transcription factors ATRX and/or DAXX was typically seen in pancreatic neuroendocrine tumors (PNETs). In this study, we evaluate the expression of ATRX and DAXX in neuroendocrine neoplasms from the pancreas and various organs to determine the diagnostic utility of these two markers in identification of pancreatic origin of a neuroendocrine neoplasm.

Design: Immunohistochemical evaluation of the expression of ATRX (rabbit polyclonal, 1:500, Sigma Aldrich Corporation) and DAXX (rabbit polyclonal, 1:200) was performed on 146 cases of NETs on tissue microarray sections, including lung carcinoid/atypical carcinoid (LNET), PNETs, stomach and duodenum (Stom/duo), ileum, appendix, and rectum/colon, urinary bladder (small cell carcinoma), skin (Merkel cell carcinoma), and liver NETs. Loss of nuclear staining was recorded as positive (no tumor cells stained with positive internal control) and negative/presence of nuclear staining (both tumor cells and internal controls stained).

Results: The loss expression of ATRX, DAXX or both ATRX and DAXX is summarized in Table 1. Two liver metastasis cases with loss of ATRX and DAXX were clinically suggestive of a gastrointestinal primary, and the remaining cases were from the pancreas.

Table 1. Summary of immunostaining results

ATRX/DAXX	Loss of ATRX	Loss of DAXX	Loss of both ATRX and DAXX	Total cases with loss of ATRX, or DAXX, or both
PNET (N=28)	3 (11%)	4 (14%)	2 (7%)	9 (32%)
LNET (N=19)	0	0	0	0
Liver (N=12)	2 (16%)	0	5 (41%)	7 (57%)
Ileum/Cecum (N=19)	0	0	0	0
Stom/duo (N=2)	0	0	0	0
Appendix (N=11)	0	0	0	0
Rectum (N=12)	0	0	0	0
Urinary Bladder (N=20)	7 (35%)	0	0	7 (35%)
Skin (N=23)	0	0	0	0

Conclusions: These data suggest that 1) loss of ATRX/DAXX is seen in 32% of PNETs and 35% of small cell carcinomas of the bladder; and 2) loss of expression of ATRX/DAXX is not evident in neuroendocrine tumors from the lung and skin; and 3) loss of expression of ATRX/DAXX is rarely seen in tumors from the gastrointestinal tract. Therefore, a metastatic NET with loss of expression of ATRX/DAXX is highly suggestive of a pancreas or bladder primary site.

1807 Molecular Characteristics of Intraductal Oncocytic Papillary Neoplasms of the Bile Ducts

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Background: "Oncocytic subtype" of intraductal papillary mucinous neoplasms of the pancreas (a.k.a. intraductal oncocytic papillary neoplasm, IOPN) is a well-known entity in the pancreas. Its distinct molecular features have also recently been discovered (PMID: 27282351). A similar, if not identical, tumor rarely occurs in the biliary tract. However, molecular characteristics of biliary IOPN have not yet been characterized.

Design: Four biliary IOPNs were identified in our institutions' surgical pathology files with matched normal tissue. All neoplasms revealed the characteristic histomorphology; uniformly exhibited typical entity-defining morphology of arborizing papillae lined by layers of cells with oncocytic cytoplasm, prominent nucleoli, and intraepithelial lumina and no obvious intracellular mucin. Formalin-fixed, paraffin-embedded specimens of all neoplasms were microdissected, and subjected to deep coverage, targeted next-generation sequencing for a panel of 410 key cancer-associated genes.

Results: The number of mutations per case ranged from 4 to 9 (median=6). None had *KRAS* mutations. Two cases had *GNAS* mutations, although not at codon 201. One case had *PIK3CB* mutation and another, *PIK3CD* mutation. Details of the individual cases are listed in Table.

	Gene	Mutation Type	Protein
1	PIK3CBMET-MLL3PREX2N-COA2WT1GNASB-COR	Splice site Missense Missense Missense Missense Missense Nonsense	p.K1025 splicep. T1116Ap.K827Ip. G1078Rp.A1294Vp. W486Lp.T1012I p.S1437
2	CDC73GSK3B-KEAP1GNAS	Missense Missense Deletion Missense	p.G416Ap.D124Np. Q620del p.A210P
3	CTNNB1IKZF-1FLT1POLD1ASX-L1CDK12FGF-3PIK3CD ZFHX3	Missense Nonsense Missense Missense Missense Missense Missense Deletion	p.D32Yp.R111p. A1319Vp.R808Hp. H194Qp.L1301Fp. S138Cp.V194Ip. G3527del
4	BLMKITLATS2 NOTCH4	Missense Missense Missense Missense	p.D239Np.P34Lp. V367Ip.R373W

Conclusions: Our preliminary data suggest that IOPN is a distinct clinicopathologic entity, not only in the pancreas, but also in the biliary tract, as alterations of common oncogenic signaling pathways appear to be uncommon in biliary IOPN, as well. Further analyses of molecular alterations in a larger series has already been initiated to discover yet unknown molecular changes, to understand the carcinogenesis of IOPN, and potentially uncover new therapeutic targets for patients whose tumors harbor these alterations.

1808 F.I.S.H.'ing to Verify the Nature of Different Epithelial Alterations in the Gallbladder: Molecular Abnormalities Are Common in Neoplastic but *Not* in Reactive Lesions, Thus Validating the Santiago Criteria and Potential Usefulness of F.I.S.H. as an Adjunct in Diagnosis

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Background: The definition and diagnosis of true dysplasia in the gallbladder (GB) is a well-known challenge. Santiago consensus meeting (2014) has proposed refined criteria.

Design: Mayo Clinic's pancreatobiliary FISH protocol devised for the diagnosis of biliary cancers was employed in 54 GBs showing various types of atypia: Low-grade dysplasia (LGD, 7), high-grade dysplasia (HGD, 17), Santiago groups 2 and 3 (focal epithelial atypia, indefinite-for-dysplasia/favor reactive; FEA; 23) and invasive ca (15).

Results: Molecular/genetic hallmark of biliary cancers at the FISH level, polysomy (copy number gain of at least 2 loci) was the most "severe" FISH finding and was seen in all 15 GB invasive carcinomas as well as more than half of HGD/CIS ("early GBC") cases whereas it was exceedingly uncommon in the Santiago-FEA (only 1 of 23) and none of the LGDs. Furthermore, the markers of early cancerous transformation (9p21 loss and single locus gain (SLG)) were seen in about a third of LGD/HGD cases but not in invasive ca (and only 1/23 FEA cases), confirming their role in non-invasive neoplastic changes in the GB epithelium.

		FISH RESULT					
Category	N	Disomy %	Hemi 9p21 Loss%	Homo 9p21 Loss%	SLG%	SLG with 9p21 loss%	Polysomy %
Control	6	100	0	0	0	0	0
Reactive atypia (Santiago groups 2/3)	23	87	0	0	4	4	4
LGD	7	72	14	0	0	14	0
HGD	17	12	24	6	6	0	53
Invasive	15	0	0	0	0	0	100
SLG, single locus gain of 1q21, 7p12 or 8q24.							

Conclusions: This study confirms that the Santiago consensus criteria for epithelial atypia do indeed identify distinct groups distinguishable not only morphologically but also at the molecular level, with the HGD group often showing polysomy and LGD/HGD groups showing early molecular alterations of 9p21. Santiago groups 2 and 3 ("focal epithelial atypia/favor-reactive/indefinite-for-dysplasia") are genetically stable proliferations not showing molecular evidence of cancerous transformation, and thus can be regarded as reactive. The Mayo Clinic FISH protocol can be used as an adjunct in the diagnosis of GB epithelial atypia in challenging cases.

1809 SMAD4 Loss in Pancreaticobiliary, Gastrointestinal and Extra-Gastrointestinal Carcinomas

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Background: SMAD4 (DPC4) is a tumor suppressor that functions as an intracellular transmitter of TGF-β signaling pathway. Loss of SMAD4 expression is seen in approximately 50% of pancreatic and 25% of colonic adenocarcinomas. In the evaluation of metastatic carcinoma of unknown primary site, loss of SMAD4 is used to suggest pancreaticobiliary origin, but there is limited data on SMAD4 immunohistochemical expression in epithelial neoplasms of other sites, particularly adenocarcinomas, and specifically CK7+ types. The goal of this study was to determine the frequency of SMAD4 loss in a large group of carcinomas from diverse anatomic sites.

Design: Immunohistochemistry for SMAD4 (Santa Cruz, clone EP618Y; 1:50 dilution) was performed on 1252 carcinomas (218 whole-tissue tumor sections and 1034 tumors in tissue microarrays) from multiple organs. Expression of SMAD4 was considered lost when all neoplastic cells lacked nuclear staining in the presence of nuclear reactivity in non-neoplastic cells, which served as internal controls.

Results: SMAD4 loss was seen in 58% (19/33) of pancreatic tumors, 27% (6/22) of appendiceal adenocarcinomas, 17% (86/522) of colorectal adenocarcinomas, 16% (6/37) of cholangiocarcinomas, 4% (2/53) of esophageal adenocarcinomas, 3% (8/266) of breast adenocarcinomas, 2% (1/45) of gastric adenocarcinomas, and 2% (1/42) of non-serous ovarian carcinomas. The remaining 232 tumors, including papillary thyroid carcinoma, lung adenocarcinoma, hepatocellular carcinoma, ovarian high-grade serous carcinoma, endometrial adenocarcinoma, and renal cell carcinoma all retained SMAD4 nuclear expression. The intensity of nuclear staining in SMAD4-intact tumors was predominantly weak to moderate in intensity.

Conclusions: SMAD4 loss is most commonly seen in pancreaticobiliary adenocarcinomas, followed by appendiceal and colonic adenocarcinomas. SMAD4 loss is also seen in a subset (2-4%) of carcinomas of other sites, specifically breast, esophageal and gastric adenocarcinomas, all of which are typically CK7 positive, similar to pancreaticobiliary carcinoma. Awareness of SMAD4 loss in carcinoma types other than pancreaticobiliary is helpful in the evaluation of metastatic carcinomas of unknown primary site.

1810 Measured Residual Tumor Cellularity Correlates with Survival in Neoadjuvant Treated Pancreatic Ductal Adenocarcinoma

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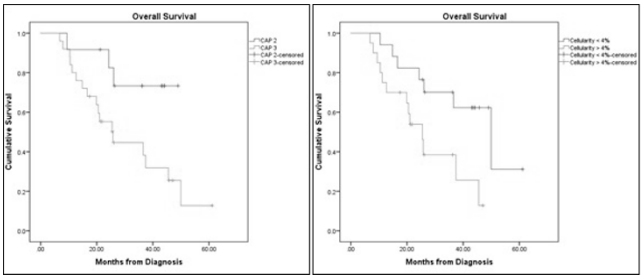
Background: Neoadjuvant chemoradiation is increasingly used to treat pancreatic ductal adenocarcinoma (PDA). Multiple histologic grading schemes are used to stratify tumor response to neoadjuvant therapy. The CAP and Evans systems are most commonly used. Each system has been shown to have prognostic value; however both have limitations. The Evans system is partially based on viability of tumor cells which is subjective. The CAP histologic grading system is based on subjective assessment of the amount of residual tumor cells and fibrosis after treatment. Our aim was to objectively assess residual tumor cellularity and determine if measured residual tumor cellularity predicts patient survival.

Design: We retrospectively reviewed slides from 76 patients who received neoadjuvant therapy for PDA from 2009 to 2014. Each case was reviewed and given an Evans and CAP tumor regression score. The most representative tumor slide was digitally scanned. The area of tumor bed (fibrosis) and tumor were selected. Residual tumor cellularity was calculated (area of tumor mm^2/area of fibrous bed mm^2 x 100%). Kaplan-Meier survival curves were constructed and Log Rank tests were used to test for statistical significance.

Results: The CAP scores were as follows: grade 1: 4 cases (5.3%); grade 2: 26 (34.2%), and grade 3: 46 (60.5%). The Evans scores were as follows: Evans 1: 2 (2.6%); Evans 2a: 44 (57.9%); Evans 2b: 26 (34.2%); Evans 3: 4 (5.3%). 39 (51%) patients had no lymph node metastases and 37 (49%) had lymph node metastases. Lymph node positive patients with residual tumor cellularity of less than 4% had significantly better overall survival than those with residual tumor cellularity greater than 4% (p=0.02). In lymph node positive patients, the CAP score also strongly correlated with survival (p=0.04). The Evans scores weakly correlate with survival (p=0.11). In lymph node negative patients, CAP score, Evans scores, and tumor cellularity did not predict survival (p=0.65, 0.65, and 0.54, respectively).



Conclusions: Tumor cellularity and CAP treatment response grade predict overall survival in patients with lymph node metastases at the time of tumor resection. Interestingly, this was not the case with the overall cohort or with lymph node negative cases.

1811 Sequential Analysis of Pancreatic Cystic Lesions Generates a Significantly Higher Diagnostic Accuracy at Molecular Than Cytologic Levels

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Background: Recent advances in diagnostic imaging have led to a large increase in the detection of pancreatic cystic lesions (PCL). Up to 10% of PCL are of neoplastic nature with potential to progress to pancreatic adenocarcinoma. Early detection of these precursors can dramatically increase the survival rate. The current diagnostic approaches rely on a combination of high-resolution imaging, cytologic analysis and tumor markers with variable accuracy. The aim of this study is to evaluate and compare the molecular and cytologic diagnostic utility of multiple sequential cyst fluid analyses.

Design: From 2010 to 2015, we prospectively evaluated 33 patients (15M and 18F, median age 64.1) for whom multiple endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of cyst fluid specimens were available (average 2.2 visits). All cyst fluid specimens were subjected to next generation sequencing (NGS) for the presence of common genes alterations and compared with cytologic and final diagnoses.

Results: Cytology diagnoses were classified into three groups: non-diagnostic (group 1, 1st visit: 30.1%. cumulative: 9.1%), negative (group 2, 1st visit: 48.5%. cumulative: 60.5%) and atypical/suspicious (group 3, 1st visit: 21.3%. cumulative: 30.3%). The mutational analysis were grouped into non-diagnostic/failure (1st visit: 0%, cumulative: 0%), KRAS/GNAS/VHL group (1st visit: 54.5% cumulative: 54.5%) and any mutation (1st visit: 57.6%, cumulative: 57.6%).

Conclusions: The final diagnoses confirm that mutational analysis identifies up to 87.9% and 87.9% and cytologic analysis detects up to 54.5% and 69.7% of neoplastic lesions in 1st and multiple visits, respectively. The power of molecular analysis, in detecting a neoplastic PCL, is significantly higher in one visit (p=0.006) with relatively similar detection rates (p=0.13) for both cytologic and molecular analyses after multiple visits.

1812 Expression and Subcellular Localization of Eukaryotic Initiation Factor 3 Subunit f (eIF3f) Is Altered in Pancreatic Ductal Adenocarcinoma (PDA) and Its Precursor Lesions

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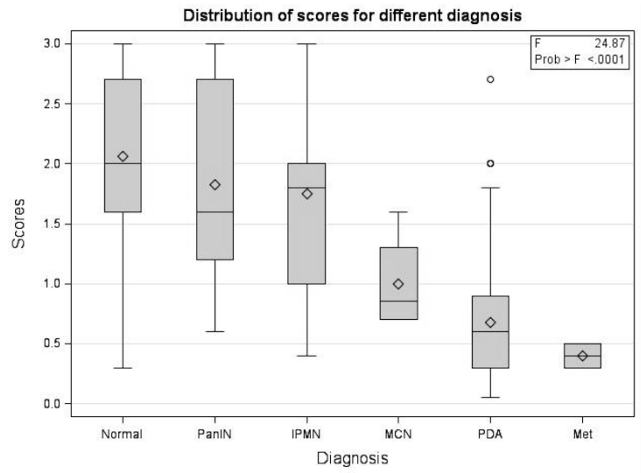
Background: eIF3f plays an important role in initiating translation and appears to have tumor suppressive functions. Previously we showed that many cancer cells, including PDA, lose eIF3f expression and eIF3f silencing led to malignant transformation of normal pancreatic ductal epithelial cells. However, it is not known if loss of eIF3f expression has diagnostic utility in distinguishing PDA from precursor lesions or benign reactive ducts.

Design: Tissue microarrays composed of 225 duplicated cases of PDA (n=83), metastatic PDA (n= 11), pancreatic intraepithelial neoplasia (PanIN; n=38), intraductal papillary mucinous neoplasm (IPMN; n=42), mucinous cystic neoplasm (MCN; n=13) and benign pancreatic tissue (n=38) were analyzed for expression of eIF3f by immunohistochemistry. We assessed subcellular localization, intensity (0, 1, 2, 3), and percentage of positive cells(score=intensity x % positive cells).

Results: There are two interesting findings. First, the subcellular localization of eIF3f was significantly altered during disease progression where there is a shift from nucleus to cytoplasm (from 8% cytoplasmic in benign ducts to 26-51% in precursors to 69-71% in PDA; p<0.0001). Second, loss of eIF3f staining occurs specifically in PDA and metastatic PDA (22 and 36%) with only rare exceptions in IPMN (7%) whereas all benign ducts retained eIF3f staining (p<0.0001). However, there is a gradual trend of decreasing eIF3f expression from benign ducts to PDA precursors and to PDA. For distinguishing between PDA and benign ducts or precursor lesions, eIF3f has a sensitivity and specificity of 23% and 98%, respectively.

	Nuclear(%)	Postivie(%)	Negative(%)
Normal	35(92)	38(100)	0(0)
PanIN	28(74)	38(100)	0(0)
IPMN	19(49)	39(93)	0(0)
MCN	9(69)	13(100)	3(7)
PDA	20(31)	65(78)	0(0)
Metastitic PDA	2(29)	7(64)	4(36)



Conclusions: Subcellular localization and expression of eIF3f is altered in PDA and PDA precursor lesions, supporting its important role in PDA development. eIF3f is a highly specific biomarker for the diagnosis of PDA, which can be very useful in the in the setting of small biopsies to distinguish benign reactive ducts or precursors from PDA.

1813 Prospective DNA Testing of 462 Pancreatic Cysts Reveals the Inclusion of Mutational Allelic Frequencies (AFs) Enhance the Detection of Malignancy in Intraductal Papillary Mucinous Neoplasms (IPMNs)

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Background: DNA testing of pancreatic cyst fluid (PCF) has emerged as an adjunct to the evaluation of pancreatic cysts. Mutations in *KRAS*/*GNAS* are highly sensitive for IPMNs, and additional alterations in *TP53*/*PIK3CA*/*PTEN* are associated with at least high-grade dysplasia (HGD). However, previous studies have been retrospective, utilizing postoperative cyst fluid, limited in sample size and/or suffer from insensitive detection strategies. A prospective study was performed to evaluate the accuracy of DNA testing in cyst classification and detection of an IPMN with at least HGD.

Design: Over 31-months, 486 PCFs obtained by EUS-FNA were prospectively submitted for molecular analysis to include *KRAS*, *GNAS*, *HRAS*, *NRAS*, *BRAF*, *VHL*, *CTNNB1*, *TP53*, *PIK3CA*, *PTEN* and *AKT1*. For all genes, except *VHL*, next-generation sequencing was performed with minimal coverage of 500X for each genomic region (Ion Torrent PGM). Sanger sequencing was used to assess exons 1 through 3 of *VHL*. Molecular findings were correlated with CEA, cytology and follow-up surgical resection material.

Results: Among 486 PCFs, 462 (95%) were satisfactory for molecular analysis. In comparison, 365 (75%) cases were sufficient for CEA analysis and 195 (25%) cases were satisfactory for cytologic diagnosis. *KRAS*/*GNAS* mutations were detected in 220 (48%) cysts with mutational AFs ranging between 3-55% and 3-92%, respectively. Alterations in *TP53/PIK3CA/PTEN* were present in 29 (6%) cases with AFs collectively ranging between 3-50%. Surgical follow-up was available for 83 (18%) patients and included: 42 IPMNs with 14 harboring at least HGD, 9 mucinous cystic neoplasms (MCNs) and 32 nonmucinous cysts. The sensitivity and specificity of *KRAS*/*GNAS* mutations for IPMNs/MCNs was 86% and 100%, respectively. The sensitivity and specificity for IPMNs alone was 100% and 95%, respectively. In conjunction with *KRAS*/*GNAS* mutations, alterations in *TP53/PIK3CA/PTEN* had 86% sensitivity and 97% specificity for IPMNs with at least HGD. However, the sensitivity and specificity improved with the inclusion of AFs. The presence of *GNAS* AFs >55% or *TP53*/*PIK3CA*/*PTEN* AFs that were equivalent to *KRAS*/*GNAS* AFs reached 100% sensitivity and specificity for an IPMN with at least HGD.

Conclusions: DNA testing of PCF for *KRAS*/*GNAS* mutations is highly sensitive and specific for IPMNs. In addition, the presence of *TP53*/*PIK3CA*/*PTEN* mutations can be a useful marker for the identification of at least HGD. However, the accuracy of DNA testing is enhanced with the inclusion of AFs.

1814 Diagnostic Utility of VHL, Maspin, IMP3 and S100P in the Evaluation of Pancreatic Resection Margins from Frozen Tissue

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Background: Pancreatectomy is a procedure used for patients with resectable pancreatic carcinomas. The margin status determines patient's outcome and whether radiation is needed. Due to cautery and frozen section artifacts, the evaluation of pancreatic resection margins can be challenging. Our previous studies demonstrated that VHL, maspin, IMP3, and S100P were a useful panel of immunomarkers in differentiating pancreatic carcinoma or high-grade dysplasia from benign reactive changes in surgical biopsy or cytology specimens, with loss of VHL expression and overexpression of maspin, IMP3 and S100P in adenocarcinoma or high-grade dysplasia. The aim of this study is to assess the utility of this panel of immunomarkers in the evaluation of pancreatic margins from frozen tissue.

Design: Thirty-nine Whipple specimens were identified from the archives of anatomic pathology at Geisinger Medical Laboratories and divided into 3 groups: Group 1 - positive margin (n=8); Group 2 - benign margin (n=10); and Group 3 - atypical margin (n=21). Immunohistochemical evaluation for VHL, maspin, IMP3, and S100P was performed. The staining results were recorded as positive (>5% of atypical cells/glands stained) or negative (<5% of atypical cells/glands stained).

Results: The staining results were summarized in Table 1. Eight of 8 cases (100%) in Group 1 expressed maspin and S100P, with loss of VHL. Ten of 10 cases (100%) in Group 2 expressed VHL and were negative for maspin, with 3 cases focally positive for S100P. In Group 3, 10 cases were negative for VHL and positive for both maspin and S100P, which confirmed true positive margins. The remaining 11 cases were confirmed to have negative margins. IMP3 was only focally positive in 1 case in Group 1.

Table 1. Summary of Immunostaining Results in 39 Cases of Frozen Surgical Resection Margins

Margin	Maspin	VHL	IMP3	S100P
Group 1 (n=8)	8/8	0/8	1/8	8/8
Group 2 (n=10)	0/10	10/10	0/10	3/10, F
Group 3 (n=21)	10/21	10/21	0/21	10/21, F

N: number of cases; F: focal

Conclusions: The immunostaining panel consisting of VHL, maspin, and S100P is useful in the evaluation of pancreatic resection margins from frozen tissue, even in cases with significant cautery and freezing artifacts. In contrast, IMP3 has little utility on frozen margin sections. It is important to interpret the immunostaining results in conjunction with histomorphology.

1815 TTF-1 and Napsin-A Staining in Pancreatic and Hepatobiliary Adenocarcinomas

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Background: Immunohistochemical (IHC) staining for TTF-1 and Napsin A is diagnostic for the majority of adenocarcinomas of pulmonary origin. However, rarely, TTF-1 positivity has been described in extrapulmonary primaries. There has been a recent publication demonstrating that TTF-1 and Napsin-A positivity in 2 of 15 (13%) biliary adenocarcinomas which could potentially lead to an incorrect diagnosis in the work-up of a metastatic malignancy (Silva et. al., IJSP, p.24-28, 2015). We evaluated TTF-1 and Napsin-A IHC in a series of resected pancreatic ductal carcinomas and intrahepatic and extrahepatic cholangiocarcinomas.

Design: We examined 15 pancreatic ductal adenocarcinomas, 8 intrahepatic cholangiocarcinomas and 12 extrahepatic cholangiocarcinomas between the years of 2011 to 2015 at a tertiary care, teaching institution. A representative section of the adenocarcinomas from the resected specimens underwent TTF-1 and Napsin-A IHC staining. The staining was scored as to 0-25%, more than 25% -75% and greater than 75% of cells with intensity rated on a 3 point scale.

Results: We identified that 3 of 12 (25%) of the extrahepatic cholangiocarcinomas and 2 of 15 (13%) of the pancreatic adenocarcinomas stained with TTF-1. The staining pattern was seen focally in up to 20% of the tumor cells with mild to moderate intensity. None of the intrahepatic cholangiocarcinomas stained for TTF-1. Napsin-A was negative in all the cases, including all the positive TTF-1 staining adenocarcinomas.

Conclusions: This study confirmed that TTF-1 positivity can be seen in approximately 25% of extrahepatic cholangiocarcinomas with usually a focalpattern with mild to moderate intensity.

TTF-1 staining was also identified in 13% of the pancreatic adenocarcinomas, but was negative in all the intrahepatic cholangiocarcinomas tested.

Napsin-A was negative in all the carcinomas, including the TTF-1 positive cases

Positive TTF-1 staining can potentially be a pitfall when evaluating metastatic adenocarcinomas of unknown primary, especially when the differential diagnosis includes a pancreatic and/or biliary primary versus a pulmonary primary. However, also staining for Napsin-A can help clarify the primary site in most of these cases.

1816 Validation of a Size-Based T-Staging Protocol for Pancreatic Ductal Adenocarcinoma After Neoadjuvant Chemoradiation Therapy

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Background: The current UICC T staging protocol does not have any prognostic value in pancreatic ductal adenocarcinoma (PDAC). Recently, a size-based T-staging protocol was newly proposed. Although a size-based T-stage protocol has prognostic value in PDAC, its significance in PDAC after neoadjuvant therapy has not been validated. Hence, we validated the applicability and prognostic relevance of a size based T staging protocol for PDAC after neoadjuvant chemoradiation therapy (NCRT).

Design: From February 2005 to December 2015, among 272 patients with cytologically/histologically proven PDAC, 142 patients with resection after NCRT at Mie University Hospital were enrolled and staged by UICC 7th T-staging protocol and a size-based T-staging protocol. Log-rank and Wilcoxon were used to test the equality of the survival distribution.

Results: As a variable, UICC T-staging was significant in predicting overall and disease-specific survival of PDAC, whereas a size-based T-staging protocol was significant in predicting overall survival.

Conclusions: Our study indicates the utility of newly proposed size-based T-staging protocol is limited to assess the survival of PDAC patients with NCRT.

1817 Prognostic Significance of PD-L1 Expression in Extrahepatic Cholangiocarcinoma

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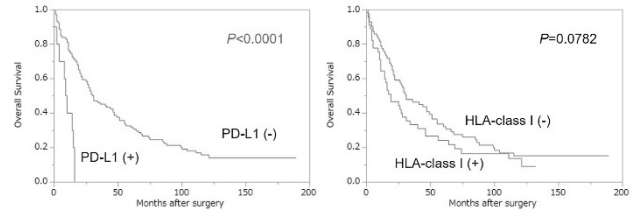
Background: Extrahepatic cholangiocarcinoma (EHCC) is one of the intractable high-grade malignancies for which further studies are needed to cure. Recently, immunotherapy, including immune checkpoint blockades, has been attracting the most attention; however, only a few studies have focused on the tumor immunity in EHCC. The present study aimed to investigate the relationship between the expression of PD-L1 and clinicopathological features including prognosis in EHCC.

Design: We analyzed the association between the expression of PD-L1, HLA-class I and clinicopathological features in 117 surgically resected EHCC specimens by immunohistochemistry using tissue microarrays.

Results: High expression of PD-L1 was significantly correlated with poorer histological differentiation (P=0.0071) and with higher expression of HLA-class I (P=0.0345). PD-L1(+) group showed worse overall survival, but HLA-class I expression did not.

figure1



Univariate and multivariate analyses revealed that N classification (P=0.0408), M classification (P=0.0266) and expression of PD-L1 (P=0.0002) were independent and significant prognostic factors.

Conclusions: These results are expected to provide useful information for selecting the appropriate patients of EHCC for immune checkpoint blockade therapies.

1818 Isocitrate Dehydrogenase Gene Mutations in Cholangiocarcinoma

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Background: Prognosis for patients with cholangiocarcinoma is poor. Surgery is the only curative option with only a minority of patients qualifying. To date there are no large randomized control trials to demonstrate the benefit of neoadjuvant or adjuvant chemotherapy and the death rate continues to rise by an estimated 2.5% per year. Our goal was to characterize potential molecular targets for earlier disease detection and prognosis in intrahepatic cholangiocarcinoma (IHCC). IHCC has been characterized by a loss of function mutation in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2). IDH mutations are also found in gliomas and acute myelogenous leukemia (AML) with IDH drugs in clinical trials for AML. Some reports have found 25% of IHCC harbor this mutation, which is not only high for a tumor mutation, but also unique in that the mutation is not identified as an important driver in other gastrointestinal malignancies.

Design: With IRB approval, we identified 57 hepatobiliary cancer specimens with concordant pathology and radiography. Of the 57 cases, 39 were classified as IHCC, 11 as extrahepatic cholangiocarcinoma (EHCC) and 7 as hepatocellular carcinoma (HCC). We collected clinical data for each case. Formalin fixed paraffin embedded

(FFPE) biopsies and resections were evaluated by a pathologist prior to microdissection. DNA was extracted using Qiagen QIAamp FFPE Tissue kits. IDH1/IDH2 mutation analysis was done by Sequenom mass spectrometry (MS) using lab developed PCR and extension primers, designed using Sequenom software. MS peaks were manually read from the MassARRAY Analyzer 4 system.

Results: The mean age of patients was 62 years (39–85). Males represented 31 of 57 (54%) of the overall specimens, and 16 of 39 (41%) for IHCC. In IHCC, 3 of 39 cases (7.6%) had an IDH1 394C>T mutation. In EHCC, 0 of 11 cases contained IDH mutations and 0 of 7 HCC cases. All 3 of the IDH mutated cases were female. In the three IDH1 mutated IHCCs, 1 patient had disease progression despite surgical resection and chemotherapy, 1 had disease progression with surgery alone and 1 is currently disease free following surgical resection alone.

Conclusions: In our patient population**,** the frequency of IDH mutations, 7.6% is lower than some of those reported in the literature. The mutation was limited to IDH1 394C>T and all 3 cases were female with different treatments and clinical outcomes. Identification of the IDH mutation may provide an additional therapeutic approach in cholangiocarcinoma patients who harbor this mutation.

1819 Expression of Estrogen Receptor beta Isoforms in Pancreatic Adenocarcinoma

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Background: There have been several limited trials in which tamoxifen efficacy was tested in patients with pancreatic adenocarcinoma (PAC). Most of these studies were small series of patients with unresectable PAC and reported mixed results. In these studies, patients were not stratified by estrogen receptor status because estrogen receptor beta (ER-b) had not yet been identified, and PAC did not express the traditional ER-alpha. Recent studies showed that the effects of estrogens, phytoestrogens and tamoxifen on PAC cell lines depended on ER-b expression. The aim of this study was to investigate ER-b expression in human PAC and whether such expression correlates with any clinicopathologic parameters.

Design: Sections of tissue microarray containing 18 formalin fixed and paraffin embedded human PAC were stained by immunohistochemistry (IHC) using monoclonal antibodies to ER-b isoforms 1, 2, and 5 (ER-b1, ER-b2, and ER-b5, respectively), and for Cyclin A. The levels of ER-b isoform expression in tumor cells and the S-phase fraction (SPF) were determined using a quantitative digital image analysis.

Results: All ER-b isoforms were expressed in PAC, although at different levels. Higher mean ER-b2 levels correlated with male sex (p=0.057), older age (p=0.005), and lower pT stage (p=0.008), but not with grade, pN stage, or SPF. Mean ER-b5 levels correlated negatively with SPF (p=0.021), but not with sex, age, grade, pT or pN. Mean ER-b1 expression did not correlate with any of the above mentioned clinicopathologic factors.

Conclusions: ER-b1, ER-b2, and ER-b5 are expressed in PAC. Higher ER-b2 and ER-b5 levels of expression are significantly correlated with lower tumor pT stage and with lower SPF, respectively, suggesting that they may play a tumor suppressive role in PAC. The association between ER-b2 levels and patient sex and age suggest that it could be influenced by endogenous/exogenous hormonal exposure.

1820 Molecular Analysis of Pancreatic Malignant Serous Cystic Tumors by Targeted Next Generation Sequencing

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Background: Pancreatic serous cystadenocarcinoma (SCAC) is a rare controversial entity that is morphologically indistinguishable from benign serous cystadenoma (SCA). Recent studies suggested that half of SCAs harbor mutations in the VHL gene and contain an average of 10 somatic mutations per tumor compared to 48 somatic mutations in pancreatic adenocarcinoma. However, little is known about the molecular landscape of SCAC and its underlying tumorigenesis.

We aimed to study the genetic alterations in SCAC using targeted next generation sequence (NGS).

Design: We retrieved cases of resected SCACs and control SCA from our records. Macrodissection of formalin-fixed paraffin-embedded (FFPE) tissue corresponding to H&E-stained slides was used to ensure least 20% neoplastic cells. Genomic DNA was extracted (Qiagen AllPrep) and subjected to targeted NGS (Illumina TruSeq Amplicon) , which includes 48 cancer-associated genes.

Results: Three SCACs (2 female, 1 male, mean age 69 years) were identified. Two of them showed local gross invasion, perineural, lymph node invasion, or liver metastasis; and the third one had malignant histomorphology

Two of three SCACs had positive results including APC and ATM mutations as well as mutations in the receptor tyrosine kinase family (ERBB-4, ABL1, KIT, CSF1R), tumor suppressor genes (PTEN), and other signal transduction genes (GNAQ, KDR, HRAS). In contrast, the benign SCA control case only showed APC and VHL mutations.

	age (yr)	sex	size (cm)	inva-sion	mets	followup	mutations
SCA	81	F	3.1	no	no	n/a	APC, VHL
SCAC1	69	F	5.5	yes	node	n/a	APC, ATM, ERBB4, GNAQ, KIT, PTEN, HRAS
SCAC2	79	F	4	no	liver, node	16yr, alive	n/a
SCAC3	60	M	3	no	node	1yr	APC, ATM, ABL1, CSF1R, KDR

Molecular alterations important in pancreatic adenocarcinoma such as Kras, p16, p53, or SMAD4 were absent from SCACs.

Conclusions: SCACs are characterized by shared APC mutations with SCAs, suggesting a possible early role in tumorigenesis. In addition, SCACs lack VHL mutations (commonly seen in SCAs) but share mutations in ATM and other genes involved in signal transduction. Further studies are warranted to confirm the role of these genes in SCACs and identify potential future treatment targets.

Pathobiology (including Pan-genomic/ Pan-proteomic approaches to cancer)

1821 Detection of Somatic Mutations in Histologically Normal Lung Tissue: Sectioning and Genomic Method

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Background: Recent studies have shown the presence of evolving clones carrying cancer-causing mutations in the benign or premalignant lesions and even histologically normal epithelium. Here, we provide proof of principle of a method for the sensitive detection of *TP53* somatic mutations within histologically normal lung tissues.

Design: Our method was as follows:

i) Appropriate sampling to obtain cells of clonal origin

Step 1: Two pieces of fresh frozen normal lung tissues of two patients with lung tumors and different smoking habits (ex-smoker and heavy smoker) were mapped microscopically to obtain fifteen 1-mm punch biopsies from each. All punches proceeded separately for DNA extraction and sequencing (n=30).

Step 2: Four more punches were taken from the ex-smoker patient, each embedded in OCT, cut to 25-30 sections of 40µm and sequenced separately (n=110). One 1mm punch biopsy of each patient's tumor was equally analyzed (n=64).

ii) Sequencing

We applied a sensitive targeted sequencing protocol specially designed for low-input DNA. 10ng DNA from each punch or section was used for targeted enrichment followed by 10,000X sequencing of *TP53* by Ion Torrent™ Proton sequencer. Each sample was sequenced as a duplicate. Only variants found in both libraries were considered for final analysis.

iii) Data analysis

We used needlestack pipeline, an ultra-sensitive caller to reliably identify variants in very low allelic fractions (AF)(https://github.com/IARCbioinfo/needlestack) and we applied a functional filter based on IARC *TP53* database (http://p53.iarc.fr/).

Results: Three different Protein-altering *TP53* mutations were detected in the ex-smoker patient, summarized below. One of them was found in the assay step 2 and in several successive sections (22-30 in punch 3 and 2-25 in punch 4) that supports a clonal expansion event. None of these mutations were detected in tumor samples.

Assay step	Punch No.	Section level	Mutation effec	Mutation type	Exon	Protein descriptiona	%AFb
1	2	N/A	Missense	G:C>A:T	8	p.C227Y	06
1	11	N/A	nonsense	G:C>A:T	6	p.Q192*	0.3
2			Missense	G:C>A:T at CpG	8	p.R273H	1-7
aReference sequence: NM_000546							
bSimilar AF between library duplicates							

Conclusions: Our findings suggest that our sectioning and genomic method is capable to detect somatic *TP53* mutations in histologically normal tissue. However, further analyses are needed with bigger sample size to confirm it.

1822 Biological Aggressiveness of Thyroid Neoplasia Depends on the Strength of the Genetic Mutation and Associated Cellular Interaction

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Background: Neoplasia of the thyroid can harbor a variety of genetic mutations displaying a range of biological aggressiveness across the benign to malignant spectrum. To better understand the mechanistic basis for this differential aggressiveness we analyzed a large database of thyroid nodule aspirates (n=3341) by cytology, mutational and microRNA (miRNA) classifier analysis.

Design: Cytology diagnosis was based on cytology reports submitted using Bethesda Diagnostic Categories (BDC- I to VI). Separate needle passes were used for molecular testing. Mutational analysis encompassed common mutations (*BRAF*, *RAS*, *PIK3CA, PAX8/PPARγ* and *RET/PTC* translocations) on next generation sequencing (NGS) platform (Illumina). Variant sequence content (%) on NGS was quantified for each detectable oncogene point mutation. The miRNA classifier utilized a 10 miRNA panel trained on 257 thyroid reactive, benign, malignant. Diagnostic modalities underwent statistical comparison using ANOVA on Ranks.

Results: miRNA classifier results yielded a quantitative measure across the benign/ malignant continuum assigned to four cancer risk categories: very low (n=830) with 99+% NPV, low (n=1962) with 94% NPV, moderate (n=372) with 74% PPV and high (n=177) with 99+% PPV. NGS mutational analysis, using the miRNA classifier as the gold standard, showed clear differences between strong (*BRAFV600E*), intermediate (i.e. other *BRAF*s) and weak (*RAS*) driver mutations. Differential properties were also demonstrated within the *RAS* gene, with *NRAS* (n=279) and *HRAS* (n=125) statistically