inhibitor and fed with high phosphate diets showed marked increases in BUN levels, which was further augmented following dehydration. Creatinine levels also dramatically increased in ACE-inhibitor treated diabetic animals following dehydration. No significant changes in urine or blood pH values were seen in diabetic or non-diabetic animals with or without ACE-inhibitor or high phosphate diet. No calcium phosphate crystals were seen either on H & E or von Kossa stained kidney tissue sections. However, tissue sections of dehydrated diabetic animals on high phosphate diet treated with ACE-inhibitor showed acute tubular necrosis.

**Conclusions:** In summary our study shows that in diabetic rats the combination of ACE-inhibitor therapy, dehydration and high phosphate diet may predispose to acute tubular necrosis.This observation may have significant public health implications since the number of diabetic patients is increasing world wide and ACE-inhibitors are commonly used in these patients to treat high blood pressure. Furthermore, a large percentage of these patients might consist of elderly diabetics who are known to be prone to dehydration for various reasons. Further studies are indicated to investigate on a cellular level the underlying mechanisms that lead to tubular epithelial cell necrosis in these patients.

### 1233 Kidney Injury Molecule-1 (KIM-1) Is a Specific and Stable Target for Identifying Proximal Tubular Injury in Humans

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**Background:** We have reported that KIM-1 is a specific injury biomarker for proximal tubules in several animal models and a limited number of human kidneys with tubular injury. We have found that the *Kim-1* gene is markedly upregulated together with a group of stress proteins (among 31,100 genes) at an early phase of renal ischemia-reperfusion injury in rats. This study was performed to further evaluate the expression of KIM-1 in several groups of human renal diseases.

**Design:** Groups consisted of normal kidneys examined away from renal tumors in nephrectomy specimens, protocol renal transplant biopsies, renal transplant biopsies with mild or moderate acute tubular necrosis (ATN), acute cellular rejection (ACR), and varying types of glomerulonephritis (GN) (total n = 93). In addition kidney sections from 19 autopsy cases were randomly identified. Paraffin embedded tissue sections were stained with monoclonal antibody against human KIM-1 using a DAKO Autostainer. The intensity score of KIM-1 and MIB-1 positive staining (along the luminal surface of proximal tubules and in nuclei, respectively) was graded from 0 to 3 (0, no staining; +/-, focal granular staining; 1+, weak granular staining; 2+, moderate granular staining and 3+, strong granular staining). Focal staining (+/- category) was scored as a positive case.

**Results:** In protocol biopsies of transplanted patients there was a low rate of positive staining for KIM-1 (see table). Kidneys from patients with a clinical diagnosis of mild ATN were positive 46% of the time, but moderate ATN, confirmed by pathology, was associated with 100% positivity. ACR and GN cases also had a high rate of positive KIM-1 staining. In contrast, these groups all had low positive staining rate of MIB-1 (Ki-67). In addition, cadaver kidneys with ATN showed positive KIM-1 staining in proximal tubules, despite prominent autolysis.

**Conclusions:** Our data indicate that KIM-1 is specific marker which can be used to indicate proximal tubular injury with varying types of renal insults and appears more sensitive than MIB-1 to highlight the injury. Furthermore, KIM-1 is a stable target that is identified even in injured kidneys with prominent autolysis.

KIM-1 and MIB-1 Expression in Human Renal Tissue.

| | Normal Kidneys | Protocol biopsies | Mild ATN | Moderate ATN | ACR | GN |
|---|---|---|---|---|---|---|
| KIM-1 positive | 1/13 | 5/24 | 6/13 | 8/8 | 11/12 | 18/23 |
| MIB-1 positive | 0/13 | 2/24 | 0/13 | 1/8 | 1/12 | 5/23 |

# Liver & Pancreas

### 1234 Von-Meyenburg Complexes Increase with Age and Are Specifically Associated with Alcoholic Cirrhosis and End-Stage Hepatitis B Infection

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**Background:** Biliary hamartomas or Von-Meyenburg complexes (VMCs) form part of a spectrum of ductal plate abnormalities that includes polycystic liver disease, congenital hepatic fibrosis, and Caroli's disease. VMCs are usually regarded as common and innocuous lesions, but cholangiocarcinoma (CCA) is known to be increased in patients with ductal plate abnormalities. Additionally, there are now a small number of case reports and series that link CCA to a background of multiple VMCs. Because cirrhosis, alcohol, and viral hepatitis have been epidemiologically linked to intrahepatic CCA, we evaluated the prevalence and associations of VMCs in a large series of liver explants.

**Design:** We studied 567 liver explants performed for cirrhosis due to Hepatitis C (HCV) (n=154), alcohol (n=112), HCV/alcohol (n=85), Hepatitis B (HBV) (n=67), and other (excluding chronic biliary tract disease) (n=149). Controls included 134 explants in non-cirrhotic conditions (e.g., metabolic abnormalities, acute liver failure). None had polycystic liver/kidney disease or congenital hepatic fibrosis. For each case, we recorded age, gender, number of VMCs, and number of histologic sections available. Statistical analysis included chi-square test for categorical data, t-test for non-categorical data, and logistic regression for multivariate analysis.

**Results:** VMCs were identified in 126 of 701 (18%) livers. Number of slides reviewed did not differ significantly between cases with and without VMCs (5.5 vs 5.0, p=0.07). In univariate analysis, older age (p<0.0001), male gender (p<0.0001), and cirrhosis of any type (p=0.012 after correcting for age and gender) were all correlated with prevalence of VMCs. In multivariate analysis, only age (p<0.0001) and cirrhosis from alcohol (p=0.001), HBV (p=0.001), and HCV/alcohol (p=0.013) were significant. VMCs were seen in only 1 of 171 (0.6%) patients ≤35 years of age. Among patients with alcoholic cirrhosis, VMCs were present in 15% overall and 5 (4.5%) had numerous (>10) lesions (range 11-98). One of these 5 also had multifocal intrahepatic papillary bile duct dysplasia.

**Conclusions:** Despite their apparent hamartomatous appearance, VMCs outside the setting of congenital hepatic fibrosis or polycystic liver disease are acquired lesions that increase with age and are associated specifically with cirrhotic-stage liver disease due to alcohol, HBV, and HCV/alcohol. A minority of patients with alcoholic cirrhosis have numerous VMCs resembling a "forme fruste" of congenital ductal plate abnormality.

### 1235 Mucinous Nonneoplastic Cysts of the Pancreas: Clinical and Immunophenotypic Analysis

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**Background:** Mucinous nonneoplastic cyst (MNC) is a recently described cystic lesion of the pancreas characterized by mucinous differentiation of the lining epithelium, a thin rim of almost acellular supporting stroma, and the absence of communication with the pancreatic ducts. MNC is difficult to distinguish from mucinous cystic neoplasms on clinical and radiographic grounds and as a result is often surgically resected. In addition, it is unclear whether MNC represents a precursor lesion to pancreatic carcinoma or truely a nonneoplastic cystic change. In an attempt to better understand the nature of MNC, we examined clinical and morphologic data and MUC1, MUC5AC, p16 and DPC4 expression in 12 patients with MNC.

**Design:** Review of archival material from the surgical pathology files revealed 12 cases of MNC that met previously established histologic criteria. All available clinical data was reviewed including aspirated fluid CEA level if available. Immunohistochemistry for MUC1, MUC5AC, p16, and DPC4 was performed on paraffin-embedded, formalin-fixed tissue using monoclonal antibodies and standard avidin-biotin technique. The percentage of positive cells was estimated for all stains.

**Results:** The patients included 8 females and 4 males ranging from 20 to 80 years of age. Six lesions were grossly unilocular, one was bilocular, and 5 were multilocular. Size ranged from 0.6 cm to 9.0 cm. Three cysts were localized to the head, two to the body, and seven to the pancreatic tail. In all ten patients where cystic fluid was analyzed for CEA, the levels of this marker were high and ranged from 160 to 11321 ng/ml (mean 2731 ng/ml). H&E stained sections showed that the cysts were lined by a single layer of mucinous epithelium without any cytological atypia or mitosis. Nuclear expression of p16 and DPC4 was observed in all the cysts. MUC5AC and MUC1 expression was observed in 10 (83%) and 4 (33%) of cysts, respectively.

**Conclusions:** Morphologic and molecular studies support the nonneoplastic nature of these mucinous cystic lesions of the pancreas. Proper classification and identification of these cysts is needed to obviate unnecessary surgery. CEA levels of the cystic fluid are high in MNC and should not be used to differentiate these cysts from other mucinous cystic neoplasms.

### 1236 Adenocarcinoma of the Minor Duodenal Papilla and Its Precursor Lesions: A Clinical and Pathological Study

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**Background:** The minor duodenal papilla, which drains the accessory pancreatic duct (of Santorini), lies proximal to the ampulla of Vater. Unlike in the ampulla and elsewhere in the duodenum, adenocarcinoma and its precursor lesions arising in the minor papilla are rare and consequently poorly defined.

**Design:** Tumors occurring in the duodenum proximal to the major papilla that were treated at our institution and had sufficient material were reviewed. Those fulfilling the following criteria were selected and regarded as tumors of the minor papilla: location at 1.5 cm – 2.5 cm proximal to the major papilla; presence of associated submucosal pancreatic ducts with peri-ductal glands and/or acinar tissue; a predominant submucosal location of the tumor; and lack of adenoma in the adjacent duodenal mucosa. Tumors thus identified were studied morphologically, immunohistochemically and clinically.

**Results:** Five cases of adenocarcinoma arising in the minor papilla were identified among 17 supra-ampullary carcinomas (29%). The results are summarized in the table.

| Case no. | Age/Sex | Size | Precursor (type) | Invasive Carcinoma (type) | Pathologic Stage | Follow up (months) | Survival Status |
|---|---|---|---|---|---|---|---|
| 1 | 75 / M | 3.0 cm | IPMN* | Colloid | pT4N0M0 | 75 | DOD |
| 2 | 77 / F | 1.8 cm | IPMN | Colloid | pT2N0M0 | 13 | NED |
| 3 | 77 / M | 1.2 cm | IPMN | Colloid | pT4N0M1 | 45 | AWD |
| 4 | 71 / F | 4.0 cm | Absent | Intestinal non-mucinous | pT4N0M0 | 85 | DOD |
| 5 | 50 / F | 3.0 cm | Absent | Pancreato-biliary | NA | NA | NA |

*intraductal papillary-mucinous neoplasm, in all cases with high grade dysplasia

Immunohistochemically, the intestinal and mucinous type tumors were positive for CK20, CDX2, DPC4, MUC2, B72.3, MLH1 and MSH2 and were negative for CK7, MUC1, p53, DUPAN-1 and CA125. Stains could not be performed for case 5.

**Conclusions:** Adenocarcinomas of the minor papilla are rare tumors occurring predominantly in the 6-7$^{th}$ decade. They may arise from IPMN precursors in the residual submucosal pancreatic tissue. Morphologically, immunohistochemically, and clinically they are similar to ampullary or IPMN-associated carcinomas and can show either intestinal-type or pancreatobiliary-type features. The proximal location with respect to the major papilla, predominantly submucosal location and presence of underlying pancreatic tissue are clues to the diagnosis of these tumors.

### 1237 Giant Cell Tumor of the Extrahepatic Biliary Tree. A Clinicopathologic Study of Four Cases and Comparison with Anaplastic Spindle and Giant Cell Carcinomas with Osteoclast-Like Giant Cells

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**Background:** Primary extraskeletal giant cell tumors have been described in a variety of sites including soft tissues of the extremities, mediastinum, larynx and skin, but to our knowledge, not in the extrahepatic biliary tree (EHBT).

**Design:** We reviewed the clinicopathologic features of four primary giant cell tumors of the EHBT, two from the common bile duct, one from the cystic duct and one from the gallbladder. Their morphologic and immunohistochemical features were compared to those of ten anaplastic spindle and giant cell carcinomas with osteoclast-like giant cells of the gallbladder. The morphologic criteria used for the identification of giant cell tumors of the EHBT were similar to those of giant cell tumors of bone. Hematoxylin and eosin stains were available for review in all cases. The following immunostains were evaluated: CD163, CD68, HAM56, AEI/AE3, CAM5.2, and epithelial membrane antigen (EMA).

**Results:** Three patients with giant cell tumors of the EHBT were men and one was a woman. None of these tumors recurred or metastasized. They consisted of a mixture of mononuclear and multinucleated osteoclast-like giant cells. The nuclei of the mononuclear cells were similar to those of the multinucleated giant cells. CD163 immunoreactivity was restricted to the mononuclear cells, whereas CD68 and HAM56 labeled only the multinucleated osteoclast-like giant cells. The mononuclear cells were EMA-positive but did not express cytokeratins. The anaplastic spindle and giant cell carcinomas contained fewer osteoclast-like giant cells, and their mononuclear cells showed marked cytologic atypia and numerous mitotic figures. The mononuclear cells were focally positive for Cytokeratins, (AE1/AE3 and CAM5.2) and did not label with CD163, CD68 and HAM56. The benign osteoclast-like giant cells showed immunoreactivity for CD68 and HAM56, but were negative for CD163 and Cytokeratins.

**Conclusions:** Our results indicate that primary giant cell tumors of the EHBT are benign neoplasms with a histiocytic phenotype. They should be distinguished from the highly lethal anaplastic spindle and giant cell carcinomas with osteoclast-like giant cells by detailed cytologic analysis and immunohistochemical stains for CD163, CD68, HAM56 and Cytokeratins.

### 1238 High Level Expression of Pak1 Is a Good Prognostic Marker in Pancreatic Ductal Adenocarcinoma

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**Background:** The p21-activated kinase-1 (Pak1) promotes cell motility and invasiveness. Pak1 is activated by the Rac, Rho, and Cdc42 small GTPases in response to a variety of stimuli including Ras and phosphatidylinositol 3 -kinase/AKT pathway activation. Recently, the neurofibromatosis type 2 associated peptide, merlin was shown to be an inhibitor of Pak1. This demonstrates the potential role of Pak1 in Ras associated carcinogenesis. Because Pak1 plays a central role in regulating cell motility and invasiveness, we sought to determine whether Pak1 may be involved in the biology of pancreatic ductal carcinomas.

**Design:** The expression level of PAK1 was tested by immunohistochemistry in 98 cases of ductal adenocarcinoma of the pancreas. Based on the degree of expression level (calculated by an established scoring system incorporating the percentage of + labeled carcinoma cells and the intensity of labeling), each case was assigned to one of 4 categories: 0-none, 1-minimal, 2-moderate, and 3-significant. Expression levels were correlated with the archival data available on some patients on DPC4, kras, p53, p21, p27 expression as well as clinical data including patient survival.

**Results:** PAK1 had a weak expression in ducts and acini while the islets were not stained. In the ductal adenocarcinomas, 21, 42, and 35 cases had weak, moderate and strong staining respectively. On statistical analysis, strong expressors had a significantly better overall survival compared to weak and moderate expressors (p=0.01). PAK1 expression had a strong inverse association with tumor grade (rho=-0.201, p=0.04). In addition, PAK1 expression had a strong correlation with DPC4 expression (p=0.03), while no significant association with p53, p27, Kras, Her2 or lymph node involvement could be demonstrated.

**Conclusions:** PAK1 expression is a good prognostic factor in pancreatic ductal carcinoma. This is in contrast to breast and colorectal cancers. Correlation of PAK1 expression with DPC4 expression suggests that PAK1 expression may be associated with TGF-beta signaling in pancreas and has a different biological role in pancreatic cancer compared to breast cancers. Further studies are needed to explore the role of Rac1/PAK1 signaling in pancreatic cancer and its relation to TGF-beta signaling.

### 1239 Late-Onset Centrilobular Necrosis in Liver Allografts: Does Humorally Mediated Rejection Play a Role?

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**Background:** Centrilobular necrosis (CLN) is a common histologic finding during the first few months following orthotopic liver transplantation (OLT), usually attributed to perioperative ischemic injury. The etiopathogenesis of late-onset CLN (occurring after 2 months without evidence of perioperative complications) is poorly understood but appears to be multifactorial. Recently, immunodetection of C4d, an cleavage product of the complement component, has been used as a marker for humorally mediated rejection, but its role in late-onset CLN in liver allografts has not been investigated.

**Design:** A total of 22 liver biopsies exhibiting late-onset CLN were obtained from 11 post-OLT patients. The laboratory data and treatment regimens were analyzed and correlated with histologic findings. Formalin-fixed tissue sections were immunohistochemically stained using a monoclonal antibody specific for C4d. The staining was considered positive if a strong (either diffuse or focal) immunoreactivity was detected in endothelial cells of the hepatic vasculature.

**Results:** The ages of the patients ranged from 2 to 67 years (median: 38), with a male-to-female ratio of 1:1.8. The original diseases included PBC (2), alcoholic cirrhosis (2), autoimmune hepatitis (1), PSC (1), hepatitis C (1), hepatitis B (1), AVM (1), cystic fibrosis (1) and biliary atresia (1). The mean time of the first post-OLT biopsy showing CLN was 19.6 months (range: 2-79 months) and all the patients were under immunosuppressive therapy. Three of the initial 11 biopsies also exhibited classic portal-based acute cellular rejection. Eight patients had adjusted their immunosuppressive therapy upon the diagnosis of CLN and the other 3 remained watchful waiting, with improvement evidenced by follow-up biopsies and/or laboratory results in 4 patients in the former and in 2 in the latter. Positive C4d immunostaining was observed in 1 case. This patient had worsening liver function despite therapy adjustment. Focal C4d deposits were first noted in hepatic arteries in the second biopsy, and diffuse positivity was detected in sinusoidal endothelium, hepatic veins and portal vessels in the third biopsy one week later. These findings were accompanied by worsening CLN despite the remission of portal-based acute rejection.

**Conclusions:** Humorally mediated mechanism may serve a role in a subset of liver allografts with worsening late-onset CLN despite adequate therapy for acute cellular rejection. C4d immunostaining should be included in the work-up list when evaluating liver biopsies to facilitate an early and accurate diagnosis.

### 1240 Clinicopathologic Correlates of C4d Staining in Pancreas Allografts

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**Background:** C4d is a well-established marker for humoral rejection in renal and cardiac allografts but has been less well characterized in pancreas allografts. The purpose of this study is to characterize and clinicopathologically correlate C4d staining in the pancreas allograft.

**Design:** All pancreatic allograft specimens were reviewed. The specimens were diagnosed for acute cellular rejection or other pathologic processes (ischemia, infection, etc.). Immunohistochemical staining with antibodies towards C4d (ARPTM Belmont MA, rabbit polyclonal antibody) was performed on a Ventana automated immunostainer. The strength of staining was graded from 0 to 3+.

**Results:** Sixteen patients (10 men; 6 women; age range 32-57 years) had material available for review from either pancreas transplant alone (5 pts), simultaneous pancreas kidney transplant (5 pts) or pancreas after kidney transplant (6 pts). The studied tissue represented 10 indication (non-protocol) biopsies (two pts with multiple biopsies), and 9 explants. Only three patients (one with multiple biopsies) demonstrated any degree of C4d staining; one pt with 3+/3+ staining had moderate acute rejection progressing rapidly to chronic rejection within 6 weeks over multiple biopsies and was demonstrated to have positive donor specific antibodies. The other two patients represented single biopsies with moderate to severe chronic rejection; donor specific antibody status currently unknown. When positive, the pattern of C4d staining was along the microvasculature of acini, islets and septae and occasionally involved the arterioles.

**Conclusions:** This survey of C4d staining in our population of pancreas transplant recipients demonstrates that it is not frequent; it is not seen in cases of acute ischemia from vascular thrombosis; it appears to be seen in chronically rejected allografts but was not seen in all cases of acute rejection. When seen in acute rejection, it was associated with vasculitis, a rapid progression to chronic rejection and donor specific antibodies. This study supports the clinical utility of C4d staining in pancreas allografts. More positive cases will need to be evaluated in order to correlate specific histologic features in allografts that predict positive C4d staining. The finding of C4d staining in our cases of chronic rejection suggests that humoral factors may play a significant role in its development, similar to that documented in renal allografts.

### 1241 Correlation of Histological Changes Is Hepatitis C with Preneoplasia and Hepatocellular Carcinoma — A Study of Liver Explants

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**Background:** We analysed explanted livers due to hepatitis C cirrhosis for incidental hepatocellular carcinoma (HCC); as well preneoplastic changes including macroregenerative nodules (MRN), small cell change (SCC) and large cell change (LgCC). Our study aimed at analyzing the relationship of microscopic changes in Hepatitis C (including features like broad scarring, inflammatory activity, fat content, bile duct proliferation, cholestasis, iron staining and apoptosis) with the presence of preneoplasia and HCC.

**Design:** A retrospective examination of all liver explants (195 cases) performed at Westchester Medical Center (WMC) from January 1996 to April 2004 was performed of which there were 50 cases of Hepatitis C end stage liver disease. ~ 20 glass slides per case stained with Hematoxylin and Eosin, Trichrome, Reticulin and Iron stains were examined. The cases were categorized as either having HCC, preneoplastic changes or neither. Each case was studied for features like broad scarring (5mm), inflammatory activity, fat content, bile duct proliferation, cholestasis, iron staining and apoptosis.

**Results:**

**CORRELATION OF HISTOLOGIOCALCHANGES OF HEPATITIS C CIRRHOSIS WITH HCC AND PRENEOPLASTIC CHANGES**

| | HCC | PRENEOPLASIC CHANGES | NO HCC OR PRENEOPLASIA |
|---|---|---|---|
| **BROAD SCARS** | 7/12 (58%) | 11/31 (35%) | 3/7 (43%) |
| **INFLAMMATION** MILD | 5/12 (42%) | 4/31 (13%) | 2/7 (29%) |
| MODERATE -SEVERE | 7/12 (58%) | 27/31 (87%) | 5/7 (71%) |
| **BILE DUCT PROLIFERATION** MILD | 7/12 (58%) | 10/31 (32%) | 3/7 (43%) |
| MODERATE-SEVERE | 5/12 (42%) | 21/31 (68%) | 4/7 (57%) |
| **FATTY CHANGE** 0-30% | 10/12 (83%) | 30/31 (97%) | 7/7 (100%) |
| >30% | 2/12 (17%) | 1/31 (3%) | |
| **IRON STAINING** | 2/12 (17%) | 18/31 (58%) | 5/7 (71%) |
| **CHOLESTASIS** | 5/12 (42%) | 19/31 (61%) | 2/7 (29%) |
| **APOPTOSIS** | 0 | 12/31 (39%) | 0 |

**Conclusions:** ♦Cases of Hepatitis C cirrhosis with HCC an increased frequency of broad scarring (58%), mild bile duct proliferation (58%), mild degree of inflammation (42%) and a lower frequency for iron staining (17%)

♦Cases of Hepatitis C cirrhosis with preneoplastic changes (SCC,LgCC, MRNs) more frequently had moderate to severe inflammation(87%) and bile duct proliferation (68%), presence of cholestasis(61%) and were positive for stainable iron (58%).

♦Apoptosis was seen exclusively in cases of hepatitis C cirrhosis with associated preneoplastic change(39%)

♦Fatty change was predominantly mild in most cases of Hepatitic C cirrhosis ,but was most frequently (100%) seen in cases without HCC or preneoplasia

### 1242 Intraductal Papillary Neoplasms of the Pancreas with Intraluminal Nodular Growth Showing Arborizing Papillae: A Subset of Neoplasms Combining Intraductal Oncocytic Papillary Neoplasms (IOPNs) and Intraductal Papillary Mucinous Neoplasms (IPMNs) of the Gastric Type

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**Background:** Intraductal oncocytic papillary neoplasms (IOPNs) of the pancreas have been proposed (Adsay, Am J Surg Pathol 1996) as an entity distinct from intraductal papillary mucinous neoplasms (IPMNs). However, the relationship between both lesions has not been fully clarified until now. We have experienced several cases that seemed to combine IOPN and gastric type IPMN.

**Design:** Five cases of intraductal neoplasms of the pancreas, which were surgically resected at three institutions, were selected and subjected to histopathological examination. Histological assessment with haematoxylin-eosin and PAS-alcian blue (pH2.5) stains, and immunohistochemistry for mucins (MUC1, MUC2, MUC5AC, and MUC6) and mitochondria were performed for each lesion.

**Results:** All neoplasmas showed intraductal nodular growth with an arborizing papillary pattern, at least partially. These argorizing papillary growths were comprised of cells with granular eosinophilic cytoplasm and round to oval nuclei with nucleoli. So-called intraepithelial lumina, and goblet cells were observed. All lesions fell into a borderline to malignant category with regard to their histologic grade. Non-nodular intraductal lesions composed of epithelia with low-grade atypia similar to gastric foveolar epithelium were present adjacent to the intraductal nodular growth lesions. The immunostaining of mitochondria revealed that oncocytic areas were more intensely immunoreactive than gastric foveolar-like areas. The former was almost diffusely positive for both MUC5AC and MUC6 except for one case, while the latter was positive for only MUC5AC or both MUC5AC and MUC6. Immunoreactivity of MUC2 was only focal if present. MUC1 was also focally positive in 3 cases.

**Conclusions:** These intraductal neoplasms of the pancreas seem to have some of the features of IOPNs as well as frequent co-existence of low-grade epithelium with a gastric foveolar feature. They showed a strong tendency toward a gastric mucin phenotype. These observations may indicate that IOPNs are closely associated with or originate from gastric type IPMNs, and may be a form of their high-grade transformation.

### 1243 Isolated Solitary Ductal Units in Adipose Tissue Is a Specific Finding of Pancreatic Adenocarcinoma and May Aid in Determining Extrapancreatic Adipose Tissue Extension

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**Background:** The distinction of ductal adenocarcinoma (DA) from chronic pancreatitis (CP) remains one of the more difficult challenges in surgical pathology. The glandular units of invasive carcinoma are often well formed with well-polarized cells, appearing deceptively benign. Conversely, the ducts of chronic pancreatitis may be atypical and pseudo-infiltrative as a result of acinar atrophy and fibrosis. We recently noted isolated ductal units in adipose tissue (ISD) to be a reliable indicator of adenocarcinoma.

**Design:** In this study, the frequency of ISDs was investigated in 105 pancreatic resections with DA and 32 with CP only. ISD was defined as a solitary gland lying individually in adipose tissue, either directly abutting adipocytes or separated from them by only a thin rim of fibromuscular tissue.

**Results:** ISD was detected in 50/105 (47 %) of pancreatic resections for DA, but not in any resections with CP only (specificity 100%; sensitivity 47%). Most of the ISDs were very well-differentiated and cytologically bland. A small subset of these units represented vascular invasion, in which the carcinoma cells epithelialized the vessel lining. Such units were almost indistinguishable from normal ducts, however, the vascular structures were confirmed by histochemical and immunohistochemical stains. ISDs were often located in histologic sections taken for the evaluation of the retroperitoneal margin and free surfaces where adipose tissue is more abundant.

**Conclusions:** Isolated solitary ductal units lying in adipose tissue unaccompanied by other elements, present in 47% of pancreatic resections in this series, is a very specific finding for carcinoma that may be instrumental in the diagnosis and staging of carcinoma as well as margin evaluation.

### 1244 Comparison and Significance of Biopsies Obtained by Percutaneous and Laparoscopic Methods in Hepatitis C Patients

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**Background:** Liver biopsy is an accurate way to assess the damage in most liver diseases. Core liver biopsy tissues can be obtained by percutaneous (PER), transjugular, laparoscopy (LAP), and fine needle aspiration methods. One of the areas subject to discussion among hepatologists and pathologists, regardless of the method used, is determining the significance of the size and number of portal tracts (PTs). We compared needle biopsy tissues obtained by LAP and PER methods.

**Design:** We studied 135 hepatitis C patients. Of these, 106 underwent LAP where a core needle biopsy of the left and right hepatic lobes was obtained. An additional 29 patients had a PER core biopsy. All cores were measured and fixed in formalin. H&E and trichrome stained slides were reviewed, and the number of PTs counted. Grading and staging of the hepatitis was obtained using the Batts and Ludwig system.

**Results:** In the LAP group, the mean number of PTs from the left lobe was 14.4 ± 4.3 while the right lobe demonstrated 13.1 ± 5.0. The size was 1.5 ± 0.2 cm and 1.4 ± 0.3 cm, respectively. For the PER group, the mean number of PTs was 16.3 ± 5.4 and the size was 1.4 ± 0.3 cm. The grade and stage for the LAP and PER groups were 2.4 and 2.3, and 2.2 and 2.2 respectively. Although, larger biopsies showed higher PTs numbers, there was no linear correlation between number of PTs and size of the tissues in either the LAP or PER group. Also, there was no difference in size, grade or stage between the LAP and the PER group. However, the number of PTs was significantly higher in PER biopsies. Comparing LAP biopsies from the left and right lobes on individual patients, grade was concordant only in 60% of the cases and the stage in 50%. However, differences of more that one grade did not occur in any of the patients and a difference of more than one stage occurred in only 3 patients.

**Conclusions:** The number of PTs in liver biopsies obtained by PER is greater than LAP biopsies despite similar biopsy size. Nonetheless, there are no significant differences in grade and stage in biopsies obtained by PER or LAP methods. The grade and stage of LAP biopsies obtained from the left and right lobes differed in a large number of patients despite the fact that the size and numbers of PTs were similar. However, differences of more than one grade or stage were infrequent. These data indicate that hepatitis C liver injury is a patchy and heterogeneous process. A single core biopsy may not accurately reflect the injury to the liver due to this viral disease.

### 1245 Autoimmune Pancreatitis Produces Monoclonal or Highly Selected Oligoclonal T-Cell Peaks in T-Cell Gamma Receptor Gene Rearrangement Studies: A Useful Adjunctive Diagnostic Assay

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**Background:** T-cell immune responses in many autoimmune diseases are antigen specific and highly selective, often resulting in monoclonal-like peaks in PCR-based T-cell receptor gene rearrangement (TCR) studies. Autoimmune pancreatitis (AIP) is often diagnostically challenging. We evaluated the diagnostic utility of TCR gene rearrangement studies in AIP and other diseases.

**Design:** Pancreas blocks from 9 AIP patients (6 pancreatectomy, 3 needle cores from steroid responsive pts), 10 chronic obstructive non-AIP pancreatitis (Chron-Panc), 4 chronic pseudocysts (P-Cyst), 5 IMPNs, and 6 invasive ductal adenocarcinomas (AdenoCA) were obtained from department files. Peripancreatic lymph nodes from the 6 AIP pancreatectomy cases were also analyzed. DNA was extracted. Two multiplex PCR reactions spanning the entire T-gamma receptor were performed. T-cell populations were assessed using fluorescence fragment analysis with estimation of clonal fragment lengths and relative peak heights on a ABI 310 instrument. T-cell monoclonality was defined as one or two isolated tall thin peaks or a tall thin peak >3x the tallest background polyclonal signal peak.

**Results:** Single tall thin monoclonal-like peaks were present in one or more markers in 8 of 9 (88.9%) AIP cases. One case had no significant T-cell population. Four cases had single peaks in 3 markers, three cases had single peaks in two markers and two or three distinct, non-overlapping oligoclonal peaks in one marker, and one case had a single tall peak in one marker and oligoclonal peaks in two markers. None of the other 25 non-AIP cases had a similar-appearing monoclonal or highly selected-oligoclonal TCR peak pattern. No significant T-cell or low-level polyclonal T-cell populations were present in 7/10 Chron-Panc, 3/4 P-Cyst, 2/5 IMPN, and 5/6 AdenoCA cases. One-primer high-level oligoclonal T-cell populations were present in 3/10 non-AIP cases, 1/4 P-Cyst cases, 3/5 IMPN cases, and 1/6 AdenoCA cases.

**Conclusions:** The T-cell population in AIP appears to be composed of a small number of highly-specific antigen responses, producing a monoclonal T-cell or oligoclonal peak pattern in TCR gene rearrangement studies. Other benign and neoplastic pancreatic processes do not produce this TCR gene rearrangement peak pattern. TCR gene rearrangement studies appear to be a useful adjunctive assay for AIP that can be used in needle core biopsies.

### 1246 Osteonectin and MUC1 Staining Are Increased in Pancreatic Cancer While Osteopontin Staining Correlates with Improved Survival

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**Background:** Pancreatic cancer is almost uniformly lethal with median survival of less than 12 months. MUC1 expression is common in pancreatic cancer and may portend a poor prognosis while MUC2 is less common in these aggressive cancers. Less is known about the expression of osteopontin (OPN) and osteonectin (OSN) in pancreatic cancer

and their role in overall survival. We compared the expression of MUC1, MUC2, OPN, and OSN in ductal adenocarcinomas of the pancreas to chronic pancreatitis and normal pancreas to determine their relative expression and association with survival.

**Design:** Cases of pancreatic cancer (N = 49) and chronic pancreatitis (N = 12) were retrieved from the archival files and tumor grade and stage were noted. Tissue microarrays of pancreatic cancer, chronic pancreatitis and normal pancreas (N = 19) were created from formalin-fixed, paraffin-embedded blocks (2 cores per block, 2 mm each). Sections were stained using antibodies against MUC1, MUC2, OPN, and OSN and evaluated. Staining for MUC1 and MUC2 was considered positive if >5% of cells stained, and OPN and OSN were scored as absent, weak or strong (0-2). Results were compared using Fisher's exact test. Survival curves were constructed using the Kaplan-Meier method and compared by Log-rank analysis.

**Results:** OSN was only expressed in stroma. MUC1 and OSN expression were significantly increased in pancreatic cancer relative to chronic pancreatitis and normal pancreas, MUC2 was rarely expressed, and OPN did not significantly differ between groups (Table). Of all pathologic (tumor grade, T stage, nodal status) and immunohistochemical factors, only strong OPN expression was associated with improved survival (median 31.0 months vs. 13.9, p = 0.008). OPN expression was independent of tumor grade, stage, or nodal status; and expression of MUC1, MUC2, or OSN.

**Conclusions:** MUC1 and OSN are significantly overexpressed in pancreatic cancer, but they are not associated with survival. OPN expression, however, imparts a survival advantage not before reported in pancreatic cancer. Its functional role in the pathophysiology of pancreatic cancer warrants further investigation.

| | Pancreatic cancer (%) | Chronic pancreatitis (%) | Normal pancreas (%) |
|---|---|---|---|
| MUC1 | 23/39 (59%)* | 0/6 (0%) | 0/18 (0%) |
| MUC2 | 2/48 (4%) | 0/6 (0%) | 0/18 (0%) |
| OPN | 29/48 (60%) | 9/12 (75%) | 14/17 (82%) |
| OSN | 44/49 (98%)* | 8/12 (62%) | 0/19 (0%) |

*p<0.01 vs. chronic pancreatitis and normal pancreas

### 1247 Osteonectin Expression but Not Osteopontin, MUC1, or MUC2 Correlates with Survival in Patients with Carcinoma of the Ampulla of Vater

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**Background:** Osteopontin (OPN) and osteonectin (OSN) are newly identified markers present in pancreatic carcinoma, but they have not been well evaluated in carcinoma of the Ampulla of Vater. We compared the expression of MUC1, MUC2, OPN, and OSN in carcinomas of the ampulla of Vater to chronic pancreatitis (CP) and normal pancreas (NP) to determine their relative expression and association with survival.

**Design:** Resection specimens for cancer of the ampulla of Vater (N=49) and CP (N=13) were retrieved from the archival files and tumor grade and stage were noted. Tissue microarrays of cancer, CP, and NP (N = 19) were created from formalin-fixed, paraffin-embedded blocks. Sections were stained using antibodies against MUC1, MUC2, OPN, and OSN and evaluated. Staining in >5% of cells was considered positive for MUC1 and MUC2; and OPN and OSN were scored as absent, weak or strong (0-2). Results were compared by Fisher's exact test. Kaplan-Meier curves were constructed and compared by Log-rank analysis.

**Results:** OSN expression was limited to the stroma, while OPN staining was seen in the epithelium of many cases. OSN was significantly increased in the stroma of ampullary cancers compared to stroma in CP and NP (Table). OSN expression was associated with a significantly decreased overall survival. When OSN was not expressed, no deaths occurred during follow-up (median 71 months) compared to 2- and 5-year survival of 67% and 32%, respectively, in tumors expressing OSN (p = 0.03). OSN correlated with MUC1 and tumor grade, but neither MUC1 nor grade were associated with survival. Nodal metastases were more common in tumors expressing OSN (48% vs. 0%, p = 0.06) and less common in those expressing MUC2 (0% vs. 48%, p = 0.03).

**Conclusions:** MUC1, MUC2, and OPN are not significantly associated with survival in ampullary cancer although MUC2 expression correlates inversely with nodal metastasis. Stromal OSN expression is associated with decreased survival, perhaps by its influence on nodal metastasis. This association demonstrates the importance of the interaction between tumor and stroma and raises the possibility that OSN may be an appropriate future target for therapy.

| | Ampullary cancer (%) | CP (%) | NP (%) |
|---|---|---|---|
| MUC1 | 9/48 (19%) | 0/6 (0%) | 0/18 (0%) |
| MUC2 | 6/48 (13%) | 0/6 (0%) | 0/18 (0%) |
| OPN | 26/49 (53%) | 9/12 (75%) | 14/17 (82%) |
| OSN | 44/49 (90%)* | 8/13 (62%) | 0/19 (0%) |

* p < 0.05 vs. chronic pancreatitis and normal pancreas

### 1248 p70s6k Expression in Pancreatic Cancer Is Associated with Survival Independent of the Akt Pathway

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**Background:** The ribosomal protein kinase, p70s6k, has been shown *in vitro* to be an important downstream effector in the mitogen-induced phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction cascade in a variety of tumors including pancreatic cancer. Activation of p70s6k is accomplished by phosphorylation by the phosphatidyl kinase-related kinase, FRAP, which can be inhibited by Rapamycin. Tumors which depend upon PI3K/Akt activation, such as those with defective PTEN (the natural lipid phosphatase antagonist of PI3K and Akt) are more susceptible to FRAP inhibition. We evaluated immunohistochemical stains for p70s6k, activated Akt (p-Akt), and PTEN expression in pancreatic cancer to determine the relationship of p70s6k expression with survival and help delineate its activation pathway.

**Design:** Pancreatic cancer resection specimens (N=48) were retrieved from the archival files and tumor grade and stage were noted. Tissue microarrays of pancreatic cancer and normal pancreas (N=18) were created from formalin-fixed, paraffin-embedded blocks (2 cores per block, 2 mm each). Sections were stained using antibodies against p-Akt, p70s6k, and PTEN and evaluated. Staining for p-Akt and PTEN were considered positive if >5% of cells stained and p70s6k was scored as absent (<5% nuclear staining), weak or strong (0-2). Results were compared using Fisher's exact test. Survival curves were constructed using the Kaplan-Meier method and compared by Log-rank analysis.

**Results:** The expression of p70s6k, p-Akt, and PTEN did not significantly differ between normal and malignant pancreatic ducts (Table). The strength of p70s6k expression was associated with improved outcome with median survivals of 7.6, 8.3, and 19.8 months seen in tumors with no, weak, or strong expression, respectively (p=0.01). No correlation was seen between p70s6k, p-Akt, or PTEN expression, and none of the stains correlated with tumor grade or stage.

**Conclusions:** Expression of p70s6k is common in pancreatic cancer, but it does not seem to be associated with p-Akt or PTEN, implying activation via an alternate pathway. The improved survival seen with increased p70s6k expression suggests a role in pancreatic cancer, and further investigation into possible therapeutic implications is warranted.

| | Pancreatic Cancer (%) | Normal Pancreatic Ducts (%) |
|---|---|---|
| p70s6k | 45/48 (94%) | 18/18 (100%) |
| p-Akt | 3/34 (9%) | 0/17 (0%) |
| PTEN | 2/18 (11%) | 0/17 (0%) |

### 1249 Multifocal Neoplastic Precursor Lesions Associated with Lobular Atrophy of the Pancreas in Patients Having a Strong Family History of Pancreatic Cancer

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**Background:** We screened 109 asymptomatic patients with a strong family history of pancreatic cancer using endoscopic ultrasound. Ten of these patients underwent surgical resection at our institution for early pancreatic neoplasia, providing a unique opportunity to define the morphology of pancreatic precursor lesions in patients with a strong family history of pancreatic cancer.

**Design:** Eight of the ten pancreata were appropriate for inclusion in this study and entirely submitted for histological examination. The number of pancreatic intraepithelial neoplasia (PanIN) lesions and intraductal papillary mucinous neoplasms (IPMNs) were determined as a function of the total number of pancreatic ducts reviewed. The parenchymal changes adjacent to these precursor lesions were defined. Immunohistochemical labeling for MUC1 and MUC2 was performed. Selected precursor neoplasms from six pancreata were microdissected and analyzed for *KRAS* gene mutations.

**Results:** PanINs were identified in all eight of the pancreata. PanINs involved a mean of 10.7 % of the duct profiles (range 1.0 to 27.3%). Different *KRAS* gene mutations were identified in separately microdissected precursor lesions in two of six pancreata analyzed. IPMNs were identified in four of the eight pancreata, including two pancreata each having two distinct IPMNs. Remarkably, both the IPMNs and the PanINs, even the low grade PanIN-1 lesions, were associated with lobular atrophy of the adjacent pancreatic parenchyma.

**Conclusions:** Some individuals with a strong family history of pancreatic cancer develop multifocal non-invasive epithelial precursor lesions of the pancreas. These precursor lesions produce obstructive lobular atrophy. This atrophy is likely the source of the chronic pancreatitis-like changes seen radiologically and pathologically in these patients. The multifocal nature of familial pancreatic neoplasia suggests that continued surveillance of these patients is warranted after partial pancreatectomy.

### 1250 EGFR Expression and Gene Copy Number in Hepatocellular Carcinoma Arising in Cirrhotic and Non-Cirrhotic Liver

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**Background:** Overexpression of epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase, is associated with tumor progression in many carcinomas. This study examines the expression and gene copy number of EGFR in hepatocellular carcinoma (HCC) arising in cirrhotic and non-cirrhotic liver.

**Design:** 20 formalin-fixed, paraffin-embedded HCC cases (10 each from cirrhotic and non-cirrhotic liver) were stained with a monoclonal antibody against EGFR (Clone 2-18C9), using the pharmDx kit (DAKO, Carpinteria, CA). Cell membrane staining was recorded as absent, 1+ (weak), 2+ (moderate) or 3+ (strong). For fluorescence in situ hybridization (FISH) analysis, Spectrum Orange- labeled EGFR probe (Vysis, Downers Grove, IL) and Spectrum Green-labeled probe against the centromeric region of chromosome 7 (CEP 7) were hybridized to 5 μm sections and counterstained with 2'-6'-diamidino-2'-phenylindole (DAPI). EGFR and CEP 7 signals were counted in 50 tumor nuclei per case and 300 normal hepatocyte nuclei. The EGFR to CEP 7 signal ratio was calculated for each case. EGFR expression and gene copy number was correlated with clinical and pathologic features including age, gender, cirrhosis, tumor size, differentiation, stage, histologic type and vascular invasion.

**Results:** EGFR was expressed in 19/20 (95%) HCC cases; 16/20 (80%) showed moderate or strong (2 or 3+) membrane staining. Normal hepatocytes were negative or showed patchy 1+ staining. EGFR expression was similar in cirrhotic and non-cirrhotic cases. FISH analysis in 10 cases showed gain in EGFR gene copy number in 3 (30%) tumors (mean 3.88; range 3.62-4.20). All 3 cases arose in cirrhotic liver and showed EGFR overexpression. The mean number of EGFR signals per cell in these cases was more than double that of normal hepatocytes (3.88 vs. 1.77). However, the mean EGFR:CEP 7 ratio in these tumors (0.99) was similar to normal cells (0.96). The remaining 7 cases showed normal EGFR gene copy number (mean 1.75). There was no significant association of EGFR expression or gene copy number with the clinical or pathologic parameters examined.

**Conclusions:** EGFR is overexpressed on the cell membrane in the majority of HCC. Gain of EGFR gene copy number in the form of balanced polysomy is present in a minority (30%) of cases. EGFR overexpression does not correlate with clinical or pathologic parameters examined. The strong expression of EGFR in HCC suggests that they may respond to treatment with EGFR antagonists.

### 1251 Histologic Iron Content Does Not Correlate with Severity of Histologic Disease in Hemodialysis Patients with Chronic Hepatitis C

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**Background:** Chronic Hepatitis C (HCV) infection is prevalent in the hemodialysis population. In non-end stage renal disease patients (ESRD) with chronic HCV hepatitis, hepatic iron is known to contribute to disease severity. Moreover, some reports suggest that phlebotomy is necessary prior to initiation of antiviral therapy to ensure adequate response rate in chronic HCV patients with iron overload. However, there are conflicting reports as to whether iron deposition relates to disease severity in ESRD patients. The aim of our study is to histologically evaluate the relationship of hepatic iron deposition to the severity of chronic HCV hepatitis in patients with ESRD.

**Design:** Liver biopsies from patients with ESRD and chronic HCV hepatitis were identified from the Surgical Pathology and Hepatology Department records. Liver biopsies were graded by a single pathologist based on the Modified Ishak Hepatic Activity Index (HAI) Scoring System. Hepatic iron content was measured using a semiquantitative method on a scale of 0 to 4+.

**Results:** There were 47 total biopsies from 45 patients with a mean age of 52 years. Nine (19%) had previously received kidney allografts that failed and were restarted on dialysis. The mean serum ALT was 28 IU/ml (range, 11-73). The mean HAI grade was 2.1, while the mean stage was 1.5. The mean hepatic iron score was 1.8, with 9 (19%) biopsies demonstrating 3+ to 4+ deposition. In all biopsies, iron was deposited in an overload pattern. There was no correlation between the hepatic iron content and the HAI grade (p=0.361) or stage (p=0.806). There was also no correlation between hepatic iron content and portal inflammation, focal necrosis, or interface hepatitis. There was a trend towards a correlation between the serum ALT and the HAI grade, but this was not statistically significant (p=0.067).

**Conclusions:** As expected, hepatic iron deposition is prevalent, but generally mild, in hemodialysis patients with chronic HCV hepatitis. In keeping with previous reports, our data show that ESRD patients generally have mild disease and low stage related to their HCV infection. These preliminary data suggest that hepatic iron deposition does not contribute to the severity of chronic HCV hepatitis in this ESRD population. Therefore, iron reduction procedures are not likely to improve the response rate to antiviral therapy in these patients.

### 1252 Immunohistochemical Expression of Vitamin D Receptor in Steatosis and Non-Alcoholic Steatohepatitis

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**Background:** The pathogenesis of nonalcoholic steatohepatitis (NASH) is complex and poorly understood. A recent abstract reported that peroxisome proliferator-activated receptor-alpha (PPARα) expression is decreased in zone 3 hepatocytes of NASH biopsies instead of its typical expression throughout the lobule in normal liver. Moreover, recent animal studies and clinical trials of PPAR agonists have demonstrated efficacy of these agents in reducing histologic severity of NASH. Vitamin D receptor (VDR) is known to inhibit PPARα transcriptional activity. Therefore, the expression of VDR in liver biopsies from NASH patients is of interest. The aim of our study was to evaluate immunohistochemical expression of VDR and its relationship to histologic activity in cases of NASH.

**Design:** From the files in the Department of Pathology, cases of NASH (n=30), steatosis (n=9), and normal liver (n=8) were identified. The cases were reviewed to confirm the diagnosis of NASH or steatosis and to grade the degree of activity in the form of hepatocyte ballooning degeneration (0-3+ scale), if present. Immunohistochemistry was performed using a rabbit polyclonal antibody to VDR (Santa Cruz, 1:250). Incubation was at room temperature for one hour. The slides were then graded on a 0-3+ scale by the degree of hepatocyte staining in the lobules. Normal liver biopsies were used as controls.

**Results:** In normal liver, reactivity for VDR was consistently evident in bile ducts, but was seen in no other cell type. Sixty percent (18/30) of the NASH biopsies showed hepatocyte reactivity for VDR compared to 33% of steatosis cases. All of these cases showed positive staining in bile duct epithelium as an internal control. In cases of NASH, VDR expression corresponded to foci of hepatocyte ballooning degeneration, largely in centrilobular regions. Linear regression analysis of steatosis and NASH biopsy results revealed a significant correlation (p=0.02) between intensity of VDR expression and severity of ballooning degeneration.

**Conclusions:** VDR expression appears to significantly correlate with the degree of hepatocyte injury in NASH biopsies. Furthermore, the pattern of expression in zone 3 hepatocytes seems to correspond to the pattern of expression of PPARα loss in zone 3 hepatocytes reported previously. Because of this expression pattern and the fact that VDR is known to repress PPARα, VDR is likely responsible for decreased PPARα expression in NASH.

### 1253 Expression of c-kit in Solid Pseudopapillary Tumor of the Pancreas

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**Background:** Solid pseudopapillary tumors (SPTs) of the pancreas are uncommon neoplasms of low malignant potential and uncertain histogenesis. A small percentage of patients develop metastatic disease and some succumb to disease. There is no effective non-surgical treatment for patients with metastatic disease, those who are not good surgical candidates, and those with an unresectable tumor. Novel therapeutic targets are needed for these patients. In this study we investigated c-kit expression in SPTs using immunohistochemical labeling.

**Design:** Fifty-two histologically confirmed SPTs from the surgical pathology files of the Johns Hopkins Hospital, the Karmanos Cancer Center and Memorial Sloan-Kettering Cancer Center formed the study group. For each case one paraffin block was retrieved and sectioned for immunohistochemical (IHC) labeling. Immunolabeling of at least 10% neoplastic cells is considered positive, and positive cases were further divided into focally positive (10-50% cells) and diffusely positive (>50% cells).

**Results:** In normal pancreatic tissue, only scattered interstitial cells of Cajal of the pancreas (J Cell Mol Med. 2005;9(1):169-90.) and mast cells showed staining of c-kit. Twenty three of the 52 SPTs showed diffuse expression of c-kit in the neoplastic cells by IHC labeling and additional 5 cases showed focal staining in the neoplastic cells. Molecular analysis of mutations in c-kit and PDGFR-alpha is in the process to further characterize these positive cases.

**Conclusions:** C-kit is expressed in a significant portion (28 of 52 cases or 54%) of solid pseudopapillary tumors of the pancreas. The molecular mechanism of c-kit overexpression in some SPTs is unknown. Further analysis of c-kit mutations and PDGFR-alpha (in the process) in these positive tumors may help us elucidate the mechanism of c-kit overexpression and better understand the histogenesis of SPTs.

### 1254 Expression of Nuclear Receptor Co-Activator PRIP (Peroxisome Proliferator-Activated Receptor Interacting Protein) in Human Hepatocellular Carcinomas

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**Background:** Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that regulate the expression of target genes. PPARs consists of 3 isotypes, namely PPAR α, β and γ that are products of separate genes. Transcriptional activation of PPARs involves not only ligand binding but also participation of nuclear receptor coactivators. PRIP is a recently identified nuclear receptor coactivator that is very important in transcriptional activities of PPARα and g. In our earlier studies we have observed overexpression of PPARγ in a majority of hepatocellular carcinomas (HCC), irrespective of the degree of differentiation. Since activation of PPARγ by synthetic ligands is increasingly considered in the prevention and treatment of HCC and coactivators are necessary for transcriptional activation of this receptor, we sought to examine the expression of PRIP in these tumors.

**Design:** Tissue microarrays containing 25 HCC were created from formalin fixed and paraffin embedded tissue blocks. HCC included 17 grade 2 tumors, 5 grade 1 tumors and 3 grade 3 tumors. Five-micron thick tissue microarrays section was evaluated for PRIP protein expression by standard immunoperoxidase procedure using rabbit polyclonal antibodies (purchased from Bethyl Laboratories, Montgomery, TX). Tumors that displayed clear nuclear staining were considered as positive for PRIP.

**Results:** Twenty one of 25 HCC (84%) showed diffuse strong nuclear staining for PRIP. One grade 1 tumor and 3 grade 2 tumors were negative for PRIP. The staining pattern was similar in grade1, grade 2 and grade 3 tumors. Interestingly, adjacent cirrhotic liver observed in 3 tumors was negative for PRIP.

**Conclusions:** For the first time, we have demonstrated the expression of PPAR coactivator PRIP in human HCC. Demonstration of expression of PPARγ and PRIP in HCC are likely to be important for chemoprevention and therapeutic intervention of HCC using PPARγ ligands.

### 1255 Expressional Disturbance of *Periods (PERs)* in Hepatocellular Carcinoma

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**Background:** In mammals, many physiological and behavioral processes follow circadian rhythm. The human circadian rhythm is controlled by at least nine circadian genes: *PER1, PER2, PER3, CRY1, CRY2, CLOCK, BMAL1, Timeless and CK1e*. Disruption of the circadian rhythm has a profound effect on a variety of human physiologic functions. Several studies have demonstrated that (1) the mice deficient in *mPer2* gene led to deregulation of some cell-cycle regulating genes (*c-Myc, Cyclin D1, Cyclin A*, and *Mdm2)*, reduced apoptosis, and increased tumor development rate, (2) the mice with a targeted mutation in the *Per1* gene displayed a shorter circadian period and reduced precision and stability in expression of *Clock* genes, (3) inactivation of the *PER1* gene and down-regulation of *PER2* and *PER3* genes resulted in deregulation of cell-cycle control. In this study, we examined the expression of 3 *PER* genes in HCC, analyzed the relationship among the expression of 3 *PERs* and some of key cell-cycle control genes *(P53, C-MYC, Cyclin A, Cyclin B, and Cyclin D1)*, and explored their roles in the carcinogenesis of HCC.

**Design:** RNA and DNA extracted from 71 primary HCCs with paired non-tumor tissue were analyzed for 3 *PER* genes by real-time quantitative RT-PCR, mutation analysis, and methylation-specific PCR analysis. Additional studies, including sequencing of methylation-specific PCR products, demethylation of HCC cell lines, and immunohistochemical examination for 3 PERs, P53, C-MYC, cyclin A, cyclin B and cyclin D1, were also conducted.

**Results:** The mRNA levels of 3 *PERs* in HCC were significantly reduced in tumor part of HCC (p<0.0004). No genetic mutation within the coding regions of 3 *PER* genes was detected. Parts (21.13%) of the CpG sites were significantly methylated in the promoter region of *PER1* gene but not in *PER2* and *PER3*. No consistent relationship among the protein expression of 3 PERs and cell-cycle regulating factors (P53, C-MYC, Cyclin A, Cyclin B, and Cyclin D1) was detected.

**Conclusions:** Disruption of the circadian rhythm due to significant down-regulation of 3 *PERs* occurred in HCC, which was partly caused by methylation at the promoter region or other factors but not genetic mutation. No definitive relationship between the disruption of *PERs* and cell-cycle deregulation was found. The potential mechanisms linking down-expression of *PERs*, disruption of the circadian rhythm, and carcinogenesis of HCC remain to be explored.

### 1256 Immunostains of Clusterin and Proliferating Cell Nuclear Antigen Help Distinguish Benign from Malignant Liver Nodules

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**Background:** It is often challenging to distinguish benign from malignant liver nodules. Using gene-chip array technique, we previously identified several candidate genes, including clusterin and proliferating cell nuclear antigen (PCNA), whose expression was significantly different between hepatocellular adenoma (HCA) and well-differentiated hepatocellular carcinoma (HCC). In the current study, we applied immunohistochemical stains of these two molecules in a large series of benign and malignant liver nodules.

**Design:** Surgical resections and needle core biopsies of liver nodular lesions, including 33 HCAs, 40 cases of focal nodular hyperplasia (FNH), 77 cirrhotic livers with macro-regenerative nodules (MRN) and 204 HCCs, were included in this study. Formalin-fixed, paraffin-embedded tissue sections were selected to include lesions of interest and adjacent normal or cirrhotic liver parenchyma. Immunohistochemical stains for clusterin and PCNA were analyzed for the staining intensity (strong or weak), percentage of positively stained cells and staining patterns (cytoplasmic, nuclear or canalicular).

**Results:** The immunostaining profiles were summarized in the table. Cytoplasmic staining for clusterin was seen in both benign and malignant liver nodules. In a small subset of well-fixed benign lesions and normal liver tissues, clusterin immunostaining highlighted intercellular canalicular spaces by forming two fine-granular parallel pericanalicular lines, generating a "railroad" pattern. However, this staining pattern was not evident in the vast majority of HCCs. Instead, a haphazardly arranged, strong linear canalicular staining pattern was observed in the majority of HCCs. PCNA immunoreactivity was stronger and more diffuse in HCCS than in benign liver nodules.

**Conclusions:** We illustrated characteristic immunostaining patterns of clusterin and PCNA in HCCs, which are potentially useful in the distinction between benign and malignant liver nodules.

| | | HCA (%) | FNH (%) | Cirrhosis/MRN (%) | HCC (%) | P Value |
|---|---|---|---|---|---|---|
| Clusterin | Strong | 90 | 94 | 67 | 52 | |
| | Weak | 10 | 6 | 33 | 48 | |
| | Canalicular | 3 | 31 | 18 | 75 | <0.0001 |
| PCNA | Strong | 6 | 13 | 27 | 96 | <0.0001 |
| | Weak | 94 | 87 | 73 | 4 | |
| | >75% | 15 | 0 | 0 | 77 | <0.0001 |
| | 51-75% | 15 | 0 | 17 | 19 | |
| | 25-50% | 58 | 6 | 35 | 4 | |
| | <25% | 12 | 94 | 48 | 0 | |

### 1257 Expression of Claudin-1, Claudin-7, and ZO-1 in Hepatocellular Carcinoma

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**Background:** Claudins 1 and 7, and ZO-1, integral tight junction proteins that serve both structural and regulatory functions, are dysregulated in a variety of malignancies. While altered expression of claudin-1 has been reported in cholestatic disorders, the role of these proteins in hepatocellular carcinogenesis remains unexplored. We hypothesized that hepatocellular carcinoma would show altered expression of these cell membrane proteins.

**Design:** A tissue microarray was constructed containing 84 hepatocellular carcinoma samples with matched non-neoplastic liver. Slides were stained for claudin-7 (Zymed, 1:400 dilution), claudin-1 (Zymed, 1:250), and ZO-1 (BD Transduction, 1:300). Membranous staining was scored as 0 (no/trace), 1+, 2+, or 3+; % cells positive were scored as 0; 1- 1-29%; 2- 30-49%; 3- 50-79%; 4- 80-100%. An expression index of 0-7 was calculated by adding the intensity score and percent positive score. Indices of 2 or 3 were considered low grade expression, 4-7 high grade expression. Medical records were reviewed for demographic data, etiology of liver disease, and clinical features.

**Results:** In most non-neoplastic livers, claudin-1, claudin-7, and ZO-1 were only weakly or focally expressed on the surface of hepatocytes. Cirrhotic liver was more likely to overexpress claudin-7 (34/60, 57%) and claudin-1 (16/21, 76%), compared to non-cirrhotic liver (3/21, 13% for claudin-7 and 29/60, 48% for claudin-1). ZO-1 expression was seen in non-neoplastic liver in only 14 cases; all but one were cirrhotic. 62 tumors showed expression of claudin-7, 70 of claudin-1, and 57 of ZO-1. Tumors arising in cirrhosis were more likely to show high grade expression of claudin-1 (29/60 tumors arising in cirrhosis [48%], compared to 5/21 [24%] tumors in non-cirrhotic liver) and claudin-7; 9/18 claudin-7 negative tumors arose in non-cirrhotic liver, compared to 14/47 in cirrhotic liver ($p<0.05$). No association of expression of claudin-1, claudin-7, and ZO-1 with etiology of liver disease, tumor grade, tumor stage, classification as early HCC, clinical outcome, sex, or age was found.

**Conclusions:** Aberrant expression of claudin-1, claudin-7 and ZO-1 is common in hepatocellular carcinoma. Non-neoplastic hepatocytes in cirrhosis also aberrantly express claudins 1 and 7, and this overexpression is also seen in tumors arising in cirrhosis. Given this association of aberrant expression with cirrhosis, further investigations of the role of these cell adhesion molecules in the pathogenesis of hepatocellular transformation and neoplasia are warranted.

### 1258 p53 and p16$^{Ink4a}$ Constrain Progression of Pancreatic Adenocarcinoma in the Mouse: Correlations between Genotype and Histologic Phenotype

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**Background:** The evolution of human pancreatic ductal adenocarcinoma (PDAC) from premalignant pancreatic intraepithelial neoplasia (PanIN) is matched by an ordered series of genetic events that includes early KRAS activation, subsequent loss of p16$^{Ink4a}$/p14$^{Arf}$, p53, and SMAD4 tumor suppressors, and marked chromosomal instability. Mouse models of PDAC have certified that activated Kras$^{G12D}$ drives PanIN development and that loss of either Ink4a/Arf or p53 results in malignant progression.

**Design:** Here, in the Kras$^{G12D}$ model, we sought to determine the histopathologic and genomic impact of mutations of p16$^{Ink4a}$, p19$^{Arf}$, and/or p53. These genetic lesions were induced through a conditional inactivation system using the pancreas-specific Pdx1-Cre transgene.

**Results:** Rapidly progressive PDAC was observed in the setting of pancreas-specific deletion either of p53 or p16$^{Ink4a}$, the latter with intact germline p53 and p19Arf sequences. Additionally, Kras$^{G12D}$, together with heterozygosity for p53 and p16$^{Ink4a}$ or for Ink4a/Arf, promoted PDAC after a longer latency and with greater propensity for distant metastases relative to mice with homozygous deletion of p53 or Ink4a/Arf. Different genotypes were also associated with specific histopathologic characteristics, most notably a trend towards increasing glandular differentiation in heterozygous models and anaplastic features in homozygous models. Tumors from p53+/- p16$^{Ink4a+/-}$ and Ink4a/Arf$^{lox/+}$ cohorts showed high frequency p16Ink4a inactivation and loss of p53 or p19$^{Arf}$, respectively.

**Conclusions:** Together, our results establish that disruptions of the p53-ARF and p16$^{INK4a}$-RB pathways play critical and cooperative roles in the progression of PDAC with specific tumor suppressor genotypes influencing the behavior, histopathologic phenotype and genomes of the resultant tumors.

### 1259 Characterization of Epidermal Growth Factor Receptor (EGFR) and HER2 Overexpression in Pancreatic Intraepithelial Neoplasia (PanIN) and Pancreatic Ductal Adenocarcinoma (PDAC): A Comparative Study Using Immunohistochemistry Correlated with Gene Amplification by FISH

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**Background:** Other studies have shown that alteration of signal molecules plays a role in progression of PDAC. Although many studies have reported that the membrane expression of EGFR and HER-2 is related to various clinicopathologic parameters, there are few studies on the localization patterns of these molecules in PDAC and PanIN. The aim of our study is using immunohistochemistry to explore the expression of EGFR and HER-2 in PanIN and PDAC. FISH was used to detect gene amplification events in PDAC and PanIN and these findings were correlated.

**Design:** Normal pancreas and representative tissue samples from 34 cases of PDAC and PanINs were examined by immunohistochemistry using the HercepTest and EGFR kits (DAKO). Positive staining for HER-2 and EGFR was quantified with ACIS. The staining patterns were detemined by pathologists using conventional light microscopy. FISH was performed for HER-2 (PathVysion dual HER-2 probe) in 28 cases, and for EGFR (Vysis LSI EGFR kit) in 34 cases. Clinicopathologic data included age, tumor size and AJCC stage.

**Results:** Membranous overexpression of HER2 protein (>2+) was seen in 17% (5/28) of the study cases. There was no significant difference in staining pattern between the invasive components of PDAC and PanIN. The HER2 gene locus was amplified in 11% (3/28) of PDAC. Neither oncogene amplification of HER2 nor protein overexpression correlated with tumor size or staging. Overexpression of EGFR (>2+) was seen in 61% (21/34) of the study cases. Cytoplasmic EGFR overexpression was more frequent in the invasive components of PDAC than in those of PanIN. EGFR gene amplification was not detected in any of the 34 PDAC's. Neither membranous nor cytoplasmic EGFR overexpression correlated with tumor size or staging.

**Conclusions:** Our results show good correlation between Her-2 gene amplification and Her-2 protein expression in PDAC. In contrast, no significant correlation was demonstrated between EGFR protein overexpression and gene amplification. However, alteration of EGFR expression, specifically, increased cytoplasmic expression, might be associated with tumor progression of PanIN to PDAC.

### 1260 Identification of Polysomy and Homozygous 9p21 Loss in PSC-Associated and Sporadic Cholangiocarcinoma with Fluorescence In Situ Hybridization

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**Background:** Relatively little is known about the chromosomal alterations that occur in PSC-associated and sporadic cholangiocarcinomas. The goal of this study was to assess paraffin-embedded cholangiocarcinoma specimens with FISH to: 1) determine if there are differences in the chromosomal alterations present in PSC-associated versus sporadic cholangiocarcinoma, and 2) examine what chromosomal abnormalities, if any, are present in biliary dysplasia.

**Design:** Histologic sections from patients with cholangiocarcinoma (N=17; 3 PSC-associated, 14 sporadic), cholangiocarcinoma and biliary dysplasia (N=5; 3 PSC-associated, 2 sporadic), and biliary dysplasia only (N=2; both PSC-associated) were selected for FISH analysis using the UroVysion probe set. The UroVysion probe set contains fluorescently-labeled probes to centromeres 3, 7, and 17 and the 9p21 (*P16*) locus. For each case, the number of signals for each probe was enumerated in 50 non-overlapping interphase nuclei and the percentage of nuclei containing 0, 1, 2, and ≥3 signals determined. Cases were considered positive for polysomy and homozygous

9p21 deletion if the percentage of cells exhibiting signal gain or loss exceeded specific cutoffs for each probe as determined from normal value studies. Additionally, p16 immunohistochemistry was performed on each case to assess for associations between 9p21 alterations and p16 expression.
**Results:** Gain of two or more chromosomes (polysomy) was observed in 13 of 16 (81%) sporadic and 4 of 6 (67%) PSC-associated cholangiocarcinomas. Homozygous loss of 9p21 was identified in 8 of 16 (50%) sporadic and 3 of 6 (50%) PSC-associated cholangiocarcinomas. Polysomy and/or homozygous 9p21 loss was also observed in 3 of 7 (43%) cases of biliary dysplasia, respectively. All invasive tumors and biliary dysplasias with homozygous loss of 9p21 in ≥ 80% of the nuclei showed complete loss of p16 expression.
**Conclusions:** Polysomy and inactivation of the *P16* tumor suppressor gene via homozygous deletion of 9p21 are common abnormalities in both sporadic and PSC-associated cholangiocarcinomas. These abnormalities are frequently detectable in high grade biliary dysplasia. Further characterization of the genetic alterations in pre-invasive and invasive biliary lesions will help our understanding of the pathogenesis of these tumors and may aid in the development of methods for early diagnosis and screening.

### 1261 Immunohistochemical Detection of IgG4-Positive Plasma Cells Helps Distinguish Lymphoplasmacytic Sclerosing Pancreatitis (LPSP) from Pancreatitis Associated with Carcinoma or Other Conditions

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**Background:** LPSP is an increasingly recognized entity, likely of autoimmune etiology. Elevated serum IgG4 levels are a characteristic finding. Two morphologic types have been reported, the usual type (with periductal lymphoplasmacytic infiltration, interstitial fibrosis and venulitis) and the "granulocytic type" (with a neutrophilic infiltrate and "granulocytic epithelial lesions"). Preoperative distinction of LPSP from pancreatic carcinoma is difficult owing to their similar clinical presentation, and a similar inflammatory infiltrate can be found on biopsies when malignant glands are not captured.
**Design:** Immunohistochemistry (IHC) for IgG4 was performed on 22 cases of usual type LPSP, 7 cases of granulocytic type LPSP, 8 of non-specific chronic pancreatitis, and 13 of peri-tumoral pancreatitis adjacent to ductal adenocarcinomas, using monoclonal IgG4 antibody (Binding site). The areas with highest density of IgG4 staining at low power were selected, and the number of IgG4-positive plasma cells in 3 high power fields was counted. The IHC results were compared with the serum IgG4 levels in10/22 usual type LPSP cases with available serum data.
**Results:** IHC results are summarized in table1. The relationship between IgG4 serum level and IHC results is shown in table 2.

| | n | Mean of IgG4-positive cells/hpf | Range of IgG4-positive cells/hpf | No. of cases with > 50 positive cells/hpf |
|---|---|---|---|---|
| LPSP, usual type | 22 | 58.6 | 0-150 | 16 (72%) |
| LPSP, granulocytic type | 7 | 7.8 | 0-40 | 0 (0%) |
| Chronic pancreatitis, NOS | 8 | 9.25 | 0-40 | 0 (0%) |
| Peri-tumoral pancreatitis | 13 | 9.92 | 0-45 | 0 (0%) |

| | Serum IgG4 > 135 mg/dl | Serum IgG4 <135 mg/dl |
|---|---|---|
| > 50 IgG4 + cells/hpf | 7 | 2* |
| < 50 IgG4 + cells/hpf | 1 | 0 |

* The serum levels for these cases were 50 mg/dl and 73 mg/dl

4 LPSP cases had only biopsy material not showing all the features of LPSP; 2 had diffuse staining for IgG4, and 2 had only focal staining despite high serum IgG4 levels. One LPSP case with only a few IgG4-positive cells had prior steroid therapy.
**Conclusions:** IgG4 immunohistochemistry may be helpful in differentiating LPSP from peri-tumoral pancreatitis and other types of pancreatitis. More than 50 positive cells / hpf is highly specific for usual type LPSP. Peri-tumoral pancreatitis can also stain with IgG4 but the staining is usually very focal. Not all cases are positive, especially those with granulocytic infiltrates.

### 1262 Anaphase Bridge Analysis as a Biomarker of Hepatitis C Virus (HCV) Hepatic Tumorigenesis

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**Background:** The causal relationship between hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) is well established. Currently, no adequate biomarkers of HCC tumorigenesis exist. We hypothesize that genomic alterations such as anaphase bridges may identify the subset of HCV patients that develop HCC. Anaphase bridges are abnormal chromosomal segments that bridge between dividing daughter nuclei at anaphase. They develop in part from telomere shortening, leading to sticky chromosome ends and subsequent end-to-end fusion with anaphase bridge formation. Telomere shortening is well-described in HCC and in chronic oxidative and proliferative injury. We therefore hypothesize that the damage imparted by HCV may induce organ-wide anaphase bridges, differentiating HCC "progressors" from "non-progressors".
**Design:** Fresh tissues from explanted livers included single random samples of non-neoplastic HCV cirrhosis from progressors (n=5) and non-progressors (n=5). Controls consisted of HCC tumor (n=1), and normal liver (n=1). Hepatocytes were macrodissected from cirrhotic nodules yielding >80% purity. Anaphase enrichment was done via flow cytometric DNA ploidy sorting of 4N cells onto slides. A MetaSystems Imaging System automated classifier was developed to identify nuclei and simultaneously count the total 4N population. Captured anaphases were reviewed to identify bridges.
**Results:** The average number of 4N cells evaluated per case was 1007 (progressors), 702 (non-progressors), 2058 (normal control), and 391 (HCC tumor). Anaphase bridges were identified in all groups, averaging 2.6 (progressors), 2.4 (non-progressors), 1.2 (normal control), and 6.0 (HCC tumor) per 1000 analyzed 4N cells. No statistically significant differences were identified between groups (p= 0.617 for progressors and non-progressors).
**Conclusions:** These data suggest that anaphase bridges in non-tumorous liver do not distinguish progressors from non-progressors. These results correlate with published reports and data from our laboratory that telomere shortening occurs at the dysplastic nodule stage of HCC formation and not in cirrhotic liver. These findings suggest that anaphase bridges do not occur early in hepatocellular carcinogenesis.

### 1263 Histologic Criteria for the "Ovarian-Type" Stroma in Mucinous Cystic Neoplasms (MCNs) of the Pancreas: A Multi-Institutional Consensus in Japan

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**Background:** Ovarian-type stroma (OS) is known as a characteristic histologic feature of MCNs forming a band of densely-packed spindle cells beneath the epithelium, and is now widely used as a criterion for their diagnosis. However, the histologic criteria of OS have not yet been fully defined.
**Design:** We collected 93 pancreatic cystic lesions which were located in the body and/or tail of the pancreas and had thick fibrous cyst wall without macroscopic communication to the duct system. Under these macroscopic criteria most of IPMNs were excluded. All patients, except for 6, were women, and the mean age was 47.1 years (14-78 yr). A meeting of experts on pancreatic neoplasms in Japan was held. Discussions took place to review the histology of the lesions brought forth, and special attention was paid to the histological characteristics of OS.
**Results:** Eighty, including 1 case of man, of the 93 cases of those lesions had a distinct OS, although their cellularity and cell density were variable. Major points for histologic recognition of OS were (1) hypercellular spindle cell bundles, (2) a layered structure (OS is located immediately beneath the epithelial layer.) and (3) enlarged nuclei; and minor points were (4) presence of luteinized cells, (5) overlapped cell nuclei, (6) being intermingled with capillaries, and (7) waviness of the spindle cells. In 10 of 13 cases without recognizable OS, epithelial cell lining on the wall was retained. More than a half of the cases without OS might be IPMN, and the remaining cases MCN, old retention cyst or congenital cyst. Other issues addressed here included thickness of cyst wall, multi-/unilocular cyst structure, and immunohistochemical markers.
**Conclusions:** We were able to apply the above-mentioned histologic criteria for OS to most cases of MCNs. However, OS may not be seen in several cases with extensive hyalinized cyst wall. In such a case, extensive histologic samplings and a careful review of the gross findings are required.

### 1264 Immunohistochemical Analysis of Stromal Cell-Derived Factor-1 (SDF-1) in Human Liver Neoplasms

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**Background:** The mechanism and pathogenesis of the high frequency of intrahepatic metastasis in hepatocellular carcinoma (HCC) has not yet been fully elucidated. The alpha-chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 have been recognized for their roles in stem cell trafficking. The SDF-1 is expressed on the surface of vascular endothelial cells and recent data suggests that the SDF-1/CXCR4 pathway may also be important for regulating tumor metastasis. Little information exists regarding the role of SDF-1 in metastasis of HCC.
**Design:** Formalin-fixed paraffin-embedded recut sections of 20 HCC, 5 hepatocellular adenoma, 19 regenerative nodules in cirrhosis and 6 normal liver tissue were immunostained for SDF-1 (1:25, RD Systems) and CD 31 using standard avidin-biotin techniques. The number of immunoreactive endothelial cells of SDF-1 and CD 31 in sinusoidal endothelial cells was scored as follows: 0 (no immunoreactive cells), 1 (<10%), 2 (10–50%), and 3 (>50%). Staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong). The expression of SDF-1 was further assessed using a numerical scoring system ranging from 0 to 6.
**Results:** The number of SDF-1 positive sinusoidal endothelial cells and the intensity of immunostaining were increased in HCC compared with cirrhotic liver, normal liver and hepatocellular adenoma ($P < 0.05$). SDF-1 showed no significant expression changes in sinusoidal endothelial cells related to grade of HCC. No significant difference of SDF-1 expression was observed between hepatocellular adenoma and cirrhotic liver, and between hepatocellular adenoma and normal liver. HCC exhibited significant increase in expression of CD 31 in numbers of stained sinusoids and staining intensity in comparison with cirrhotic liver, normal liver and hepatocellular adenoma ($P < 0.01$).
**Conclusions:** Increased expression of SDF-1 in sinusoidal endothelial cells in HCC suggests that the SDF-1/CXCR4 pathway may be important for regulating tumor metastasis. Additional studies will be needed to dissect the exact role of SDF-1 in HCC metastasis and progression.

### 1265 Cytoplasmic Immunoreactivity of Thyroid Transcription Factor – 1 (TTF-1) in Hepatocytes: True Positivity or Cross Reaction?

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**Background:** TTF-1, a 41-kd homeodomain containing DNA-binding protein, was first described in thyroid follicular cells as a regulator of thyroid-specific genes. Subsequently, it was identified in lung (type II cells & Clara cells) and ventral forebrain. The nuclear immunoreactivity for TTF-1 is useful for the identification of carcinomas of thyroid and lung origin. Recently, cytoplasmic immunoreactivity for TTF-1 in

hepatocytes and its utility as a marker to differentiate hepatocellular carcinoma from metastatic carcinoma have been reported. Our aim is to determine whether the staining is a result of cross reaction of TTF-1 antibody or true positivity resulting from aberrant expression of TTF-1 or products of alternative slicing of TTF-1 gene.
**Design:** Six cases of fresh tissue-3 explanted livers (2 cirrhosis, 1 rejection), 1 liver lobectomy for metastatic colon adenocarcinoma, 1 thyroid goiter, 1 lung lobectomy for carcinoma-were obtained for H&E sections, TTF-1 immunostain and for RNA and protein extraction. Western blot using monoclonal antibody anti-TTF-1, and anti-β-actin as internal control for the quality and quantity of the protein was performed. TTF-1 mRNA level was measured by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using 3 pairs of primers to detect TTF-1 and its possible alternative splicing products. Also, a pair of primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a house keeping gene, was used as an internal control for the mRNA.
**Results:** Cytoplasmic immunoreactivity for TTF-1 was detected in hepatocytes of normal liver and cirrhotic liver (strong), and of liver transplant with rejection (weak). Thyroid and lung tissues showed strong nuclear staining. Metastatic adenocarcinoma was negative. Western Blot analysis revealed a very strong band corresponding to an approximately 170-kd protein only from liver tissue and not from either thyroid or lung tissue. mRNAs of TTF-1 were not detected in liver tissues while the positive controls including lung and thyroid tissues yielded expected RT-PCR products. GAPDH mRNA can be easily amplified by RT-PCR from livers, thyroid and lung tissues with similar intensity of the expected band.
**Conclusions:** TTF-1 immunoreactivity in the hepatocyte cytoplasm is due to an approximately 170-kd protein. This protein seems to be not an alternative slicing product of TTF-1. The identity of this high molecular weight protein is currently unknown. Nevertheless, it is not expressed in either thyroid or lung tissue.

### 1266 *All-Trans* Retinoic Acid Suppresses TGF-β1-Induced Activation of Rat Hepatic Stellate Cells

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**Background:** Being activated by cytokines such as transforming growth factor beta 1 (TGF-β1), hepatic stellate cells (HSCs) are the major source of extracellular matrix (ECM) in fibrotic liver. Therefore, they play an important role in the development of liver fibrosis/cirrhosis. Activated HSCs lose most of intracellular retinoids resulting a diminished signal intracellularly. It is known that exogenous retinoids such as *all-trans* retinoic acid (atRA) can inhibit proliferation and activation of HSCs. However, the mechanisms remain to be defined. The purpose of this study is to examine the effects of atRA on TGF-β1-stimulated cultured rat HSCs and explore its related molecular mechanism.
**Design:** HSCs were isolated from healthy male rat livers and cultured. HSCs proliferation was determined by MTT assay; alpha-smooth muscle actin (α-SMA) and $p16^{INK4a}$ (one of cyclin-dependent kinases inhibitors) protein by immunocytochemistry (IHC); mRNA levels of precollagen type I and III, nuclear retinoic acid receptor beta 2 (RAR-β2), and $p16^{INK4a}$ by nuclear acid *in situ* hybridization (ISH); and gelatinase activities by zymography.
**Results:** 1 ng/ml TGF-β1 stimulated HSCs to proliferate and enhanced expression of α-SMA protein and precollagen type I and III mRNA, but 1 μM atRA inhibited the above effects of TGF-β1 (by 55.26%, 70.42%, 21.12% and 22.07% respectively; $P<0.05$, n=3), and atRA suppressed TGF-β1 and reduced the activity of matrix metalloproteinase type 2 in the supernatant of HSCs only when atRA was added to the cultures prior to the TGF-β1 treatment. RAR-β2 mRNA was not detected in the control or TGF-β1-stimulated HSCs when using ISH, but HSCs treated with atRA did induce RAR-β2 mRNA expression. IHC showed that TGF-β1 completely inhibited the expression of $p16^{INK4a}$ protein, while atRA reduced its expression. However, the differences between the mRNA levels of $p16^{INK4a}$ in these cells were statistically negligible.
**Conclusions:** atRA can inhibit HSCs activation induced by TGF-β1, in which intracellular retinoid signaling and up-regulation of $p16^{INK4a}$ gene expression are involved, and atRA may hamper hepatic fibrogenesis through the following means: (1) to suppress HSCs proliferation induced by TGF-β1; (2) to reduce ECM production of HSCs and (3) to prevent HSCs from TGF-β1 induced inhibition of ECM degradation.

### 1267 Paired Wedge and Needle Biopsies in Histological Evaluation of Fatty Liver Disease

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**Background:** Fatty liver disease is increasing in the United States and much of Europe. Biopsies are often performed to assess the amount of fat accumulation, inflammation, and fibrosis in the liver. There is little information on whether the histological findings in wedge biopsies (often performed at the time of bariatric surgery as a "baseline") are directly comparable to needle biopsies, which may be subsequently obtained to follow disease progression.
**Design:** Simultaneous paired wedge and needle biopsies were obtained from the same lobe of the liver at the time of bariatric surgery for morbid obesity. The biopsy sizes were compared by calculating the $mm^2$ of tissue for each biopsy. The liver biopsies were blinded and scored using the NAS score (Hepatology. 2005 Jun; 41(6):1313-21), a modification of the Brunt score. Variables were compared using Wilcoxon Signed Rank tests and paired t-tests.
**Results:** Biopsies were obtained from 17 women and 5 men. The needle biopsies averaged $17\pm7$ $mm^2$ in size and the wedge biopsies $109\pm91$ $mm^2$. Overall, the biopsies showed mild fatty liver disease, with a median fatty grade of 1, lobular inflammation grade of 1, and fibrosis stage of 1. There was no statistically significant difference between wedge and needle biopsies for any histological variable including grading of macrovesicular steatosis, balloon cell change, lobular inflammation, and fibrosis stage. For fatty change, 13/22 pairs had identical macrovesicular steatosis score, 6/22 had one grade higher steatosis on the wedge biopsy, and 3/22 had 1 grade higher steatosis on the core biopsy. Mild pericellular or portal fibrosis was identified in 10/22 cases, and bridging fibrosis in 2/22 cases. Fibrosis stages were identical in 13/22 biopsy pairs. Of the cases with a discrepancy on staging, 4/22 wedge biopsies had mild (stage 1a) pericellular fibrosis that was not observed on needle biopsy, while 2/22 cases had more pericellular fibrosis evident on needle biopsy. In addition, 2/22 cases had mild portal fibrosis only apparent on the wedge biopsy. There was no differences in the sizes of the wedge or needle biopsies between those with and without staging discrepancies ($p>0.05$).
**Conclusions:** In this cohort of individuals with generally mild fatty liver disease, both wedge and needle biopsies show comparable histological findings, including the amount of fat, balloon cell changes, lobular inflammation, and fibrosis. However, wedge biopsies tended to identify more steatosis and mild pericellular fibrosis.

### 1268 Acute Hepatitis C in Non-Immunosuppressed Adults

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**Background:** Acute hepatitis C in immunocompetent individuals is rarely symptomatic and rarely biopsied. Thus the histology of acute hepatitis C is poorly described. We describe the histology of four cases of acute symptomatic hepatitis C in adults.
**Design:** Cases were selected from the consult and surgical pathology files of a single institution. Clinical, serological and histological findings were reviewed.
**Results:** The four individuals, three males and one female, had an average age at biopsy of $56\pm11$ years. None were immunosuppressed. The time interval from clinical presentation to biopsy ranged from 41 to 141 days. Individuals presented with a variety of symptoms including jaundice, abdominal pain, chest pain, fatigue, decreased appetite and rash. Clinically, acute hepatitis C was initially suspected in none of the cases. The average ALT/AST/alkaline phosphatase at the time of biopsy was 308/73/85 U/L. The average total bilirubin was 5.2mg/dL. The histological findings in three cases showed cholestatic hepatitis with portal changes that raised the possibility of down-stream biliary tract disease: all three showed bile ductular proliferation in the background of portal tracts containing mixed infiltrates of lymphocytes and neutrophils. In the lobules, mild to moderate lobular inflammation and spotty necrosis was seen. Interestingly, a significant number of neutrophils were present in the lobular infiltrates in two cases. In one case, bridging necrosis was present. The fourth case, biopsied 70 days after presentation, showed only minimal portal and lobular chronic inflammation, with the most striking finding being numerous lobular clusters of pigmented macrophages. The clinical diagnosis in this case had been autoimmune hepatitis and the biopsy was interpreted as non-specific but potentially consistent with that diagnosis. Serological findings (Hepatitis C antibody seroconversion and HCV RNA levels) over time led to the proper diagnosis in all cases.
**Conclusions:** Acute hepatitis C in the immunocompetent adult can have a surprisingly cholestatic histological pattern with features that mimic down stream biliary tract disease.

### 1269 Long Term Histological and Clinical Follow-Up of Anti-Mitochondrial Antibody (AMA) Positive Autoimmune Hepatitis (AIH): Another Variant of Autoimmune Hepatitis or a Well Controlled Overlap Syndrome?

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**Background:** AMA are the serological hallmark for Primary Biliary Cirrhosis (PBC). It has been proposed that the detection of AMA in individuals otherwise thought to have AIH, represents an "overlap" of AIH and PBC. Identifying the primary disease is important to address because if PBC and AIH are present simultaneously, treatment may need to be modified. **Aim**: To determine if positive AMA in patients with overt AIH has pathological or clinical significance.
**Design:** Patients who met the criteria for "probable" or "definite" AIH according to the International AIH Group were included. Patients consistently AMA-positive by immunofluorescence and ELISA for PDH-E2 and confirmed by immunoblotting (IB) were reviewed. Liver biopsies were performed in all patients at the time of initial presentation and repeated in most to reevaluate disease status. All biopsies of the study patients were evaluated by a hepatopathologist for features of AIH and PBC. For those in whom AMA was persistently detected, their biopsies were also reviewed by a second hepatopathologist.
**Results:** Ten of 126 patients with typical features of AIH had detectable AMA in serum (ELISA). None had any features suggestive of PBC. None had detectable anti-LKM. Of 10 patients who were AMA positive at the time of diagnosis, 6 remained persistently AMA positive by ELISA and IB. All responded to standard immunosuppressive therapy, were followed long term and their clinical course (7-26 years) remained typical for AIH. No bile duct damage was seen on initial or follow-up liver biopsies. All but one patient presented with jaundice and had complete resolution upon initiation of steroid therapy.
**Conclusions:** None of the patients given a diagnosis of AIH with persistent AMA developed histological clinical, or biochemical features of PBC in long term follow up. Either this group of patients represents another subtype of AIH, i.e. AMA positive AIH, that responds well to corticosteroids, without concurrent or subsequent features of PBC or perhaps early treatment with high dose prednisone prevents the development of subsequent PBC in patients with AIH who tested AMA.

### 1270 Increased Liver Cell Steatosis and Apoptosis Represent a Mechanism towards Increased Liver Injury in Experimental Acute Liver Failure

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**Background:** Liver cell steatosis seems to have a generally benign prognosis, either because most hepatocytes are not significantly damaged, or mechanisms that replace injured hepatocytes are induced. Apoptosis has been linked to liver cell depletion and

ensuing liver fibrosis. This study assesses the effect of liver cell steatosis and apoptosis on the disease severity, in an experimental model of acute liver failure.
**Design:** The study comprised 120 Wistar rats that received simultaneously allyl-alcohol (intraperitoneally 0.05ml/kg) and carbon tetrachloride (rhinogastric 1.9ml/kg). Rats were sacrificed 2, 4, 6, 12, 24, 48, 81, and 153 hr, after chemicals institution. SGPT values were measured in blood samples. Steatosis (% of hepatocytes affected) was graded as follows: 0 (<5%), 1 (5%-30%), 2 (31%-70%), 3 (>70%). Liver tissues were evaluated for **I)** Bax, Bcl-2, TNFα, and caspase-3 mRNA (RT-PCR) and protein (Western blot), **II)** immunohistochemical expression of antigens CK7, HEPAR and **III)** apoptosis (TUNEL method). Results were expressed following computerized analysis.
**Results:** The death rate of animals was 80% within 48 hrs after chemical institution. Liver sections developed areas of steatosis and areas of combined (periportal+pericentral) parenchymal necrosis; the latter developed at 2 hr peaking at 48 hr. Liver regeneration originated from zone 2 and accomplished (at 153 hr) mainly by non-necrotic mature hepatocyte proliferation; most of them were HEPAR+ and less CK7+. Apoptosis reached the peak at 48 hr. TUNEL stain revealed the presence of apoptotic bodies within steatotic hepatocytes. A direct correlation between liver cell apoptosis and degree of steatosis was recorded (r =0.58, p=0.004). Increased steatosis was associated with: **I)** decreased Bcl-2 mRNA levels (r =-0.34 p=0.032), **II)** increased Bax/Bcl-2 mRNA and protein ratio (r=0.63 and 0.74 p=0.0038 and 0.0024), **III)** active caspase-3 (r=0.64, p=0.0041). In addition a direct correlation was revealed between TNFα levels and active caspase-3 (r=0.68, p=0.0027).
**Conclusions:** In cases of experimental acute liver failure, liver cell steatosis and apoptosis may contribute to hepatocytic injury. Further research is warranted in order to clarify the molecular pathways responsible for the proapoptotic effect of steatosis and whether this increase in apoptosis contributes directly to progression of liver injury in cases of acute liver failure.

### 1271 Increased Bax/Bcl-2 Ratio Upregulates Caspase-3 and Increases Apoptosis in Experimental Fatty Liver Disease

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**Background:** Apoptosis has been linked to liver cell depletion however, its regulating mechanisms have not been fully elucidated. Bax/Bcl-2 ratio has been considered as the best regulator of apoptosis. This study investigates the alterations of bax/bcl-2 ratio in relation to changes in the apoptosis co-ordination enzyme, caspase-3, in experimental non-alcoholic (NAFLD) and alcoholic (AFLD) fatty liver disease.
**Design:** The study included 50 male C57Bl/6 wild type mice aged 7-8 w: 25 mice were ob/ob (group A-NAFLD) and 25 their lean littermates (group B-AFLD). Twenty mice were used as controls. In group A, mice received standard mouse chow ad libitum. In group B, mice were fed with liquid diets containing ethanol, for 5 w. Ethanol concentration was gradually increased by 1%, every 3 days, until reaching 4% ethanol (vol/vol). Controls were fed with equal volumes of similar liquid diet in which dextrin substituted ethanol. All mice were sacrificed at 5 w. SGPT values were measured in blood samples. Liver tissues were evaluated for **a)** bax and bcl-2 mRNA (semiquantitative real time PCR assay), **b)** bax and bcl-2 protein levels (Western blot), and distribution (immunohistochemistry), **c)** caspase-3 activity (substrate cleavage assay), and **d)** apoptosis [apoptotic index(AI)-TUNEL method]. Results were expressed following image analysis and the bax/bcl-2 ratio was calculated.
**Results:** Mice of groups A and B, displayed higher SGPT values compared to controls (p<0.001). In groups A and B, a significant increase of bax mRNA and decrease of bcl-2 mRNA, compared to controls, was recorded (group A: +253% and -65%, group B: +234% and -77%). Similar results were observed for bax and bcl-2 proteins. Also, the bax/bcl-2 ratio was increased in groups A and B compared to controls (A: +201% and B:+197%). In addition, AI was higher in groups A and B compared to controls (p<0.001). No significant differences regarding the bax or bcl-2 levels, bax/bcl-2 ratios and AI were observed between groups A and B. In mice with NAFLD and AFLD, bax/bcl-2 ratios were correlated with *up-regulated caspase-3 activity* (r=0.741 and 0.621-p<0.01), *apoptosis* (r=0.567 and 0.359 p<0.01 and p<0.05), and *SGPT values* (r=0.581 and r=0.612, p<0.01)
**Conclusions:** This study provides evidence that the changes in the bax/bcl-2 ratio may contribute to caspase-3 activation and increase of liver apoptosis in experimental fatty liver disease. These results may have prognostic and therapeutic implications in fatty liver disease.

### 1272 Resistance of Mice Fed Methionine and Choline Deficient Diet to Acetaminophen Hepatotoxicity

*P Kashireddy, MS Rao.* Feinberg School of Medicine, Northwestern University, Chicago, IL.
**Background:** Acetaminophen (AP) is a widely used analgesic that can cause severe liver injury when ingested at higher doses. The mechanism of AP-induced hepatotoxicity remains unclear or controversial. The hepatotoxic dose of AP is quite variable and unpredictable between individuals and is influenced by several factors (bioactivation, detoxification, GSH depletion, protein adducts etc.). Chronic alcohol consumption, a common cause of steatohepatitis (ASH), is considered as a risk factor for AP toxicity. Alcohol mediated AP hepatotoxicity appears to be mediated through induction of cytochrome P-450 enzymes. However, the effect of nonalcoholic steatohepatitis (NASH), a common liver disease in the Unites States, on AP-induced hepatotoxicity is not studied. Here, we investigated the hepatotoxicity of AP in mice treated with methionine and choline deficient diet (MCDD), an experimental model of steatohepatitis, very similar to that of NASH and ASH in humans both morphologically and biochemically.
**Design:** C57BL/6 male mice aged 8 to 10 weeks were fed MCDD (10 mice) or normal chow (8 mice) for 4 weeks. 24 hours before sacrifice 6 mice from MCDD group and 4 from normal chow group were given AP, ip, at a dose of 500mg/ Kg body weight. AP hepatotoxicity was evaluated by measuring serum alanine transaminase (ALT) and histological examination of liver.
**Results:** In mice fed normal chow and treated with AP the serum ALT levels were 30 fold higher (compared to control animals on normal chow) and livers showed extensive centrilobular necrosis. Livers of mice fed MCDD and treated with AP or MCDD alone showed only steatohepatitis without any centrilobular necrosis. Serum ALT levels in both these groups were only 2.5 to 3 fold higher compared to control mice.
**Conclusions:** Results of these experiments indicate that mice with MCDD-induced steatohepatitis are resistant to the hepatotoxic effect of acetaminophen. The protective effect of MCDD against AP-induced toxicity may be due to inhibition of cytochrome P-450 enzymes that metabolize AP to N-acetyl-p-benzoquinone (NAPQI), a reactive quinone.

### 1273 Telomere Signals in Genotoxic and Nongenotoxic Carcinogen-Induced Hepatocellular Carcinomas in Rat: Evaluation by Quantitative Fluorescence In Situ Hybridization

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**Background:** Telomere dysfunction is probably an important mechanism for the generation of chromosomal instability, a common finding in many types of malignant tumors. It is not known whether telomere dysfunction is dependent on the causal factor or common to all tumors irrespective of etiology. To resolve the issue of the role of etiological agent (causal factor) in causing telomere dysfunction, we have examined hepatocellular carcinomas (HCC) induced in rats by aflatoxin B1, a genotoxic carcinogen, and ciprofibrate, a non-genotoxic carcinogen that induces liver tumors through oxidative stress, for telomere shortening (telomere dysfunction) using quantitative fluorescence in situ hybridization procedure.
**Design:** HCC were induced in male F-344 rats by feeding a diet containing aflatoxin (2mg/Kg diet) for 50 weeks or ciprofibrate (250mg/Kg diet) for 60 weeks. Formalin fixed paraffin sections of 10 HCC from aflatoxin treated rats and 7 HCC and adjacent nontumorous liver from ciprofibrate treated rats were hybridized with a Cy3-labeled telomere-specific peptide nucleic acid probe, and counterstained with 4-6-diamidino-2-phenylindole. Visualization and photography were performed using Zeiss axiophot epifluorescence microsope. Images were taken at x100 magnification and photographs were prepared with the use of photoshop 7.0. Telomere signals were counted in 20-25 nuclei of tumor cells (each tumor) and normal liver (5 specimens).
**Results:** Telomere flourescence signals were easily identified as discrete red spots within each nucleus. The mean number of telomere signals in the nuclei of non-tumorous liver was 17 (range 15-22). The mean number of telomere signals in aflatoxin and ciprofibrate-induced tumors was 1 (range 0-3.6) and 0.4 (range 0-2.1), respectively (significantly less than in non-tumorous liver).
**Conclusions:** Aflatoxin-induced HCC are morphologically higher grade compared to ciprofibrate-induced tumors. Phenotypic properties of HCC-induced by these 2 groups of carcinogens are also different. In spite of morphological and phenotypic differences between HCC induced by aflatoxin and ciprofibrate, telomere shortening is similar in these tumors. The results presented here indicate that telomere shortening is common to HCC-induced by both genotoxic and non-genotoxic carcinogens.

### 1274 Intraductal Tubular Carcinoma of the Bile Ducts

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**Background:** Although most carcinomas of the bile ducts are predominantly invasive, some have an exophytic pattern within the bile ducts; these intraductal papillary carcinomas (papillary cholangiocarcinomas) usually have well-formed papillae at the microscopic level. We describe a distinct type of intraductal carcinoma of the bile ducts with a predominantly tubular growth pattern.
**Design:** Eight cases of intraductal carcinoma involving the bile ducts were identified in our pathology files (1983-2005). We studied clinical presentations, histologic and immunohistochemical (in 7 cases) features, and clinical follow-ups.
**Results:** Patients (3 M and 5 F, 38 to 78 yrs), presented with obstructive jaundice or abdominal pain. Six of the patients underwent a partial hepatectomy; two underwent a laparoscopic bile duct excision, followed in one by a pancreatoduodenectomy. The tumors measured 0.7-8.0 cm and were grossly intraductal in 6/8 cases. The intraductal (intrahepatic in 7/8 cases, CBD in 1) portions of the tumors were densely cellular and composed of back-to-back tubular glands and solid sheets with minimal papillary architecture. The cells were cuboidal to columnar with mild-moderate cytologic atypia. Foci of necrosis were present in some cases. An extraductal invasive component was present in 2/6 cases with insufficient resected bile duct wall for evaluation; the invasive component composed less than 25% of the tumor in 3 cases, and more than 75% in one. The immunohistochemical features were as shown below:

| case / marker | MUC1 | CA19-9 | Smad4 | CK19 | CEA | B72.3 | HePar-1, MUC5AC, MUC2, p53, Synaptophysin, Chromogranin, CA125 |
|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | - | - | - |
| 2 | + | + | + | + | - | - | - |
| 3 | - | + | weak + | + | - | - | - |
| 4 | + | focal + | + | + | - | - | - |
| 5 | - | + | + | + | focal + | weak + | - |
| 6 | + | - | + | + | - | + | - |
| 7 | + | focal + | weak + | + | - | + | - |

Four patients were free of tumor recurrence after 4-21 mos. One patient presented with metastases but was stable with tumor regression after 4 mos. of chemotherapy. One patient developed lung metastases 60 mos after surgery, and one had recurrence in the distal bile duct at 48 mos followed by distant metastases 24 mos later. Clinical follow-up could not be obtained for one of the patients. All patients with metastases had invasive carcinoma.

**Conclusions:** Intraductal tubular carcinoma of the bile ducts is likely a variant of papillary cholangiocarcinoma with a distinct histologic pattern resembling a malignant counterpart of pyloric gland adenoma. Immunohistochemical features are similar to other pancreatobiliary-type carcinomas. This tumor may be hard to recognize as intraductal. When the tumor is entirely intraductal, the outcome appears to be favorable, but metastases can occur when invasive, even after many years.

### 1275 MUC6 Expression Distinguishes Oncocytic and Pancreatobiliary Type from Intestinal Type Papillae in Pancreatic Neoplasia: Delineation of a Pyloro-Pancreatic Pathway

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**Background:** The differential expression of MUC proteins has been instrumental in determining the direction of differentiation in pancreatic neoplasia, and has helped identify distinct pathways of carcinogenesis. The expression profile of MUC6 ("pyloric" type mucin, which is known to be up-regulated by various signaling pathways, in particular NFkappa-b) in pancreatic neoplasia, especially in the subtypes of intraductal and cystic mucinous neoplasia is not known.

**Design:** A panel of pancreatic neoplasms including intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms (MCNs) and infiltrating ductal adenocarcinomas was immunolabeled using an antibody to MUC6, and the percent of cells labeling was classified as 0-negative, minimal (<10%), low (10-49), moderate (50-80) and diffuse (>80%).

**Results:** In normal tissue, MUC6 was expressed in intercalated ducts of the pancreas, but not in the larger ducts or islets. Focal granular labeling was noted in some acini. Among non-invasive IPMNs and MCNs, MUC6 expression was detected consistently in flat (non-papillary) mucinous areas without any atypia but disappeared once the cells became more proliferative and stratified. In the papillary components of non-invasive IPMNs, MUC6 expression was highly specific for oncocytic type of epithelium (diffuse in 8/9 cases) and pancreatobiliary type (low or moderate in 5/5), whereas it was rarely expressed in intestinal type papilla (minimal in 1/9). Interestingly, the papillary nodules in mucinous cystic neoplasia (MCNs) were also mostly negative (minimal in 1/7). MUC6 was uncommon in invasive carcinomas: 39/112 conventional ductal adenocarcinoma showed expression (low-15, moderate-13, diffuse-11). Only 1/10 colloid type invasive carcinomas showed expression.

**Conclusions:** The preferential expression of MUC6 in oncocytic and pancreatobiliary but not intestinal type papillae of IPMNs confirms the presence of a (pyloro-pancreatic) pathway distinct from the MUC2/CDX2 expressing intestinal pathway in intraductal neoplasia. Lack of MUC6 expression in the papillary nodules of MCNs, despite their pancreatobiliary appearance, is intriguing.

### 1276 Loss of Rap1GAP Expression in Pancreatic Ductal Adenocarcinoma and Its Association with Decreased Survival

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**Background:** Rap1-specific GTPase-activating protein (rap1GAP) is significantly downregulated in pancreatic cancer and is a candidate tumor suppressor gene located on chromosome 1p35-36. We hypothesized that loss of rap1GAP correlated with negative histological parameters and decreased patient survival.

**Design:** Immunohistochemical staining for Rap1GAP was performed in 112 cases of conventional pancreatic ductal adenocarcinoma (PDA). Based on the degree of expression level (calculated by an established scoring system incorporating the percentage of + labeled carcinoma cells and the intensity of labeling), each case was assigned to one of 4 categories: 0-none, 1-low, 2-moderate, and 3-significant. Expression levels were correlated with the archival data available on some patients on DPC4, kras, p53, p21, p27, and Fas ligand expression as well as clinical data including patient survival.

**Results:** Rap1GAP was normally expressed in all components of normal pancreas. A decrease in the expression was noted in 75% of PDA cases: The expression was lost or low in 60, moderate in 13, and normal in 25 cases. The expression tended to be higher in patients <50 yrs old (average IER 1.85 *vs.* 1.40), in smokers (1.71 *vs.* 1.21), carcinomas in the head (1.55 *vs.* 1.21 in tail) and those with no family history of pancreatic cancer (2.0 *vs.* 1.62). No significant correlation was identified with expression and tumor size (> vs. <2cm: 1.42 *vs.* 1.62) or lymph node status (pos *vs.* neg: 1.48 *vs.* 1.45). With Spearman rank correlation test p27 and Rap1GAP expression had a significant correlation (p=0.05). When Kaplan-Maier survival curves were analyzed, the most significant grouping was between 0 (no expression) vs. 1, 2, or 3 expressors of Rap1GAP. In univariate analysis, loss of expression was associated with a shorter survival (p=0.003). In a Cox-proportional Hazard model incorporating tumor stage, grade, lymph node status and Rap1 GAP, the significance of Rap1GAP expression persisted (p=0.03).

**Conclusions:** Decreased or loss of Rap1-GAP expression correlated with poor histological parameters and decreased survival in patients with PDA, supporting the role of Rap1GAP as a functionally important tumor suppressor gene in pancreatic cancer.

### 1277 BRAF and KRAS Mutations in Intrahepatic and Extrahepatic Cholangiocarcinoma

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**Background:** The RAS/RAF/ERK signaling pathway is an important mediator of cellular growth and malignant transformation. The association of KRAS mutation and the biliary tract carcinogenesis has been well documented, but the role of BRAF mutation in biliary tract carcinoma has not been well established. For the understanding of the oncogenesis, KRAS and BRAF mutation assessment should be performed altogether. Our purpose is analyzing the role of RAS/RAF/ERK pathway in cholangiocarcinoma (CC)s and differences in carcinogenesis of intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcionmas (ECC).

**Design:** We obtained DNA samples from tumor tissues in paraffin blocks, each of 69 ICCs and 65 ECCs. BRAF and KRAS mutation was evaluated by enriched PCR /RFLP analysis and sequencing. Mutation analysis was carried out on the BRAF codon 600 and KRAS codon 12 and 13, the most frequent sites of mutation.

**Results:** Mutations of KRAS codon 12 and 13 were identified in 10 (14%) and 13 (19%) of 69 ICCs, respectively, while those were found in 6 (9%) and 7 (11%) of 65 ECCs, respectively. BRAF codon 600 mutation was identified in 18 (26%) of ICCs and 16 (25%) of ECCs. BRAF and KRAS mutations were not seen in the same specimen. We failed to observe a correlation between BRAF or KRAS mutations and histopathological factors or prognosis of patients.

**Conclusions:** KRAS and BRAF mutations are relatively common in CCs and show no significant difference in intrahepatic and extrahepatic CCs. As the whole, the disruption of RAS/RAF/ERK pathway plays an important role in the carcinogenesis of CC.

### 1278 Is In-Situ Hybridization Testing Helpful in Detecting Hepatitis C Recurrence in Liver Transplant Patients?

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**Background:** Recurrent hepatitis C(HCV) is almost universal after liver transplantation(LT), confirmed by demonstrating viremia using polymerase chain reaction(PCR). We studied the utility of in-situ hybridization for HCV(HCV ISH) on posttransplant liver biopsies (LBxs) by analyzing histologic and clinical F/U data.

**Design:** 21 patients (18M,3F; 39-70yrs) receiving LT for HCV were selected for HCV ISH due to histologic hepatitis without viremia, or hepatic dysfunction of uncertain cause. These were considered index biopsies (IB). 5 had been retransplanted (24-260 wks) due to recurrent HCV(3), chronic rejection(1), and cryptogenic hepatitis(1). F/U LBxs in 10 patients were also studied. Grading and staging for hepatitis was on a scale of 0-4. HCV ISH was performed using sense and antisense probes, with controls. Patient charts were reviewed for clinical information.

**Results:** HCV ISH was performed on 21 IBs obtained at 1-327 wks from LT. ISH was positive in 11 (52%), negative in 8 (38%) and equivocal in 2 (9.5%). Positive IBs: 10 patients had negative viremia and 6 were on interferon(IFN) at the time of IB. All 11 LBxs showed hepatitis (mean grade/stage 1.7/2.0). 7 patients had F/U serum and histologic data. All had negative viremia at 17-79 wks from IB. 5 remained on IFN. Repeat LBxs (23-85 wks) all had persistent hepatitis (mean grade/stage 1.6/1.6). HCV ISH was performed on 5/7: 3 positive, 1 negative and 1 equivocal. Negative IBs: 6 patients at IB had negative viremia, 3 were on IFN. 4 patients were tested for viremia at 12-22 wks from IB; 1 remained negative, 1 remained positive and 2 converted from negative to positive. 5/8 IBs showed hepatitis (mean grade/stage 1.4/1.0); 3 had mild acute rejection and were not graded or staged. 1(of 8) patient had F/U LBxs demonstrating grade 2/stage 0 hepatitis in both F/U LBxs. ISH was not performed on these LBxs. Equivocal IBs: Both patients had negative viremia at IB and F/U. IBs showed hepatitis (mean grade/stage 2/2), and F/U LBxs revealed persistent hepatitis (mean grade/stage 2/2.3), at 52 and 58 wks. Both follow-up LBxs were tested with HCV ISH, and 1 was positive.

**Conclusions:** 1) HCV detected by ISH may not correlate with viremia. 2) The impact of IFN therapy on HCV ISH is unclear. 3) Nonviremic patients with positive HCV ISH and IFN treatment do not appear to have progressive hepatitis. 3) The role of HCV ISH in diagnosis and management of recurrent HCV needs further study.

### 1279 Galectin-1 as a Pancreatic Cancer Biomarker: Proteomics to Tissue Microarray Validation

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**Background:** Early detection biomarkers for pancreatic cancer are greatly needed to to improve patient survival, which currently is abysmally low for this disease.

**Design:** In the search for such early biomarkers, we performed quantitative proteomic profiling followed by tissue microarray (TMA) immunohistochemical validation of candidate markers. Proteomics was performed using isotope-code affinity tag (ICAT) technology and tandem mass spectrometry on pancreatic tissue from patients with pancreatic cancer vs. pancreatitis and normal pancreatic controls. The microarray was constructed from 106 patients, including 43 sporadic pancreatic ductal adenocarcinomas, 30 with primary (1º) chronic pancreatitis without cancer, and 33 normal pancreatic controls. Immunohistochemistry (IHC) was performed using galectin-1 polyclonal antibody (1:100 dilution; Fitzgerald Industries International, Concord, MA) and heat-induced antigen retrieval. Staining intensity was analyzed semiquantitatively from 0 to 3+.

**Results:** Galectin-1, among other peptides, displayed marked and distinctive over-expression by quantitative proteomic analysis in pancreatic cancer relative to controls. IHC validation demonstrated that only pancreatic stroma stained by galectin-1 and that there was marked over expression in stroma from pancreatic ductal cancer patients relative to controls (see table). Using the proportional odds model, the three states are significantly different with respect to the intensity of staining ($p < 0.0001$).

**Conclusions:** Galectin-1 is a galactoside-binding protein previously reported to yield strong immunoreactivity in cancer-associated extracellular matrix and fibroblasts, as is further confirmed by these results. We demonstrate galectin-1 overexpression in pancreatic stroma accompanying ductal adenocarcinoma, relative to controls, by ICAT-tandem mass spectrometry quantitative proteomics followed by TMA immunohistochemical validation ($p < 0.0001$). Galectin-1 is therefore a promising biomarker candidate for pancreatic cancer.

Galectin-1 TMA IHC on Pancreatic Stroma

| Diagnosis | Normal Pancreas Control | 1º Chronic Pancreatitis Control | Pancreatic Adenocarcinoma |
|---|---|---|---|
| # of patients | 33 | 30 | 43 |
| 0 no staining | 6 (18%) | 2 (7%) | 0 |
| 1+ mild | 27 (82%) | 15 (50%) | 2 (5%) |
| 2+ moderate | 0 | 10 (33%) | 9 (21%) |
| 3+ marked | 0 | 3 (10%) | 32 (74%) |

### 1280 Prevalence and Determinants of Gallbladder Neoplasia in Patients Undergoing Liver Transplantation for Primary Sclerosing Cholangitis

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**Background:** Primary sclerosing cholangitis (PSC) is associated with an increased frequency (10-20%) of hepatobiliary malignancy, especially cholangiocarcinoma. Dysplasia, neoplastic polyps, and carcinoma of the gallbladder have been described in PSC but appear to be less common than bile duct carcinomas. However, the prevalence and risk factors for gallbladder neoplasia among PSC patients undergoing orthotopic liver transplantation (OLT) have not been well-studied.

**Design:** Among 78 consecutive liver explants for PSC, there were 54 patients who had not undergone prior cholecystectomy and these gallbladders were submitted in their entirety for histologic examination. We evaluated the following histologic features: presence of diffuse lymphoplasmacytic chronic cholecystitis; pyloric metaplasia; intestinal metaplasia; dysplasia (low- or high-grade); and adenocarcinoma. Gallbladder dysplasia and carcinoma were correlated with several clinicopathologic parameters using Fisher exact test and t-test, including: 1) gender; 2) age; 3) PSC duration; 4) inflammatory bowel disease at time of OLT; 5) concomitant intra- or extrahepatic biliary dysplasia/carcinoma.

**Results:**

Histologic abnormalities of 54 gallbladders in end-stage PSC

| | Number (%) |
|---|---|
| Lymphoplasmacytic chronic cholecystitis | 25 (46%) |
| Pyloric metaplasia | 52 (96%) |
| Intestinal metaplasia | 26 (48%) |
| Mucosal dysplasia | 20 (37%) |
| Low grade dysplasia | 8 (15%) |
| High grade dysplasia | 12 (22%) |
| Adenocarcinoma | 8 (15%) |
| Invasion into lamina propria | 2 (4%) |
| Muscle or adventitia invasion | 6 (11%) |

Clinicopathologic correlates with gallbladder neoplasia

| | Gallbladder carcinoma | Gallbladder dysplasia |
|---|---|---|
| Gender | NS | NS |
| Duration of PSC | NS | NS |
| Older age at transplant | NS | p<0.05 |
| Inflammatory bowel disease | NS | p=0.01 |
| Intrahepatic biliary dysplasia | p=0.0015 | p<0.0001 |
| Cholangiocarcinoma* | p=0.009 | p=0.005 |

*Cholangiocarcinoma present at explant or history of chemoradiation for cholangiocarcinoma pre-transplant. NS, not significant

**Conclusions:** Complete histologic evaluation of gallbladders in patients undergoing liver transplantation for PSC yields a high frequency of inflammatory, metaplastic, and neoplastic changes. Both older age at transplant and presence of inflammatory bowel disease were weakly correlated with gallbladder dysplasia. Mucosal dysplasia and adenocarcinoma of the gallbladder were both significantly correlated with intrahepatic bile duct dysplasia and with cholangiocarcinoma, supporting the concept of a "field effect" in the extra- and intrahepatic biliary tract in PSC.

### 1281 Characterization of Biliary Dysplasia and Metaplasia in Liver Explants Removed for Primary Sclerosing Cholangitis

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**Background:** Patients with primary sclerosing cholangitis (PSC) are at increased risk for developing cholangiocarcinoma (CCA). However, the prevalence of biliary dysplasia among patients undergoing orthotopic liver transplantation (OLT) for PSC is incompletely defined. In addition, there are no large studies which compare the frequency of dysplasia among PSC cases removed for CCA with those removed for nonneoplastic conditions.

**Design:** We evaluated 78 consecutive PSC liver explants by randomly sampling the intrahepatic biliary tree, with a mean number of 20 sections per case (range 12-41). The following histologic features were evaluated: biliary dysplasia (low or high grade); intestinal metaplasia; pyloric metaplasia; and mucinous metaplasia. Using Fisher's exact test and t-test, we correlated these features with the presence or absence of CCA and with the following clinicopathologic parameters: gender; age; PSC duration; and inflammatory bowel disease (IBD) at time of OLT.

**Results:** Among PSC OLT explants, 24 (31%) were removed for/contained cholangiocarcinoma (CCA), while 54 (69%) were removed for other reasons (non-CCA). Dysplasia was identified in 20 (83%) of CCA cases and 17 (31%) of non-CCA cases (p=0.000024). Among livers with dysplasia, papillary dysplasia was seen in 8 (40%) of CCA and 5 (29%) of non-CCA (p=0.73). In 6 of 78 cases (8%) there was extensive papillary dysplasia resembling pancreatic intraductal papillary neoplasms (IPMN). Intestinal metaplasia was the only metaplastic lesion seen more commonly in CCA than non-CCA cases (p=0.011). There was no statistically significant difference between the CCA and non-CCA groups with respect to gender, duration of PSC, age at transplant, and IBD status.

Histologic abnormalities in 78 PSC liver explants

| Histologic feature | Carcinoma N=24 Number (%) | Non-carcinoma N=54 Number (%) |
|---|---|---|
| Biliary dysplasia | 20 (83) | 17 (31) |
| Low grade | 17 (71) | 17 (31) |
| High grade | 15 (63) | 6 (11) |
| Intestinal metaplasia | 11 (46) | 9 (17) |
| Pyloric metaplasia | 17 (71) | 40 (74) |
| Mucinous metaplasia | 18 (75) | 42 (78) |

**Conclusions:** Livers removed secondary to complications of PSC often contain metaplastic and neoplastic biliary changes. These changes are not confined to livers explanted for CCA, but are also frequently found in those removed for other reasons. Papillary dysplasia, either alone or in conjunction with flat dysplasia, is a relatively common form of dysplasia seen in both groups. Intestinal metaplasia appears to be unique among metaplastic changes in that it is more commonly seen in the setting of CCA.

### 1282 Long Term Survival in Orthotopic Liver Transplant Patients with Hepatopulmonary Syndrome

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**Background:** Hepatopulomanry syndrome (HPS) is a rare syndrome defined as hypoxemia in patients with end stage liver disease (ESLD) resulting from intrapulmonary vasodilation. HPS is associated with poor prognosis and previously was considered as an absolute contraindication for orthotopic liver transplants ( OLT). This trend is changing in recent years. There are few studies which have evaluated factors associated with survival in patients of ESLD with HPS. The aim of this study is to determine long term outcome of patients and grafts for those with ESLD and HPS who underwent OLT.

**Design:** We identified 13 patients with HPS who had OLT based on the presence of intrapulmonary shunting and abnormal $PaO_2$ (<80 mmHg) and also included 43 age and disease matched OLT patients. Patient history, biopsies and outcomes were reviewed to determine the graft survival and patient outcome.

**Results:** The patients with HPS ranged from 35-68 years old (mean 55 yrs) and the age range of the control group were from 5-71 years (mean 52 yrs). The underlying diseases in patients with HPS included viral Hepatitis C (Hep C, n=5, 38.4%), steatohepatitis (n=3, 23%), cryptogenic (n=2, 15.3%), 1 each for viral Hepatitis B (Hep B, 7.6%), Primary Biliary Cirrhosis (PBC, 7.6%) and Alpha 1 Antitrypsin deficiency (7.6%). The underlying diseases in control group included Hep C (n=14, 32.5%), steatohepatitis (n=10, 23.2%), cryptogenic (n=8, 18.6%), Hep B (n=2, 4.7%), PBC (n=4, 9.3%), primary sclerosing cholangitis (n=2, 4.7%), and alpha 1 antitrypsin deficiency (n=3, 7.0%). The median survival (33 months vs. 34 months) and death within 3 months post transplant (7.6% vs. 7.0%) did not show significant differences between HPS and control groups (P>0.05). HPS resolved in all except 1 (7.6%) patient. Interestingly, a higher (P = 0.01) frequency of recurrent Hepatitis C with bridging fibrosis or cirrhosis was noted in 2/5 (40%) allografts within 1 year in patients with HPS in comparison to the control group in which 1 allograft (out of 14, 7.1%) developed recurrent Hepatitis C with cirrhosis. One of the two patients with advanced fibrosis in HPS group demonstrated features of fibrosing cholestatic hepatitis.

**Conclusions:** Patients with HPS could be transplanted with similar survival benefits as non HPS group. Hepatitis C patients with HPS may be associated with faster progression to advanced fibrosis in comparison to non HPS patients.

### 1283 Loss of Heterozygosity in Hepatocellular Neoplasia

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**Background:** The prognosis of hepatocellular carcinoma (HCC) is usually poor, with 3-year survival rates estimated at 44.9% after hepatic resection. Chromosomal losses have been identified using various technologies in 25-45% of patients. The loci most frequently involved are 1p, 4q, 5q, 6q, 8p, 9p, 13q, 16p, 16q, and 17p. Gains have also been described, occurring in 30-55% of the time at 1p, 6p, 8q, and 17q. There have been several studies suggesting that assessment of loss of heterozygosity (LOH) in HCC can assist in staging and prognosis for HCC. This study analyzed a set of well-characterized hepatocellular tumors for a panel of known tumor suppressor genes.

**Design:** Cases with diagnosis of either HCC or dysplasia were retrieved from the archives and histologic review confirmed the diagnosis in each case. Microdissection and DNA extraction were performed from paraffin embedded tissue. Seventeen polymorphic microsatellite loci (1p, 3p, 5q, 8p, 9p, 10q, 17p and 17q) were tested for loss of heterozygosity based on literature review. Semi-quantitative capillary electrophoresis was used to analyze LOH by comparing the ratios of peak heights between normal and tumor tissues in heterozygous cases

**Results:** Two cases of low-grade dysplasia, one case of multiple hepatocellular adenomas, four cases of well-differentiated HCC, and seven cases of moderately differentiated HCC were included in this study. The genes most frequently lost in the panel were 8p23, 9p21, and 3p26 (60%, 38%, and 27%, respectively). The average fractional allelic loss (FAL) was relatively low for the low-grade tumors (14.8%), and was higher for the moderately differentiated hepatocellular carcinomas (mean FAL of 36%)

**Conclusions:** It has been suggested that the Tumor-Node-Metastasis (TNM) staging system can be an inaccurate predictor of recurrence-free survival in patients with HCC and that genotyping may be of assistance in differentiating low-grade from high-grade tumors. Our results suggest that low- and high-grade tumors do have different genetic profiles and that this analysis may be of relevance in the workup of HCC.

### 1284 KOC (K Homology Domain Containing Protein Over-Expressed in Cancer) Distinguishes Metastatic Pancreatic Adenocarcinoma from Benign Ductal Lesions of the Liver

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**Background:** Patients with potentially resectable pancreatic adenocarcinoma usually undergo surgical exploration prior to definitive resection. These patients are often found to have incidental hepatic lesions, such as bile duct adenomas or hamartomas, that may mimic metastatic carcinoma, particularly when material is limited. KOC (K homology domain containing protein over-expressed in cancer), is an oncofetal RNA-binding protein that is highly expressed in pancreatic adenocarcinomas, but not in benign pancreatic ducts. The purpose of this study was to assess KOC immunoexpression among a series of benign biliary-type epithelial lesions of the liver in order to evaluate the utility of this marker in distinguishing these entities from metastatic pancreatic adenocarcinoma.

**Design:** Five micron tissue sections cut from 32 routinely processed liver biopsy specimens (10 bile duct hamartomas, 2 bile duct adenomas, 10 cirrhotic livers with exuberant ductular proliferations, 10 cases of metastatic pancreatic adenocarcinoma) were stained with a monoclonal antibody to KOC using standard techniques. For each case, the intensity of the staining reaction within the lesion was graded (absent, weak, moderate or strong) and recorded.

**Results:** KOC expression was present in 9/10 (90%) metastatic pancreatic carcinomas and was of moderate or strong intensity in 7/10 (70%) cases. In contrast, all of the benign ductular proliferations (n=22) were negative for KOC (Table 1).

**Conclusions:** KOC is an oncofetal protein that is expressed in the majority of pancreatic carcinomas (>90%), but not in benign biliary ductal tumors or non-neoplastic pancreaticobiliary ducts. Thus, this marker may be helpful in distinguishing metastatic pancreatic carcinoma from its potential mimics in liver biopsy specimens.

Table 1: KOC Immunoexpression Among Benign Biliary-Type Lesions of the Liver and Metastatic Pancreatic Carcinoma

| | Staining Intensity | | | |
|---|---|---|---|---|
| Lesion | Strong | Moderate | Weak | Absent |
| Pancreatic Carcinoma | 3/10 (30%) | 4/10 (40%) | 2/10 (20%) | 1/10 (10%) |
| Bile Duct Hamartoma | 0/10 (0%) | 0/10 (0%) | 0/10 (0%) | 10/10 (100%) |
| Bile Duct Adenoma | 0/2 (0%) | 0/2 (0%) | 0/2 (0%) | 2/2 (100%) |
| Proliferating Ductules in Cirrhosis | 0/10 (0%) | 0/10 (0%) | 0/10 (0%) | 10/10 (100%) |

### 1285 Zone 3 Hepatocyte Giant Cell Transformation in the Setting of HCV/HIV Coinfection

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**Background:** Syncytial giant cell change has been reported in a number of organ systems in the setting of viral infections, such as HIV. We report a unique giant cell transformation of zone 3 hepatocytes in the setting of chronic Hepatitis C/HIV co-infection, and less commonly in HCV infection alone.

**Design:** The prevalence of hepatocyte giant cell transformation was determined in liver biopsies performed for clinical management of chronic HCV infection. All cases were needle core biopsies performed between 2001-2005. Clinical and laboratory data were reviewed. Biopsies were reviewed and graded and staged with the ISHAK scoring system. In addition, four cases were immunostained for Ki67.

**Results:** Amongst 856 liver biopsies preformed for clinical management of HCV, 22 biopsies (2.6%) showed giant cell transformation, representing 18 individuals. The median ALT was 37 IU, the median AST 49 IU, and the median alkaline phosphatase was 97 IU. Eleven cases had HCV RNA loads available, with a median HCV RNA of 334,00 copies per ml. Twelve of eighteen individuals (67%) were also HIV positive. Histologically, the findings were not that of giant cell hepatitis of the more usual sort. Giant cell changes were found exclusively in zone 3 hepatocytes and the accompanying histological findings were otherwise typical of chronic HCV. Giant cell changes were focal in 19/21 cases and diffuse in 2 biopsies. Giant cell change was characterized by multinucleated hepatocytes (3 or more nuclei) with a gray amorphic cytoplasmic change that resembled smooth endoplasmic reticulum proliferation. The nuclei were all peripherially located. Most cases had mild inflammation and fibrosis (median ISHAK grade 3, median ISHAK stage 1). Ki-67 immunolabeling showed minimal hepatocyte cycling in all cases and was negative in giant cells. Five individuals (4 HIV positive) had follow-up biopsies and all continued to have giant cell change. Two of the 18 individuals had previous biopsies, which did not show giant cell transformation. Most individuals were on multiple anti-viral medications, but there was no correlation with any single medication.

**Conclusions:** Giant cell transformation occurs most commonly in the setting of HCV/HIV co-infection, but can also rarely be seen in chronic HCV infection alone. This change is seen in zone 3 hepatocytes, can be persistent, and does not correlate with the level of ISHAK grade or stage, or laboratory values.

### 1286 Mucinous Cystic Neoplasm: Unique Ovarian Stroma but Heterogeneous Epithelial Lining

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**Background:** Pancreatic mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm of branch duct variant are both peripheral cystic lesions lined by a mucinous columnar epithelium. However, the presence of an ovarian stroma and the lack of communication with the main duct are discriminators of MCNs. However, the morphologic and immunophenotypic characteristics of the epithelium have not been fully investigated.

**Design:** The study group consisted of 25 MCNs (20 adenomas and 5 adenoma with focal borderline malignancy) resected in 23 women and 2 men (mean age 47.6 yr., range 23 - 79). The lack of communication with the main duct and the ovarian stroma was confirmed in all cases. The lining epithelial cells were classified as cuboidal cells (CCs), low mucinous columnar cells (LCs) and tall mucinous columnar cells (TCs). The distribution and extent of these cell types as well as the presence of pyloric gland-like structure (PG) and goblet cell (GC) were evaluated. The expression of MUC1, MUC2, MUC5AC, MUC6 and CDX-2 was also examined by immunohistochemistry and correlated with the epithelial morphology.

**Results:** All but one MCN which was completely lined by CCs, showed more than one epithelial type. In 11 cases, the various epithelial components were evenly represented. CCs and LCs were predominant in 6 cases and TCs in 7. Sixteen MCNs showed PGs, which were prominent in 6. Scattered GCs were noted in 15 cases. Reflecting the epithelial morphology, the expression of mucins was also heterogeneous.

| | MUC Immunophenotype | | | | |
|---|---|---|---|---|---|
| | MUC1 (apical) | MUC2 | MUC5AC | MUC6 | CDX2 |
| Cell Type | CC, LC | GC | LC, TC, PG | CC, LC, TC, PG | all |
| Focal (n) | 9 | 6 | 10 | 13 | 5 |
| Diffuse (n) | 10 | 0 | 9 | 8 | 4 |

**Conclusions:** The epithelial cells of MCNs are morphologically and immunophenotypically heterogeneous. Notably, MUC1 does not correlate with an invasive phenotype. Also, most MCNs are almost invariably positive for MUC5AC and MUC6. Interestingly, 36% of MCNs display a intestinal phenotype as evidenced by CDX2 positivity. These features are also markedly different from the reported immunophenotype of IPMN.

### 1287 EphA2 Protein Expression in Pancreatic Carcinoma Correlates with Invasive Potential, Tumor Differentiation and Organ Site of Metastasis

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**Background:** EphA2 is a transmembrane receptor tyrosine kinase that functions in the regulation of cell growth, survival, angiogenesis, and migration. In pancreatic cancer cells, EphA2 overexpression has been correlated with increased invasive and metastatic ability. However, the patterns of EphA2 expression in the normal pancreas, pancreatic intraepithelial neoplasia (PanIN), primary infiltrating and metastatic pancreatic cancer tissues is unknown.

**Design:** Clinicopathologic data and paraffin-embedded materials from 143 patients with primary (102 specimens) and/or metastatic pancreatic cancer (373 specimens) were collected from the Surgical and Autopsy Pathology Files or the Gastrointestinal Cancer Rapid Medical Donation Program (GICRMDP). Immunohistochemical labeling for EphA2 protein was performed using anti-EphA2 mAb (Santa Cruz, clone H-77)) at a 1:25 dilution following standard techniques. Slides were scored by both authors.

**Results:** Review of the histopathology of each patient's material revealed that 139 were conventional infiltrating duct adenocarcinoma, and 4 were adenocarcinomas arising in an IPMN. EphA2 protein immunolabeling of any intensity was detected in 16 of 20 normal ducts, in 9 of 10 PanINs, in 102 of 103 primary cancers and in 360 of 373 metastases. The expression was predominantly cytoplasmic, with membranous staining also observed in some cases. When evaluated related to labeling intensity, primary carcinomas were more strongly positive compared to normal ducts ($p<0.02$) and PanIN lesions ($p<0.003$), poorly differentiated carcinomas were more strongly positive for EphA2 than well-and moderately-differentiated tumors ($p<0.02$), and non-mucinous carcinomas were more strongly positive than mucinous carcinomas ($p<0.03$). Overall, EphA2 expression was present in more primary cancers than the metastases ($p<0.03$), although EphA2 labeling in pancreatic cancers with strong EphA2 expression was maintained in their matched metastases ($p<0.003$). An exception was liver and soft tissue metastases that showed less labeling than for primary carcinomas ($p<0.05$ and $p<0.02$, respectively) as compared to other organ sites.

**Conclusions:** Our data indicate that EphA2 overexpression correlates with invasive disease, with the greatest labeling seen in poorly differentiated or non-mucinous carcinomas with widespread metastases at autopsy. Differential expression in various metastases derived from the same primary carcinoma suggests a role for EphA2 in organ specific metastasis.

### 1288 Total Parenteral Nutrition Therapy and Liver Injury in Infants and Adults, a Comparative Study

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**Background:** Total parenteral nutrition (TPN) is a well-known cause of liver injury. The type and degree of histopathologic change vary widely. We describe the histologic changes of TPN associated liver injury in infants and adults, and determine whether there is correlation with various clinical studies.

**Design:** We performed a retrospective study of 126 patients who underwent biopsy or transplantation between 1/97 and 7/04 while on TPN. 37 cases were excluded due to underlying or comorbid conditions. There were 53 infants (<1 yr. at TPN start) and 36 patients >1 yr. at TPN start. Sections were evaluated and graded for the presence of portal and lobular inflammation, cholestasis, steatosis, apoptosis, ballooning/feathery changes, bile duct proliferation, ductopenia, portal and perivenular fibrosis. Clinical parameters including duration of TPN at biopsy and laboratory values were collected by chart review.

**Results:** Previously described TPN associated histologic findings were confirmed. Ductopenia was observed in 23% of infants and 25% of adults. Cholestasis was seen in 91% of infants and 67% of adults; steatosis was seen in 26% of infants and 58% of adults ($p=0.01$). Severe ballooning/feathery change was seen in 28% of infants but no adults. Extensive hepatocellular apoptosis was seen in 21% of infants but only 0.03% of adults ($p=0.02$). Severe perivenular fibrosis was seen in 17% of infants but only 0.06% of adults. 60% of infants developed stage 3 or 4 portal fibrosis compared to only 19% of adults. None of the patients who started therapy after one year developed cirrhosis

(p<0.001). The mean duration of TPN in months for stage 1, 2, 3, and 4 fibrosis was 0.2, 3, 6, and 6.4 for those biopsied <1 year of age and was 64, 41, 51, 56 for those biopsied >1 year of age (p=0.04). We found no correlation between biochemical studies and the degree of hepatocellular injury or fibrosis.

**Conclusions:** Infants are much more susceptible to TPN related hepatocellular injury, are more likely to develop fibrosis, and progress to high stage fibrosis more rapidly than adults. Ductopenia, a previously unreported finding, is seen in both infants and adults. Perivenular fibrosis is seen in patients with high stage portal fibrosis. Cholestasis, although more common in infants, is the most common change in all age groups. Steatosis occurs less frequently than cholestasis and is more common in adults. Progression to fibrosis in infants may be dependent on the length of therapy. There is no correlation between biochemical markers and the biopsy estimates of liver injury.

### 1289 Clinicopathologic Review of Transplant Patients with Adenocarcinoma of the Biliary System

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**Background:** We undertook this study to define the clinicopathologic features and outcome of liver transplant patients with intra (ICC) and extrahepatic (ECC) cholangiocarcinoma and adenocarcinoma of the gallbladder (AGB) and to attempt to quantify the success of transplantation for these tumors.

**Design:** Cases were identified by a database search of explanted livers with the diagnosis of adenocarcinoma or cholangiocarcinoma. Available tissue slides were retrospectively reviewed for confirmation of diagnosis and determination of pathologic features. The clinical charts were also retrospectively reviewed for associated clinical conditions at the time of transplant and for tumor recurrence and outcome.

**Results:** A total of 20 cases were identified, 9 ICC, 6 ECC and 5 AGB. The male/female ratios were 5/4, 5/1 and 5/0 respectively. The age range(mean) was 40-60(49.9) for ICC, 25-57(43.5) for ECC, and 34-61(50.8) for AGB. 33.3% of ICC cases were associated with primary sclerosing cholangitis (PSC), vs. 66.7% of ECC and 80% of AGB. Prior to transplantation tumors were identified radiographically in 88% of ICC, 50% of ECC and 0% of AGB, although some gallbladder abnormality was described in 2 cases (40%). The range(mean) of tumor sizes were 1.4-7.5(4.2cm) for ICC, 0.9-8.0(4.6cm) for ECC and 1.6-9.0(4.0cm) for AGB. At transplant, 2 patients (22%) with ICC had lymph node metastasis. The same patients also had metastasis to the small bowel and peritoneum. One patient (17%) with ECC had LN metastasis and 2 others (33%) had metastasis to the pancreas and peritoneum. Only one patient (20%) with AGB had LN metastasis and none had other metastasis. 66 % of patients with ECC had pathologic stage of T3 and T4 vs. 20% of AGB and 11% of ICC. 3 patients (50%) with ECC died of disease recurrence at 11, 14 and 37mo after transplantation. One was alive with disease recurrence(18mo) and 2 with no disease (6, 11mo). One patient (11%) with ICC died of disease recurrence (9mo), one was alive with disease recurrence(35mo), and 7 were alive without disease (5-39mo). All patients with AGB were alive without disease 2-45mo after transplant.

**Conclusions:** 1) In this study, patients transplanted with ECC and AGB were more often also diagnosed with PSC than patients with ICC. 2) Patients with AGB are less likely to be recognized before transplantation. 3) Patients with ECC were transplanted with higher stage tumors and subsequently had a worse outcome than the other groups.

### 1290 Characterization of Peripheral Blood Lymphocyte Chimerism in Orthotopic Liver Transplant (OLTX) Recipients: Potentials for Early Detection of Graft Versus Host Disease (GVHD) in Liver Transplant Patients

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**Background:** GVHD following liver transplantation is a rare but potentially fatal occurrence. Early diagnosis is difficult to achieve. The "normal" persistence of "trafficing" donor lymphocytes in recipient peripheral blood (PB) for several weeks following OLTX makes it difficult to establish abnormal cut offs for post OLTX chimerism. This study aims to better characterize the "normal" chimerism expected post OLTX in order to facilitate early diagnosis of GVHD in such pts.

**Design:** Prospective donor/recipient T lymphocytes chimerism analysis was performed weekly, for a median of 8 weeks post transplantation, in 49 consecutive pts who underwent OLTX at our center between 12/2003 and 6/2004. Chimerism analysis was performed on PB using quantitative short tandem repeat (STR) assay. Informative STR markers identified in pre-transplant donor and recipient samples were used for post-transplant monitoring of chimerism. All PB samples were CD 3+ immunobead enriched prior to STR analysis.

**Results:** Donor T-cells were detected in PB from 40/49 pts post OLTX. Clinical diagnosis of GVHD was established in 1/49 pts who became symptomatic on day 12 and died on day 63 post OLTX. Data from remaining 48 pts lacking definitive clinical evidence of GVHD were used to establish "normal" limits for donor T cell levels post OLTX. The mean level of donor T cells consistently decreased in the 8 weeks post OLTX. The following table summarizes mean and upper limits of the 99% confidence intervals (CI) for the 48 pts. Donor T cell levels for the single acute GVHD pt are also shown.

| Time post OLTX : | 2 days | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk |
|---|---|---|---|---|---|---|
| Mean Donor T cells Levels: | 4.8% | 2.4% | 1.1% | 0.5% | 0.3 | 0.2 |
| Upper limit of 99% confidence intervals: | 16% | 11% | 6% | 3% | 2% | 2% |
| GVHD pt levels: | 5.5% | 5% | 26.5% | 73.7% | 83.9% | 57.3% |

By reapplying above derived "normal" limits to the entire cohort we were able to identify an additional pt with persistent chimerism levels above the upper limits of the 99% CI for more than 16 weeks. This latter pt clinically demonstrated neutropenia. Both his chimerism and neutropenia resolved following immunosuppression reduction.

**Conclusions:** Our findings suggest that STR donor CD3+ T-cell levels above 20%, one week or more post OLTX, support the diagnosis of GVHD. Levels <20% but higher than suggested upper limits of 99% CI could also be significant and should be cautiously interpreted in light of other clinical parameters.

### 1291 Cystic Squamoid Transformation of Intercalated Ducts: A Common Incidental Finding and a Rare Cause of Cystic Tumor in the Pancreas

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**Background:** Cystic and intraductal tumors of the pancreas form an important category with a challenging differential diagnosis. Some of these are low grade malignancies or precursors of adenoca, and therefore it is important to recognize the types of lesions that fall in their differential diagnosis.

**Design:** The clinicopathologic features of a hitherto unrecognized cystic tumor of the pancreas, and its possible relationship to a more common incidental microscopic lesion is analyzed.

**Results:** 3 patients (2 female, 1 male; ages 53, 56, and 77) underwent resection specifically for this cyst (tumors measuring 2.5, 9, 3.5 cm, located in the tail, head and body, respectively). Its microscopic version was detected incidentally in normal components of 6/72 (8%) of pancreata resected for various tumors. The cyst linings ranged from attenuated, flat squamoid cells to transitional (predominant pattern), to stratified squamous without keratinization. The cells in the basal/parabasal region expressed p63 (transitional/squamous cell marker, not detected in any normal pancreas or non-squamous neoplasia) and the superficial cells were positive for MUC1 and MUC6 (markers present in intercalated duct cells), and negative for GLUT-1 (consistent marker of serous adenomas). Larger cysts were unilocular and separated from the unremarkable parenchyma by a fibrous wall devoid of lymphoid or skin adnexal elements. Microscopic ones had abortive septae , irregular contours and were found lying within compact acinar tissue, and appeared to be transforming from intercalated ducts, often showing adjacent tightly packed clusters of ducts with similar morphology. The cysts typically contained distinctive acidophilic acinar secretions that formed concretions, more evident in medium-sized examples, confirming their communication with the acinar system, and suggesting a localized obstruction in their pathogenesis. There was no evidence of pancreatitis.

**Conclusions:** The distinctive morphologic, immunophenotypic and clinical characteristics of this cystic lesion warrant its classification as a separate entity. We propose to refer to it as metaplastic squamoid cystic transformation of intercalated ducts. It is a relatively common incidental finding in pancreas, and may occasionally present as a cystic mass.

### 1292 Pancreatic Mucinous Neoplasms: Analysis of Apomucin and Tumor Suppressor Gene Expression by Tissue Microarray

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**Background:** Mucinous tumors of the pancreas include ductal carcinoma (DCA), mucinous cystic neoplasm (MCN) and intraductal papillary mucinous tumor (IPMT). The aim of this study is to investigate expression of apomucins and tumor suppressor genes in pancreatic mucinous tumors to explore their diagnostic value in distinguishing these entities.

**Design:** A tissue microarray with 234 tissue cores (measuring 1 mm), including duplicate cores of 55 pancreatic tumors (15 DCA, 23 IPMT, 17 MCN) and 53 cases of pancreatitis and mucinous neoplasms of various sites was constructed in the usual fashion. The markers analyzed were apomucins (MUC1, MUC2, MUC5AC), tumor suppressor genes (p53, p16, CDX-2), CK 7 and 20, and villin. Staining was interpreted as weakly or strongly positive, negative or uninterpretable.

**Results:** Immunohistochemical profiles are listed in the table below.

Immunohistochemical Profiles of Pancreatic Mucinous Tumors*

| Immunostain | DCA | IPMT Adenoma | IPMT Borderline | IPMT Carcinoma | MCN Adenoma | MCN Borderline | MCN Carcinoma |
|---|---|---|---|---|---|---|---|
| MUC1 | 10/15 (67%) | 6/6 (100%) | 12/15 (80%) | 2/2 (100%) | 10/12 (83%) | 3/3 (100%) | 2/2 (100%) |
| MUC2 | 1/14 (7%) | 1/6 (17%) | 7/14 (50%) | 1/2 (50%) | 0/11 (0%) | 1/3 (33%) | 0/2 (0%) |
| MUC5AC | 7/15 (47%) | 5/6 (83%) | 12/14 (86%) | 2/2 (100%) | 6/11 (55%) | 3/3 (100%) | 2/2 (100%) |
| p53 | 13/14 (93%) | 5/6 (83%) | 11/14 (79%) | 1/2 (50%) | 5/10 (50%) | 3/3 (100%) | 2/2 (100%) |
| CDX2 | 0/15 (0%) | 0/5 (0%) | 1/15 (7%) | 2/2 (100%) | 0/13 (0%) | 2/3 (67%) | 0/2 (0%) |
| p16 | 2/13 (15%) | 2/6 (33%) | 1/14 (7%) | 0/2 (0%) | 5/10 (50%) | 1/3 (33%) | 0/2 (0%) |
| Villin | 7/15 (47%) | 1/6 (17%) | 6/15 (40%) | 1/1 (100%) | 5/12 (42%) | 2/3 (67%) | 1/2 (50%) |
| CK 20 | 0/14 (0%) | 0/6 (0%) | 3/14 (21%) | 1/2 (50%) | 2/11 (18%) | 2/3 (67%) | 0/2 (0%) |
| CK 7 | 13/15 (87%) | 6/6 (100%) | 13/15 (87%) | 1/2 (50%) | 10/12 (83%) | 3/3 (100%) | 2/2 (100%) |

*The denominator reflects the number of evaluable cases.

**Conclusions:** DCA and MCN are MUC1+/MUC5AC+/MUC2- while IPMT often express MUC2, CDX-2, and CK20 with increasing grade. This finding supports the intestinal pathway of carcinogenesis in IPMT. However, apomucin and tumor suppressor gene expression fails to provide diagnostic utility in distinguishing these entities.

### 1293 Pancreatic Intraepithelial Neoplasia: Analysis of Apomucin and Tumor Suppressor Gene Expression by Tissue Microarray

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**Background:** Pancreatic intraepithelial neoplasia (PanIN) is considered the precursor of invasive ductal carcinoma of the pancreas (DCA). The aim of this study is to investigate expression of apomucin subtypes and tumor suppressor genes in various grades of PanIN to explore the relationship between PanIN and invasive ductal carcinoma and provide insight into the pathway of carcinogenesis.

**Design:** A tissue microarray with 234 tissue cores (each measuring 1 mm), including 34 cases PanIN (12 PanIN grade 1, 17 PanIN grade 2, and 5 PanIN grade 3) and 15 cases of DCA was constructed in the usual fashion. Duplicate cores were included of the PanIN cases as well as 53 cases of normal pancreas, chronic pancreatitis, and mucinous neoplasms of the breast, appendix, colon, and ovary. The markers analyzed were apomucins (MUC1, MUC2, and MUC5AC), tumor suppressor genes (p53, p16, and CDX-2), cytokeratins 7 and 20, and villin. Staining was interpreted as weakly or strongly positive, negative or uninterpretable. Two pathologists (KCJ and RKP) jointly scored all cases.

**Results:** Immunohistochemical expression profiles for the varying grades of PanIN and DCA are presented in the table below.

Immunohistochemical Profiles of PanIN and Ductal Carcinoma*

| Immunostain | PanIN Grade 1 | PanIN Grade 2 | PanIN Grade 3 | Ductal Carcinoma |
|---|---|---|---|---|
| MUC1 | 9/12 (75%) | 12/17 (71%) | 4/4 (100%) | 10/15 (67%) |
| MUC2 | 0/12 (0%) | 0/16 (0%) | 0/5 (0%) | 1/14 (7%) |
| MUC5AC | 9/12 (75%) | 12/17 (71%) | 2/5 (40%) | 7/15 (47%) |
| p53 | 3/12 (25%) | 9/16 (56%) | 4/5 (80%) | 13/14 (93%) |
| p16 | 1/12 (8%) | 2/17 (12%) | 0/5 (0%) | 2/13 (15%) |
| CDX-2 | 0/12 (0%) | 0/17 (0%) | 0/5 (0%) | 0/15 (0%) |
| Villin | 0/11 (0%) | 3/16 (19%) | 3/5 (60%) | 7/15 (47%) |
| CK20 | 1/12 (8%) | 2/17 (12%) | 0/5 (0%) | 0/14 (0%) |
| CK7 | 11/12 (92%) | 16/17 (94%) | 5/5 (100%) | 13/15 (87%) |

*The denominator reflects the number of evaluable cases.

**Conclusions:** PanIN and DCA have identical immunohistochemical expression profiles with expression of apomucins MUC1 and MUC5AC, lack of expression of MUC2 and CDX-2, and a CK7+/CK20- phenotype. Increasing expression of p53 was noted with increasing grades of PanIN with similar p53 expression seen in PanIN grade 3 and DCA. Our data support the hypothesis that PanIN is a precursor lesion of invasive pancreatic ductal carcinoma.

### 1294 The Role of Movat Pentichrome Stain in the Diagnosis of Lymphoplasmocytic Sclerosing Pancreatitis

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**Background:** Lymphoplasmacytic sclerosing pancreatitis (LPSP) is a recently established entity that is important to recognize, not only because it closely mimics pancreatic cancer and leads to unnecessary Whipple resections, but also because it is treatable medically. LPSP may respond dramatically to steroid therapy, unlike other forms of chronic pancreatitis. It mimics cancer clinically via production of pancreatic head masses that obstruct both the pancreatic and bile ducts. Diagnostic criteria have been described but they have not been rigorously evaluated for specificity. Although dense lymphoplasmacytic infiltration, fibrosis and duct damage are characteristic findings of LPSP, they are not specific. Venous lesions (lymphocytic phlebitis and obliterative phlebitis) have been reported in the majority of LPSP; however, their specificity is unknown. Movat pentichrome staining to enhance detection of easily missed vascular pathology has also not been rigorously addressed.

**Design:** Movat pentichrome stains were evaluated on pancreatic tissue from 13 patients meeting the clinicopathologic criteria for LPSP (including 9 with resections and 4 with biopsies), 13 patients with usual forms of chronic pancreatitis, and 13 patients with pancreatic ductal adenocarcinoma-associated chronic pancreatitis.

**Results:** Marked lymphocytic venulitis and/or obliterative venous change with elastin fiber destruction was detected in all 13 cases of LPSP. Moreover, venous pathology was diffuse in distribution in all LPSP cases, involving virtually all veins. In contrast, none of the control cases revealed diffuse venous involvement. Rather, 18 of 22 controls had no venous pathology at all. Only patchy venous changes were observed in the remaining controls [1 control case of usual-type chronic pancreatitis and 3 control cases malignancy-associated chronic pancreatitis controls, for a total of 4 of 22 controls (18%) with limited venous pathology]. Diffuse, marked lymphocytic venulitis/obliteration by Movat staining was thus 100% sensitive and 100% specific for LPSP and 82% specific considering any degree of venous involvement.

**Conclusions:** This study documents the high specificity of diffuse lymphocytic venulitis/obliteration in the diagnosis of LPSP, as detected by Movat pentichrome staining, and confirms the previously described sensitivity of this finding. This stain should be considered in the evaluation of any type of chronic pancreatitis clinically mimicking pancreatic carcinoma.

### 1295 Hepatocellular Carcinoma in Explanted Livers – Clinicopathological Features of 101 Patients and Development of a Tumor Recurrence Pathological Score

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**Background:** Orthotopic liver transplantation (OLT) is used to treat hepatocellular carcinoma (HCC), and Milan and UCSF criteria are used for OLT patient selection. Tumor recurrence is the main cause of death; post-OLT pathological predictors of outcome are needed. This study examines liver explants with HCC and compares patients with and without recurrent HCC (rHCC) to identify those at risk for recurrence.

**Design:** 101 explanted livers with HCC were reviewed blindly to assess: number of tumors; largest tumor size; tumor edge, stroma, inflammation, microvascular invasion, necrosis, architecture; satellitosis; nuclear atypia; mitoses; cell types; non-neoplastic liver. Clinical data was reviewed. Statistical analysis included chi-square and t-tests. Using variables that were commoner with recurrent HCC, a pathological score was employed to determine the probability of HCC recurrence (min. score=1; max. score=21).

**Results:** 20 cases had rHCC. rHCC was more common in patients who met Milan and/or UCSF criteria (75%, 80% vs 30%, 60%; p=<0.001, 0.078). HCC recurrence was more common in patients who met UCSF criteria alone compared to those who met both Milan and UCSF criteria (67% vs 12%; p<0.001). Serum alpha-fetoprotein >1000ng/mL was specific for recurrent HCC (20% vs 0%; p<0.001). Background inflammation was greater in the absence of rHCC (p=0.030). Tumor size was greater with rHCC (mean 4.1 vs. 2.3 cm; p<0.001). Infiltrative edge (85% vs 24%; p<0.001), satellitosis (70% vs 18%; p<0.001), microvascular invasion (60% vs 9%; p<0.001), necrosis (55% vs 29%; p=0.039), macrotrabecula (45% vs 7%; p<0.001), 10 or more mitoses/10 hpfs (50% vs 24%; p=0.029) and bizarre cells (55% vs 11%; p<0.001) were more common with rHCC. Mean pathological score was greater with rHCC (12 vs 5; p<0.001). With a threshold score of 8, the sensitivity and specificity for selecting patients with rHCC was 95% and 82% respectively.

**Conclusions:** Liver explants with rHCC and non-recurrent HCC show clinicopathological differences. While Milan and UCSF criteria help in OLT patient selection, use of a post-OLT pathological score may help to identify patients at risk for tumor recurrence who may be candidates for adjuvant therapy.

### 1296 CXCL10: An Important Determinant of Inflammation in Chronic HCV Infection?

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**Background:** Chemokines, chemotactic cytokines, may promote hepatic inflammation in chronic HCV. We assessed peripheral blood and intrahepatic expression of non-ELR CXC chemokines and their common receptor CXCR3 in chronic HCV.

**Design:** We measured plasma CXCL9 and CXCL10 by ELISA on HCV-infected patients undergoing pretreatment liver biopsy. Mean numbers of portal and lobular CD3+, CD4+, CD8+, and CXCR3+ cells were detected by immunohistochemistry and quantified by counting five high power fields (HPF). Intrahepatic chemokines (CXCL9, CXCL10, and CXCL11) were detected using immunohistochemistry and the expression was determined semiquantitatively. Inflammation and fibrosis were assessed using a modified Scheuer's system.

**Results:** Samples were obtained from 9 men and 5 women, median age 52.8 (range 27-70) years. Five patients had mild (<2) portal inflammation, 7 had mild (<2) lobular inflammation and 7 had mild (<2) fibrosis. CD3+ and CXCR3+ cells were significantly increased in the portal, compared with the lobular rareas (CD3: 61.4+/-27.2 vs. 17.0+/-10.5 cells/HPF, p=0.001; CXCR3+: 40.0+/-14.0 vs. 5.8+/-3.0/HPF, p<0.001). The majority of portal CD3+ cells were CD4+ as opposed to CD8+ while the converse occurred in the lobules (portal CD4+ 49.1+/-22.7, CD8+ 37.9+/-17.1/HPF, p=0.09; lobular CD4+:3.1+/-2.0, CD8+ 16.5+/-12.6 cells/HPF, p=0.01). CXCL10+ hepatocytes and lobular CXCR3+ lymphocytes were significantly increased in patients with more severe lobular inflammation (p=0.004 and p=0.048, respectively).

**Conclusions:** In chronic HCV infection, CXCL10 and CXCR3+ lymphocytes are significantly associated with intrahepatic inflammation. Thus, CXCL10, by attracting CXCR3+ lymphocytes to the liver lobules may promote lobular/parenchymal inflammation in chronic HCV.

### 1297 Does hTERT Expression in Nontumorous HCV Cirrhosis Identify HCC Patients?

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**Background:** Patients with HCV cirrhosis are at increased risk for hepatocellular carcinoma (HCC), but no adequate screening tests yet exist to identify the approximately 10% minority who develop HCC. hTERT (human telomerase reverse transcriptase gene) is the catalytic subunit of telomerase, an enzyme that synthesizes telomeric DNA, compensating for the telomeric loss that occurs with each cell division. Although detection of telomerase activity is laborious and requires fresh tissue, hTERT expression can be detected immunohistochemically (Yan et al, Histochem Cell Biol 2004). Telomerase activity and hTERT expression have both been demonstrated in HCC and in nontumorous cirrhotic liver. We hypothesize that hTERT expression in cirrhotic liver could serve as a biomarker to differentiate patients with HCC ("progressors") from those without it ("nonprogressors").

**Design:** We studied formalin-fixed, paraffin-embedded tissue sections of nontumorous cirrhotic liver from 31 pts with HCV cirrhosis who underwent orthotopic liver transplantation. Cases were anonymized as to HCC progressor or nonprogressor. Immunohistochemical staining for hTERT (NCL-hTERT; Novacastra, UK) was performed on a Ventana XT automated stainer at a 1:10 dilution using standard heat-based antigen retrieval. Positive staining was scored as the number of positive nuclear signals per 200 consecutive hepatocyte nuclei. Results were compared between HCC progressors and nonprogressors, and an unpaired means comparison was performed.

**Results:** 14 HCC progressors and 17 nonprogressors were studied. Positive nuclear staining was present in internal control lymphocytes. The mean number of positive signals in 200 hepatocyte nuclei was 177 in nonprogressors and 208 in HCC progressors (unpaired means comparison P<0.17).

**Conclusions:** Although expressed at low levels in cirrhotic liver, hTERT activity may be greater in HCC progressors than nonprogressors. These results suggest that hTERT expression by immunohistochemistry in non-tumorous liver may be a useful biomarker to distinguish progressors from non-progressors, although the results did not achieve statistical significance in this preliminary set of 31 cases. These findings suggest that

hTERT expression may be an important factor in early HCC tumorigenesis and telomere maintenance.

### 1298 The Diagnostic Utility of CK19 and CD99 in Pancreatic Endocrine Tumors (PET)

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**Background:** Several pathologic features, such as tumor size, necrosis, lymphovascular invasion, perineural invasion, gross invasion into peripancreatic tissue, lymph node spread and distant metastases have all been used as prognostic parameters in a variety of classification schemes. Recently, it has been suggested that CK19 (positivity) and CD99 (negativity) immunohistochemistry is of use in predicting aggressive behavior in PET.

**Design:** The computer records and personal consultation files of one of the authors (SLA) were accessed for all cases of PET from 1985 to 2005. Relevant clinical information was recorded and follow-up obtained in a proportion of cases. All cases had a routine panel of immunohistochemical markers for PET performed, including stains for CK19 and CD99. Pathologic parameters such as tumor size, mitotic count, MIB-1 labeling, lymphovascular invasion, perineural invasion, lymph node involvement and spread to liver were recorded.

**Results:** Fifty four PET in 21 males and 33 females, ranging in age from 23 to 80 years, were retrieved. Thirteen cases had lymph node spread while 6 cases showed liver metastasis; 1 case showed both lymph node and liver involvement. Twelve cases displayed lymphovascular/perineural invasion (without obvious lymph node spread). Tumor size ranged from 0.5 to 11cm and 18 cases were larger than 3.0cm in diameter. Thirty one cases were CK19 positive and 32 were CD99 positive. Eleven of 13 cases with lymph node spread were CK19 positive ($p<0.001$). The CK19 immunoprofile did not show any statistically significant correlation with any other clinicopathologic parameter. CD99 was positive in an almost equal number of cases with and without lymph node spread, liver metastases and lymphovascular invasion. Follow-up was obtained on 27 patients ranging from 1 month to 63 months, with 14 patients followed up for 1 year or more. No correlation with survival was evident with either marker.

**Conclusions:** CK19 positivity is statistically correlated with those cases showing lymph node spread, irrespective of tumor size. CD99 does not appear to correlate with any clinicopathologic feature and its loss was not associated with aggressive PET.

### 1299 Quantitative Histological-Hemodynamic Correlations in Cirrhosis

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**Background:** While the definitive diagnosis of cirrhosis is histological, it is the degree of portal hypertension, as determined by the hepatic venous pressure gradient (HVPG), that is an important determinant of the severity of cirrhosis. An HVPG > 10 mmHg (termed clinically significant portal hypertension or CSPH) is predictive of the development of complications of cirrhosis, including death. Using a semiquantitative scale, we previously found that nodule size and septal thickness are independent predictors of CSPH. This study aimed to confirm these findings using quantitative analysis by digital imaging.

**Design:** Forty-two patients with biopsy-proven cirrhosis and HVPG measurement within six months of the biopsy were included in the study. Trichrome-stained slides were analyzed using digital image analysis (Bioquant®). The following parameters were measured without knowledge of HVPG results: fibrosis area (FA), expressed as a percentage of the total biopsy area; median septal width (SW) of all fibrous septa per slide, median nodule size (NS) as determined by measuring the diameter of cirrhotic nodules, number of nodules (NN) per mm length of liver biopsy. Using non-parametric statistics, HVPG and the presence (or absence) of CSPH were correlated to each parameter, along with the appropriate use of multivariable models

**Results:** HVPG correlated significantly with FA ($p<0.001$) and NS (0.006). On stepwise linear regression, only FA independently correlated with HVPG ($r=0.606$, $p<0.001$). On univariate analysis (Table), biopsies of patients with CSPH had a significantly greater FA, NS and NN compared to those without CSPH. On binary backward logistic regression, FA and NN were the only histological parameters predictive of the presence of CSPH ($r=0.606$, $p=0.008$).

**Conclusions:** In this study, using an objective, quantitative methodology, we confirm that there is a significant relationship between HVPG and a combination of small nodularity and the amount of fibrosis. This confirmatory study validates our proposed subclassification of stage 4 (cirrhotic) liver biopsies into a "mild" and a "severe" form.

| | No CSPH (n=18) | CSPH (n=24) | p |
|---|---|---|---|
| FA (%) | 18.4 (4.3-42.2) | 27.6 (14.2 45.9) | 0.01 |
| Median NS (μ) | 829 (596-1845) | 602 (236-1561) | 0.001 |
| Median SW (μ) | 314 (48-922) | 283 (77-1195) | 0.65 |
| NN | 0.72 (0.35-1.25) | 0.95 (0.51-1.91) | 0.05 |

### 1300 The Role of GLUT-1 in "Clear-Cell" Ductal Tumors of the Pancreas

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**Background:** GLUT-1, a glucose transporter molecule, has been found to have an important role in the up-regulation of various cellular pathways, and has been implicated in neoplastic transformation. It is also known that GLUT-1 is inducible by HIF1-alpha which is considered to be a key factor in the development of von Hippel-Lindau related neoplasms.

**Design:** The immunohistochemical expression of GLUT-1 was tested in normal pancreas and a variety of pancreatic neoplasia including 12 serous cystadenomas, 10 mucinous cystic neoplasms, 6 intraductal papillary mucinous neoplasms and 94 ductal adenocarcinomas. Percent of cells labeled was graded as minimal (<10%), focal (10-80%) or diffuse (>80%) and the intensity was scored as weak or strong.

**Results:** In normal pancreas, GLUT-1 was expressed in the islets and perineurial cells, but not in the ducts or acini. All 12 of 12 (100%) serous cystadenomas showed GLUT-1 expression (diffuse, strong in 9, and diffuse, weak in 3). However, it was mostly absent or only minimal in mucinous neoplasia: 1 of 6 (16%) intraductal papillary mucinous neoplasms and 3 of 10 (30%) mucinous cystic neoplasms showed minimal and weak immunolabeling. In the 94 ordinary ductal carcinomas analyzed, 8 (6%) showed diffuse, strong expression and there was focal, weak expression in 75 cases (70%). All 4 (100%) clear-cell variants of ductal adenocarcinomas showed diffuse, strong expression, as did areas of clear cell change seen in ordinary ductal carcinomas.

**Conclusions:** Consistent Glut-1 expression is seen in serous cystadenomas, but not in mucinous cystic or intraductal papillary mucinous tumors of the pancreas. Glut-1 may give rise to the distinctive clear-cell appearance of these tumors by inducing the accumulation of glycogen in the cytoplasm ("glycogen-rich adenoma"), and may also be a factor in their pathogenesis through its known interaction with several pathways including HIF1-alpha. Furthermore, Glut-1 may also be responsible for the clear cell pattern in invasive ductal adenocarcinoma.

### 1301 Epstein-Barr Virus Hepatitis: Diagnostic Value of Immunohistochemistry, In Situ Hybridization and Polymerase Chain Reaction on Liver Biopsy

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**Background:** Epstein-Barr virus (EBV) hepatitis is an uncommon, almost always self-limited disease in immunocompetent patients. Rarely, atypical clinical presentations and equivocal laboratory tests make the clinical diagnosis difficult and warrant a liver biopsy. While EBV hepatitis may show characteristic histology, this is not always the case. Confirmation with ancillary techniques is thus valuable. The aim of this study was to compare the effectiveness of 3 available methods for EBV detection in liver biopsies.

**Design:** Formalin-fixed, paraffin-embedded liver biopsies from 10 cases of EBV hepatitis and 5 controls of chronic hepatitis C were evaluated by immunohistochemistry (IHC) for EBV latent membrane protein (LMP), in situ hybridization (ISH) for EBV encoded RNA (EBER) and polymerase chain reaction (PCR) for EBV DNA. A house-keeping gene (HLADQ) was used to verify the presence of amplifiable DNA extracted from paraffin blocks.

**Results:** The ages of the patients with EBV hepatitis ranged from 5 to 75 years (mean: 50 years; median: 60 years), with a male-to-female ratio of 1:1. None of the patients was immunosuppressed. Histologic evaluation of the liver biopsies revealed a spectrum of findings ranging from nonspecific portal and lobular inflammation to characteristic sinusoidal lymphocytic infiltration with atypical lymphocytes. PCR for HLADQ was positive in all 10 cases, confirming DNA detectability in the samples. EBV DNA was positive in 9 biopsies. EBER was positive in 8 cases, with scattered positive cells individually distributed in portal tracts and sinusoids, typically few in number. In 7 biopsies, EBV was detected by both PCR and ISH. In the other 3, EBV was detected by PCR but not by ISH in 2, and vice versa in 1 (Table). IHC for LMP was negative in all 10 cases. The 5 control cases of chronic hepatitis C were negative by all detection methods.

**Conclusions:** ISH and PCR are equally sensitive in detecting EBV in routinely processed liver biopsies. The ready implementation of ISH in pathology laboratories makes it a useful ancillary tool in confirming the diagnosis of EBV hepatitis in equivocal cases. However, EBER-positive cells can be very sparse and easily overlooked. IHC for LMP has no utility in the diagnosis of EBV hepatitis.

| PCR-positive (n=9) | EBER-positive (n=8) | LMP-positive (n=0) | No. of Cases (n=10) |
|---|---|---|---|
| positive | positive | negative | 7 |
| positive | negative | negative | 2 |
| negative | positive | negative | 1 |

### 1302 Vasculogenic Mimicry Is Associated with Shorter Survival in Hepatocellular Carcinomas

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**Background:** Vasculogenic mimicry (VM), a special form of angiogenesis found in melanomas, breast carcinomas, and sarcomas, manifests cell fate alterations and is associated with poor prognosis. In this study, we investigated the presence of VM in hepatocelluar carcinoma (HCC) and correlated with clinical outcomes.

**Design:** Paraffin-embedded tissue of ninety-nine cases of HCC from 1980 to 2000 with complete clinical and pathological data were retrieved from Tianjin Cancer hospital. Immunohistochemical staining for CD31, CD105 (endoglin), Hepatocyte, VEGF, MMP-2, MMP-9 was conducted.

**Results:** The mean age of the patients was 56 + 12. Forty cases were classified as Edmondson pathologic grade I-II and 59 cases were graded as III-IV. VM was detected in 12 of the 99 HCC cases (12%). Among the positive cases, only 1 VM was found in 40 grade I-II cases (2.5%), and 11 in 59 grade III-IV cases (18.6%) of HCC ($P=0.036$). The cells forming VM were Hepatocyte and VEGF positive, CD31 and CD105 negative, which suggesting these cells were arising from the tumor cells of hepatocellular carcinoma, not from endothelial cells. The group of patients with VM had a higher rate of hematogenous metastases ($P=0.003$). MMP-2 and MMP-9 were present in 100% of the patients with VM and less frequently in the patients without VM (83.9% for MMP-2 and 82.8% for MMP-9) ($P<0.05$). Result from Kaplan-Meier Survival analysis showed that HCC with VM had a significantly shorter survival time compared with HCC cases without VM.

**Conclusions:** Vasculogenic mimicry also exists in hepatocelluar carcinoma. The presence of VM is associated with a higher grade of HCC, higher rate of hematogenous metastases, and a shorter survival.

### 1303 Bile Duct Dysplasia in the Setting of Chronic Hepatitis C Infection

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**Background:** Intrahepatic cholangiocarcinomas are rare and risk factors remain poorly understood. The risk factors for intrahepatic cholangiocarcinoma appear different from those for extra-hepatic cholangiocarcinoma and two recent epidemiological studies from Europe and the United Stages have identified chronic hepatitis C infection (HCV) as a major risk factor. To further evaluate this association, we searched for dysplasia in the intrahepatic bile ducts in native livers removed at transplantation for chronic HCV.

**Design:** We reviewed explants from 3 transplant centers to determine the prevalence of dysplasia in the intrahepatic bile ducts. All cases of chronic biliary tract disease were excluded. The location and morphology of the dysplastic foci were studied.

**Results:** A total of 1058 explants were reviewed: HCV (N=511), alcohol alone (112), HCV and alcohol (85), HBV (67), cirrhosis from other causes (149), and non-cirrhotic livers, e.g. cases transplanted for acute liver failure (134). Dysplasia of the intrahepatic bile ducts was seen in 19/1058 (1.8%) of cases and was associated with chronic HCV infection and alcohol use, p = 0.012. 10/19 cases of dysplasia were in the setting of chronic HCV alone, while 5/19 were in the setting of alcohol cirrhosis alone, and the remaining 4/19 were in the setting of combined HCV and alcohol cirrhosis. Dysplasia was not identified in other causes of cirrhosis and no dysplasia was seen in non-cirrhotic livers. 17/19 cases were classified as low grade dysplasia and 2/19 as high grade dysplasia. One case of high grade dysplasia was accompanied by intrahepatic cholangiocarcinoma. In all cases of dysplasia, the lesions were multifocal and involved septal sized bile ducts. No lesions involved the smaller interlobular ducts or the large extra-hepatic bile ducts. In 16/19 cases, the dysplasia was papillary and in 3/19 cases the dysplasia was flat.

**Conclusions:** Dysplasia can develop in the intrahepatic bile ducts in cirrhosis from chronic HCV, supporting recent epidemiological studies identifying chronic HCV as a major risk factor for intrahepatic cholangiocarcinoma. Alcohol also appears to be a risk factor. The dysplastic foci were multiple, involved septal sized bile ducts, and generally had a papillary morphology.

### 1304 Prognostic Significance of Cell Cycle Proteins p53, p21, p27, p14, p16 and mdm2 in Hepatocellular Carcinomas

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**Background:** Hepatocellular carcinomas (HCC) are devastating and aggressive human tumors and molecular pathogenesis has been under intense investigation as a part of the effort to develop more effective therapeutic strategies for these tumors. Cell cycle control is a crucial event in the regulation of human normal tissues functions, and abnormalities of regulatory cell cycle genes have been observed to contribute to the development of many human malignancies. This study investigates the immunohistochemical expression of 6 essential cell cycle proteins (p53, p21, p27, p14, p16 and mdm2) in HCC and their correlation with classic pathologic tumor features, proliferating index (Ki67 positivity) and patients' survival.

**Design:** Formalin-fixed, paraffin-embedded 4μm sections obtained from 76 HCC hepatectomy specimens, were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal and polyclonal antibodies for p53, p21, p27, p14, p16, and Ki67. Results were expressed as % of positive cells. Mean follow up time was 27.19 months (range 1.5-83 months).

**Results:** The number of cases in which the expression of p53, p14, p16, p21 p27 and mdm2 were detected was: 56%(43/76), 70%(53/76), 65%(49/76), 48%(36/76), 82%(62/76) and 93% (70/76) respectively. The mean index for Ki67 was 16.58±10.24. Highly significant relationship was observed between a) vascular invasion presence and lower disease-free survival (p=0.0007) and b) advanced tumor stage and lower disease-free survival (p=0.0008). Log-rank tests showed that with division of Ki67 indices into those above and below 10% there was an inverse association between proliferating index and disease-free survival (p=0.0075). p53 and p21 were more frequently expressed in advanced stage tumors (p=0.014 and 0.002 respectively). p53 expression was associated with lower disease-free survival only in p21(+) tumors (p=0.021). Neither p14 nor p16 expression were associated with grade, Ki67 index or overall survival. Cox regression analysis revealed that only vascular invasion and tumor stage were independent prognostic factors (CI: 0.032-0.502, p=0.03 and CI:1.167-5.408, p=0.019).

**Conclusions:** The study confirms that in cases of HCC, vascular invasion and tumor stage are independent predictors of the outcome of the patients. p53 was associated with poor prognosis only in tumors overexpressing p21; that means high levels of p21 in tumor cells associated with aberrant p53 protein expression, may result in tumor recurrence.

### 1305 Activated Caspase-3 and Survivin Expression and Cell Proliferation in Hepatocellular Carcinomas and Their Relationship with Disease Outcome

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**Background:** Caspase 3 is a downstream effector cysteine protease in the apoptotic pathway. Survivin, a member of the inhibitor-of-apoptosis proteins family, suppresses apoptosis and stimulates cell division. In this study we investigated activated caspase-3 (AC-3) expression in hepatocellular carcinomas (HCC) and related it to survivin expression, cell proliferation, prognostic parameters, and outcome.

**Design:** Formalin-fixed, paraffin-embedded 4μm sections obtained from 76 HCC hepatectomy specimens, were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal antibodies for AC-3 and Ki67 (cell proliferation) and polyclonal antibody for survivin. Human tonsils were used as positive controls. Immunostaining was evaluated in the cytoplasm and nucleus of hepatocytes and Kupffer cells. Results were expressed as % of positive cells. Mean follow up time was 27.19 months (range 1.5-83 months).

**Results:** AC-3was expressed within hepatocytes in 69/76 (90.7%) and within Kupffer cells in 52/76 (68,4%) cases. The stain was nuclear. AC-3 expression was correlated with lower tumor grade (p=0.012), higher disease-free (p=0.008) and higher total survival (p=0.007). Survivin was present in 34/76 (44.7%) HCC and the stain was cytoplasmic and nuclear. Non-neoplastic liver tissue was negative. There was a trend of higher nuclear survivin expression towards advanced tumor grade and stage, presence of vascular invasion, and higher mitotic index (p=0.04, p=0.003, p=0.002 and p=0.0019 respectively). Survivin expression was correlated with lower disease-free and total survival (p=0.024 and 0.032 respectively). Spearman rank correlation revealed that a) AC-3 expression was reversibly correlated with cytoplasmic survivin expression (p=0.024), b) nuclear survivin expression was directly correlated with Ki67 expression (p=0.00426). No correlation was recorded between AC-3 and Ki67 expression.

**Conclusions:** In HCC, activated caspase 3 expression is correlated with higher disease-free survival and higher total survival and displays a reverse correlation with survivin expression which is considered as a poor prognostic parameter. The higher caspase-3 expression probably reflects the fact that apoptosis is associated with good prognosis in cases of HCC. Thus, methods stimulating apoptosis (i.e. specific chemotherapies or anti-survivin therapies) may be useful in the attempt to increase the disease-free and total survival in patients with HCC.

### 1306 Hepatic Progenitor Cell Activation and Periportal Ductular Reaction Presence, in Chronic Hepatitis C. Correlation with Disease Severity, Impaired Hepatocyte Replication and Response to Treatment

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**Background:** Hepatic progenitor cells (HPC) are liver stem cells that involved in the progress of liver disease. In experimental models, HPC undergo activation when mature liver-cell proliferation is suppressed. This study investigates the potential correlation of HPC activation and the resultant periportal ductular reaction (PDR) with a) disease severity and response to treatment, and b) impaired liver cell replication, in patients with HCV.

**Design:** The study included 154 liver biopsies obtained from 77 patients with HCV. All patients were PCR(+), received therapy and assigned as responders (A=29), non-responders (B=29), relapsers (C=19). 77 biopsies were obtained before treatment (A1, B1, C1) and 77 after (A2, B2, C2). Paraffin sections were stained with anti-CK7, anti-LCA, anti-CD34, and anti-p21. Cells with morphologic features of HPC (Roskams T et al Hepatology 39:1739, 2004) that were CK7+ *and* LCA(-)/CD34(-) were scored. The aforementioned reference was used to define PDR. The presence of HPC was also determined by gene analysis for AFPmRNA, performed on microdissected liver tissue samples. Microdissection targets included areas with HPC expression and microdissected tissue was amplified with PCR. PDR was quantified as % of biopsy area.

**Results:** The table lists the results for HPC and PDR presence. Statistical analysis revealed significant correlation between **a)** HPC with fibrosis (p=0.0028) and inflammation (p=0.0035), **b)** PDR and fibrosis (p=0.0017). Impaired hepatocyte replication (%p21+ cells) was independently associated with **a)** %HPC (p=0.0031) and **b)** PDR degree (p =0.0042). Multivariate analysis showed that PDR is an independent factor for the prediction of fibrosis.

**Conclusions:** This study shows that in HCV cases, HPC activation and degree of PDR, are significantly decreased in patients with response to treatment. These results support the hypothesis that HPC numbers and PDR degree are strongly correlated with disease severity. The fact that these factors are associated with impaired hepatocyte replication implies the presence of an alternative pathogenetic pathway during liver regeneration that leads to ductular reaction and progressive liver fibrosis, with consequent liver failure.

| | | HPC expression and PDR presence | | |
|---|---|---|---|---|
| Groups | | HPC-CK7+ | AFPgene | PDR |
| A | A1 | 30.7±1.9* | 32.5±2.1£ | 28.8±4.5† |
| | A2 | 8.3±1.4* | 9.2±1.8£ | 4.6±1.4† |
| B | B1 | 53.4±6.3 | 54.2±8.1 | 48.3±7.1 |
| | B2 | 51.2±5.4 | 52.7±6.2 | 47.5±6.3 |
| C | C1 | 49.7±1.8 | 50.6±3.4 | 46.6±7.1 |
| | C2 | 47.3±4.3 | 46.9±7.2 | 43.5±5.2 |

*, £,† :p<0.001

### 1307 A Histopathological Study on Combined Hepatocellular and Cholangiocarcinoma: Cholangiocarcinoma Component Is Originated from Hepatocellular Carcinoma

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**Background:** Combined hepatocellular and cholangiocarcinoma of the liver is relatively infrequent, and its pathogenesis remains obscure. This study is for investigation of the pathologic features and pathogenesis of combined hepatocellular and cholangiocellular carcinoma.

**Design:** In this study, we investigated the pathological features, Ki-67 labeling index, and p53 immunohistochemistry of 18 surgically resected cases of combined hepatocellular and cholangiocarcinoma among 1102 consecutive cases of surgically resected primary liver cancers. All tumors were compatible with the WHO definition of this tumor. Microscopically, we classified the cases into the following three categories according to the arrangement of the hepatocellular carcinoma and cholangiocarcinoma components; (i) Type I in which hepatocellular carcinoma and cholangiocarcinoma formed nodules that could easily distinguish to each other, (ii) Type II in which the both components were finely mixed, so that the two components were almost indistinguishable, and (iii) Type III in which the tumors had lobular structures with

hepatocellular carcinoma existing centrally and cholangiocarcinomas existing peripherally.
**Results:** Microscopically, the tumors were classified into type I 7 tumors, type II 5 tumors, and type III 6 tumors. In one case of type I, well differentiated hepatocellular carcinoma demonstrated cholangiocarcinoma in a "nodules-in nodules" fashion. The average of Ki-67 labeling index of hepatocellular carcinoma component of combined hepatocellular and cholangiocarcinoma was 3.4±2.1% and the index of cholangiocarcinoma component was 10.4±9.9%, which is significantly higher than that of the hepatocellular carcinoma component. On p53 immunohistochemistry, 5 of 18 cases (29.4%) were positive. In one case, the cholangiocarcinoma component was positive for p53, but the hepatocellular carcinoma component was negative. In the other 4 cases, both the hepatocellular carcinoma and cholangiocarcinoma component were positive.
**Conclusions:** Microscopically, type III seems to be a feature of metaplasia or proliferation of bipotential stem cells. Metaplasia of hepatocellular carcinoma to intrahepatic cholangiocarcinoma is assumed to be one of the pathogenic pathways of combined hepatocellular and cholangiocarcinoma.

### 1308 HNF-4α Expression in Human Liver Regeneration after Massive Hepatic Necrosis

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**Background:** Submassive hepatic necrosis usually leads to proliferation of ductular hepatocytes, which are considered to be bipotential progenitor cells that contribute to liver regeneration. Ductular hepatocytes express both biliary (Cytokeratin 19) and hepatocyte (HepPar1) markers and are similar to the oval cells in the rodent, where studies have shown that oval cells differentiate into hepatocytes and express hepatocyte specific transcription factors. In liver tissue sections with submassive necrosis, we studied expression of HNF-4α, which regulates the expression of genes critical for hepatocyte differentiation.
**Design:** Explanted livers from 21 patients with fulminant hepatic failure were examined using immunohistochemical staining of 4μm paraffin sections with normal liver control. The percentage of CK19, HepPar1 and HNF-4α positivity in 500 ductular cells was recorded. HNF-4α and HepPar1 expression were compared using a Pearson correlation coefficient; regional variation was determined in 4 cases based on a count of 300 ductular cells both adjacent to and away from nodules of hepatocytes. Double immunohistochemical staining with HNF-4α and CK19 was performed on 2 cases.
**Results:** HNF-4α positivity ranged from 2.9% to 43.5% (mean 16.2%, median 12.3%.) HepPar1 positivity ranged from 0% (in one case) to 48.1% (mean 8.6%, median 7.1%.) CK19 positivity was >95% of ductular cells in all cases. There was a weak positive correlation between HNF-4α and HepPar1 expression (Pearson correlation coefficient, 0.326.) HepPar1 and HNF-4α expression increased in ductular structures adjacent to nodules of regenerative hepatocytes compared to those away from the nodules (average positive HNF-4α: 23.9% vs. 9.3%, HepPar1: 26.7% vs. 0.9%.) HNF-4α positive cells were seen within CK19 positive ductules using double immunohistochemical staining. In the normal liver, HNF-4α diffusely stained the nuclei of nearly all hepatocytes; whereas, few regenerative hepatocyte nuclei were positive in the submassive necrosis cases.
**Conclusions:** Strong staining of HNF-4α and HepPar1 was seen in CK19-positive ductular hepatocytes adjacent to nodules of regenerating hepatocytes, which showed an altered pattern of HNF-4α expression. These results suggest that viable hepatocytes may induce hepatocyte differentiation in adjacent ductular hepatocytes. In conclusion, this study shows HNF-4α expression in ductular hepatocytes, supporting the concept that they differentiate into hepatocytes and contribute to human liver regeneration after submassive necrosis.

### 1309 The Regulation between AM and TGF-β1 and AM Regulates TGF-β1-Stimulated MMP-2 Expression in Hepatic Stellate Cells

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**Background:** Adrenomedullin (AM), a peptide isolated from human pheochromocytoma, can be produced by a variety of cells including hepatic stellate cells (HSCs) and the expression of AM can be regulated by many cytokines including transforming growth factor beta-1 (TGF-β1). TGF-β1 is the key cytokine in hepatic fibrogenesis which induces the activation of HSCs by upregulating the expression of matrix metalloproteinase-2 (MMP-2). It was determined that the effect of TGF-β1 on AM synthesis seems to be paradoxical dependent on cell types. However, it is unclear so far how AM interacts with TGF-β1 and if AM has any effect on TGF-β1-regulated MMP-2 expression in hepatic stellate cells. The aim of our study was to investigate, in cultured HSCs, the regulation between TGF-β1 and AM as well as the effect of AM on TGF-β1-induced MMP-2 expression and its possible mechanism.
**Design:** All the experiments were performed using the HSCs line T6. Transcript levels of AM and TGF-β1 were determined by real time RT-PCR; protein expression of AM and TGF-β1 by radioimmunoassay (RIA) and Western blot, respectively; MMP-2 expression and phosphorylation level of extracellular singal-regulated kinase (ERK) by Western blot.
**Results:** AM transcription level in HSCs was inhibited by TGF-β1 in a dose- and time-dependent fashion, but TGF-β1 was of no effect on AM protein secretion. Interestingly, TGF-β1 in both transcription and protein levels was suppressed by AM also in a dose- and time-dependent manner. MMP-2 protein expression in HSCs was increased in response to TGF-β1 and upregulation of MMP-2 expression stimulated with TGF-β1 was suppressed by AM again in a dose-dependent manner. AM decreased the upregulated phosphorylation level of ERK in HSCs treated with TGF-β1 and TGF-β1-upregulated MMP-2 expression was suppressed by adding MEK inhibiotor $U_{0126}$.
**Conclusions:** Our results suggest that AM may have an anti-fibrosis role by inhibiting TGF-β1 production and AM may inhibit the activation of HSCs by suppressing the upregulation of MMP-2 stimulated with TGF-β1, which is partially mediated through ERK pathway.

### 1310 Pseudo-Ground Glass Changes in Liver Biopsies

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**Background:** Ground glass cytoplasmic change in hepatocytes is typically associated with chronic hepatitis B infection. We report a series of cases of psuedo-ground glass change that closely mimicked HBV inclusions, but appeared in a uniquely different clinical setting.
**Design:** Clinical and laboratory data were reviewed. The proportion of hepatocytes involved was scored as 1+ (1-30%), 2+ (31-60%), 3+ (>61%). The livers were staged and graded with the Ishak scoring system. Immunohistochemistry was used to further characterize the pseudo-ground glass change. Electron microscopy was performed from paraffin embedded tissue in 2 cases.
**Results:** Ten cases of psuedo-ground glass change were found in 6 males and 4 females including 4 children, average age 7 ± 4 years, and 6 adults, average age 45 ± 11 years. 8/10 individuals were immunosuppressed secondary to bone marrow or solid organ transplant (N=4), HIV infection (N=2), kidney dialysis (N=1), or inflammatory bowel disease (N=1). Medication histories, available in 8 cases, indicated that all were on multiple medicines (range 2 to 33). No single common medicine was identified, though 6/8 were on at least 1 steroid. Histologically, the psuedo-ground glass change was essentially identical to the ground glass change seen in chronic HBV, with well circumscribed, gray-glassy inclusions surrounded by a rim of normal cytoplasm. Pseudo-ground glass change was present in zone 1 (N=4), zone 3 (N=2), and azonal (N=4). The proportion of hepatocytes involved was 1+ (N=4), 2+ (N= 4), and 3+ (N=2). Most cases showed mild inflammation (all Ishak scores less than 4/18) and no fibrosis (7/10) or mild portal fibrosis (3/10). All cases were negative for HBsAg immunostaining and/or HBV serological testing. The pseudo-ground glass change was PAS positive and diastase sensitive. EM on 2/2 cases showed abnormally organized glycogen. No evidence for viral particles or endoplasmic reticulum proliferation was seen. Three cases had follow-up biopsies (at months 1, 1, 36), and the psuedo-ground glass was persistent in 2 cases and resolved in one case (1 month biopsy interval), with resolution temporally correlating with tapering of steroids.
**Conclusions:** Psuedo-ground glass change is typically seen in immunosuppressed individuals on numerous medications and closely mimics the ground glass change of chronic HBV. However, the pseudo-ground glass is unrelated to HBV and appears to represent abnormally deposited glycogen.

### 1311 Telangiectatic Focal Nodular Hyperplasia: An Appraisal of Its Diagnostic Features in Comparison to FNH and Hepatocellualr Adenoma

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**Background:** Telangiectatic focal nodular hyperplasia (TFNH) of the liver is an uncommon neoplasm that was initially believed to be a variant of focal nodular hyperplasia (FNH). However, recent molecular studies indicate that TFNH is a monoclonal neoplasm, similar to hepatocellular adenoma. Because of the therapeutic and pathogenetic implications, the accurate diagnosis of TFNH is critical. To help clarify its diagnostic features, we report on 6 cases of TFNH and compare the findings to both classical FNH and hepatocellular adenoma.
**Design:** Review of the surgical pathology database at Mount Sinai Medical Center between 2002 and 2005 revealed 6 cases of TFNH, 4 surgical resections and 2 needle biopsies. Ten patients with FNH and hepatocellular adenoma were also identified. All available H+E slides for TFNH were reviewed. Histochemical stains including reticulin, masson trichrome, and Verhoeff's elastic stain were performed on all cases.
**Results:** In contrast to FNH and hepatocellular adenoma, all 6 cases of TFNH show marked dilatation of sinusoids, and dystrophic/fibrotic portal tracts. Some of the histomorphological findings, including ductular proliferation, thick fibrous septa, and unpaired dystrophic arteries were similar to FNH. However, central scar with fibrous bands was absent in all 6 cases. Other histomorphological features were similar to hepatocellular adenoma, such as greater than 2 cell-thick plates, hemorrhage and necrosis.
**Conclusions:** Despite many similarities between TFNH and either FNH or hepatocellular adenoma**, c**ertain morphologic criteria are useful in identifying TFNH as a unique neoplastic entity. Further ancillary studies including immunohistochemical stains and molecular techniques should further aid in elucidating the underlying pathogenesis and prognosis.

### 1312 Incidence and Pathological Features of Malignancies Developing in Liver Allografts Post Orthotopic Liver Transplantation

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**Background:** The development of malignancy in liver allografts following orthotopic liver transplantation is a disastrous complication. Reported malignancies include recurrent hepatocellular carcinomas (HCCs), de novo HCCs, de novo sarcoma, de novo posttransplantation lymphoproliferative disorder (PTLD), and metastatic non-HCC carcinomas (mets) from either donor or recipient origin. The incidence and the pathological features of those malignancies have not been well characterized. We report our observations based upon a single institutional experience.
**Design:** Patients who underwent orthotopic liver transplantation for HCCs at the Mount Sinai Medical Center from 1994-2005 were retrieved from the database of the Department of Pathology. Only patients with malignancies in the allografts confirmed

by histological examinations were included in this study. The clinical information and pathological diagnosis were reviewed and analyzed. To distinguish donor versus recipient origin, some malignancies were studied using sex chromosomal determination by fluorescent in situ hybridization (FISH) test (sex-FISH).
**Results:** Fifteen of 385 (3.9%) studied recipients transplanted with HCCs developed malignancy in the allografts 7 to 101 months (median 22 months) posttransplantation. Among them, seven patients had recurrent HCCs (47%), four PTLDs(27%), two de novo HCCs (13%), and two mets (13%) (lung adenocarcinoma and squamous cell carcinoma, SCC). Morphologic features and sex-FISH confirmed the 7 recurrent HCCs and 1 metastatic lung cancer to be of recipient origin, two HCCs and 4 PTLDs were arising de novo in the allografts, and one SCC was of donor origin. All recurrent HCCs and mets showed similar or lower grade differentiation compared to the original tumors. The original tumors in patients with recurrent HCCs were multifocal in most of cases (6/7) and measure 2.2 to 5.8cm (median 2.5cm). Five of them (71%) had microvascular invasion.
**Conclusions:** Liver allografts are prone to develop malignancy due to the immunosuppressant post transplantation. The most frequent malignancies developing in the allografts are recurrent HCCs followed by de novo PTLDs and de novo HCCs, but metastatic non-HCC carcinomas also occur. Lymphovascular invasion appears to be a risk factor for recurrent HCCs in the allografts.

### 1313 Centrizonal Ductular Reaction in Subacute and Chronic Liver Disease

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**Background:** Ductular reaction (DR) and CK7+ hepatocytes, also known as intermediate cells (ICs) are typically associated with cholestatic, portal-based chronic liver disease. The origin of the CK7+/CK19+ ductules may likely be the hepatic progenitor cells (originating in canals of Hering) in periportal zones. DR may also be present in subacute stages of acute hepatitis and in chronic centrizonal damage, but the origin of this form of DR is not clear. We studied the morphology and immunoperoxidase profile of DR and ICs in cases of subacute and chronic liver disease with centrizonal injury.
**Design:** We reviewed centrizonal liver injury (CZ) in 3 subacute lesions (2 biopsies, 1 explant, approx 1 mon post injury due to acetaminophen) and 3 chronic lesions (explants with alcoholic cirrhosis). Immunohistochemical staining for CK7, CK19, MIB1, and anti-hepatocyte Ab (HAb) was performed. In addition, 20 serial sections cut at 5μ and stained for CK7 were examined on one case of alcoholic cirrhosis.
**Results:** *Subacute CZ injury*. CK7+ ductular reaction was prominent, and correlated well with staining of CK19+ ductules. Essentially no ICs were present. MIB1 was positive in numerous hepatocytes and in a few ducts, but rarely in DR. DR with mixtures of cells either positive for HAb or CK19 were easily seen (and designated "hepatoductules"). *Chronic CZ injury.* CK7+ ductular reaction was prominent. In contrast, CK19 showed numerous ductule-like structures with weak to absent staining. HAb showed some DR with focally positive cells ("hepatoductules"). ICs were focally present within cirrhotic nodules. MIB1 was essentially negative in ductular reaction and hepatocytes. Serial sections of numerous scarred CZs did not reveal obvious origination of ductular reaction from periportal areas or interlobular bile ducts. Focal hepatocyte differentiation of an interlobular bile duct was also rarely noted.
**Conclusions:** The morphology and staining pattern of ductular reaction supports a metaplastic process in response to injury wherein the hepatocytes convert to ductular structures in both centrizonal subacute and chronic hepatocyte damage. The absence of MIB1 staining in DR argues against a proliferative response of cells of ductular type. Hepatocytes may transition to ductular reaction via "hepatoductules" without necessarily involving a periportal/canal of Hering stem cell component. ICs may also be a "transitional" route to ductular reaction in chronic injury.

### 1314 Morphologic Characterization of Explanted Liver-Derived Insulin-Producing Beta-Like Cells

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**Background:** Transdifferentiation of liver stem cells into insulin-producing cells (IPC) may provide potential cell source for islet cell transplantation in treatment of patients with type 1 diabetes. We previously reported the feasibility of converting the liver cells into functional IPC by expressing Pdx1-VP16, a modified pancreatic transcription factor essential for pancreas development and maintenance of beta-cell function. Transplantation of these cells into the left renal capsule of diabetic mice restores euglycemia. However, no study has systematically addressed morphologic, immunophenotypic, and ultra-structural characteristics of these liver-derived IPC after transplantation and their interaction with diabetic microenvironment on the process of further differentiation and maturation into functional beta-like IPC.
**Design:** In this study, we made a systematic cytologic, histologic, and ultrastructural examination of the explanted liver-derived IPC (using native beta cells as reference) by Hematoxylin and Eosin staining, immunohistochemsitry, and electron microscopy combined with immunoglod-labeling.
**Results:** We found that the genetically modified liver-derived IPC resemble the native beta-cells based on the following: 1) Cytological feature of these cells are typical of the neuroendocrine cells with salt and pepper chromatin and abundant cytoplasm. 2) These cells expressed several key pancreatic transcription factors (Pdx1, Nkx6.1, and Islet-1). 3) They produced insulin exclusively but not glucagon. 4) The cellular insulin content is directly related to proximity to the capillaries. 5) The insulin-secretory granules are situated in the side near the capillary endothelium, a features characteristic of endocrine cells.
**Conclusions:** In conclusion, our studies indicate that genetically engineered liver-derived IPC can faithfully recapitulate the physiologic and anatomic features of the native beta-cells, thus commending their further study as beta-cell surrogates for cell replacement therapy.

### 1315 Preclonal p53 Loss and Telomere Lengthening in Random Hcv Cirrhosis Identify Hepatocellular Carcinoma Patients

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**Background:** Patients with HCV cirrhosis are at increased risk for hepatocellular carcinoma (HCC), but no adequate screening tests yet exist to identify the approximately 10% minority who develop HCC. We hypothesize that the diffuse inflammatory damage imparted by HCV induces similarly organ-wide genomic injury that could serve as a biomarker to differentiate patients with HCC ("progressors") from those without it ("nonprogressors").
**Design:** Thirty-nine patients with HCV cirrhosis who underwent liver transplantation were studied, including 23 progressors with HCC and 16 nonprogressors. Hepatocyte preparations from random non-tumorous cirrhotic liver, 3 HCC, and 6 normal control livers were analyzed by FISH chromosome 17 centromere and p53 arm probes (Vysis). Telomere length was measured by quantitative PCR, generating a ratio between the quantity of telomeric DNA and the quantity of a single copy control gene. Logistic regression and receiver operating characteristics (ROC) curve analyses were done to determine whether these assays discriminate HCC progressors from nonprogressors.
**Results:** FISH demonstrated p53 loss in a mean of 3.8% of cells from nonprogressors versus a mean of 8.0% of cells from progressors (two-sided Wilcoxon rank sum test $P<0.005$). Gains of p53 and centromere abnormalities did not differ significantly between the groups. Average telomere length values for nonprogressor cirrhotic liver was 0.922 versus 1.183 in progressors (Satterthwaite t-test $P<0.001$). Using logistic regression with 1000 bootstrap re-sampling for variable selection, p53 loss percentage and telomere length were the significant predictors, yielding an area under the ROC of 0.929 and a sensitivity of 87% and specificity of 81% with optimal choice of threshold (likelihood ratio chi-squared $P<0.0001$). Normal liver controls demonstrated p53 loss in a mean of 1.5% of cells and average telomere length value of 1.138. Mean HCC telomere length was 0.55.
**Conclusions:** These results demonstrate that HCC progressors in HCV cirrhosis harbor genetic alterations that are detectable *in a random biopsy* with high sensitivity and specificity. These data not only provide important insights into the organ-wide genetic changes that accompany the development of HCC in HCV cirrhosis but also show great promise for improved biomarkers of HCC risk.

### 1316 Panin in Heterotopic Pancreas: Evidence for the Progression Model

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**Background:** Morphologic, clinical and genetic evidence suggests that pancreatic intraepithelial neoplasia (PanIN) is a precursor to ductal adenocarcinoma. A progression model proposes that the change from normal epithelium to PanIN to infiltrating cancer is accompanied by the accumulation of genetic changes. But studying precursor lesions in a pancreas with existing tumor is hampered by the fact that obstructrion by a mass can lead to low-grade PanIN. Further, cancerization of ducts can mimic high-grade PanIN. Finally, chronic pancreatitis is commonly associated with carcinoma, and the possible interactions between tumor, pancreatitis and PanIN are complex. Heterotopic pancreas is a relatively common anomaly usually found in the upper gastrointestinal tract. Because such heterotopias have a genetic make-up, physiologic function and local environmental exposure similar to that of the pancreas, they offer an opportunity to study putative precursor lesions in a setting free of confounding factors.
**Design:** We identified five pancreatic cancer patients between 1975 and 2004 who had heterotopic pancreas removed at the time of surgery for ductal adenocarcinoma in the head of the pancreas. Twenty-eight examples of pancreatic heterotopia from patients without known ductal adenocarcinoma served as controls. Four controls had other pancreatic neoplasms (ampullary carcinoma, serous cystic adenoma, islet cell tumor and IPMN); the others had apparently normal pancreas. All slides were reviewed independently by two pathologists blinded to patient background. Pancreatic ducts were classified as normal, PanIN1A, 1B, 2 or 3 according to published criteria. All 5 cases from cancer patients were stained for p53, cyclin D1 and p16, as were intrapancreatic PanIN from the corresponding ductal adenocarcinomas.
**Results:** All five cancer-associated heterotopias had PanIN 1A or 1B; three had PanIN 2 and one had PanIN 3. Two of 28 controls had PanIN 1A. The PanINs in all 5 heterotopias showed focal nuclear accumulation of p53, overexpression of cyclin D1 and loss of p16 expression. Intrapancreatic PanINs from the 5 corresponiding carcinomas had a similar pattern of abnormalities. There was minimal fibrosis, no inflammation and no acinar destruction in the cancer-associated heterotopias, despite the presence of chronic pancreatitis in all 5 cancers.
**Conclusions:** The presence of PanIN in heteropic pancreas from patients with ductal cancer supports the progression model and provides an opportunity to study it in the absence of confounding factors. Chronic pancreatitis is not a cause of PanIN in heterotopic pancreas.

### 1317 IgG4 Stain for the Diagnosis of Autoimmune Pancreatitis: Helpful in Pancreatic Biopsies, but Not in the Duodenum

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**Background:** Autoimmune pancreatitis (AIP) produces an enlarged pancreas that can mimic carcinoma. Because AIP usually responds to corticosteroid therapy, it is important to distinguish it from carcinoma. Our previous work has shown that IgG4 stain can be a useful aid to the diagnosis in resected specimens; here we apply it to needle biopsies in an effort to establish a preoperative diagnosis. It has been suggested that AIP is actually a systemic "IgG4 disease," with increased numbers of IgG4-positive plasma cells in a variety of organs, including the gut. We stained duodenal mucosa from patients known to have AIP, and compared the results to a broad range of control specimens.

**Design:** Needle bioipsies of pancreas were obtained prospectively from patients suspected of having AIP on clinical grounds. Duodenal mucosa was obtained retrospectively by sampling mucosa from Whipple resections done for pancreatic masses later confirmed as AIP. For controls, we selected a series of duodenal biopsies, including 20 diagnosed as normal, 19 with Giardiasis, 20 with celiac disease, 24 with peptic duodenitis and 10 associated with adenoma. Immunohistochemical stain for IgG4 was scored semiquantitively as previously described: 0 = 1 to 5 positive cells/high power field; 1 = 6-10 positive cells; 2 = 11-30 positive cells; 3 = >30 positive cells.

**Results:** All needle biopsies of pancreas had increased staining for IgG4, helping to confirm the diagnosis of AIP. Duodenal mucosa from patients with known AIP did not show more IgG4-positive cells than controls.

IgG4 stain in pancreatic biopsy and duodenal mucosa

| | Pancreas | Duodenal mucosa | | | | | |
|---|---|---|---|---|---|---|---|
| IgG4 score | AIP | AIP | Normal | Giardia | Celiac | Duodenitis | Adenoma |
| 0 | 0 | 7 | 18 | 17 | 14 | 20 | 3 |
| 1 | 2 | 1 | 2 | 2 | 5 | 4 | 5 |
| 2 | 2 | 1 | 0 | 0 | 1 | 0 | 2 |
| 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |

**Conclusions:** Immunohistochemical staining for IgG4 is a useful adjunct to the diagnosis of AIP in needle biopsies, particulary since helpful histologic clues such as periductal lymphoplasmacytic infiltrates and obliterative phlebitis may not be sampled by needle biopsy. IgG4 staining of duodenal biopsies will not help identify patients with AIP.

# Neuropathology

### 1318 CADASIL: New Evidence of Vascular Degeneration

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**Background:** CADASIL, or Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy, is a hereditary form of vascular dementia caused by mutations in the Notch 3 gene which is located on Chromosome 19. The clinical course is characterized by early onset of migraine headaches, progressive occurrence of transient-ischemic attacks and strokes, depression, dementia, and premature death. The target structures in CADASIL include mainly medium and small vessels within the cerebral white matter, but extra-CNS sites such as skin may also be affected. Although the Notch 3 mutations are present from birth, the disease does not manifest itself until the third or fourth decade of life. To understand the possible mechanisms of disease progression, we examined gene expression related to Notch signaling in postmortem brain tissue from patients with CADASIL or normal aging.

**Design:** Formalin-fixed paraffin-embedded tissue sections were immunostained to detect Notch 1, Notch 3, smooth muscle actin, ubiquitin, and insulin-like growth factor, type 1 receptor. RNA was extracted from fresh frozen brain tissue to perform real time quantitative RT-PCR analysis of the same genes to determine if their altered levels of expression were mediated at the level of transcription.

**Results:** Immunostaining studies demonstrated that the granular degenerative changes in the media of white matter and leptomeningeal arterioles was associated with increased smooth muscle actin fragmentation and ubiquitination of proteins. In addition, the levels of insulin-like growth factor (IGF-I) receptor expression in the vessels were reduced. Real time quantitative RT-PCR studies using RNA isolated from white matter vessels confirmed the significantly reduced IGF-I receptor expression, as well as down-regulation of smooth muscle actin and both Notch 1 and Notch 3. Further in vitro experiments showed that Notch expression was regulated by IGF-I signaling.

**Conclusions:** Vascular degeneration in CADASIL is associated with down-regulation of genes encoding the IGF-I receptor, smooth muscle actin, Notch 1, and Notch 3. Since IGF-I regulates Notch expression, and Notch regulates cytoskeletal function, impaired IGF-I signaling in vessels may contribute to the progression of CADASIL vasculopathy with increasing age.

### 1319 Evaluation of NF2 Gene Deletion in Sporadic Schwannomas, Meningiomas and Ependymomas by Chromogenic In Situ Hydridization

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**Background:** Neurofibromatosis type 2 (NF2) is an autosomal dominant cancer syndrome characterized by the development of bilateral vestibular schwannomas and other central nervous system (CNS) tumors, in particular meningiomas, gliomas and ependymomas. The NF2 gene has been isolated from chromosome 22 and germ line mutations have been identified in NF2 patients. Fluorescence in situ hybridization (FISH), loss of heterozygosity (LOH)-testing, and comparative genomic hybridization (CGH) have been used to detect NF2 gene alterations in both sporadic and NF2-associated CNS tumors. In this study, we performed chromogenic in situ hybridization (CISH) to evaluate for NF2 deletion in a group of sporadic schwannomas, meningiomas and ependymomas.

**Design:** Eighteen sporadic lesions, including nine ependymomas, six meningiomas and three schwannomas were included in this study. CISH was performed utilizing the NF2 deletion probe (Zymed Laboratories). Deletion of NF2 gene was identified when the NF2 gene copy number was less than the centromeric copy number in more than 50% of tumor cells. Cases were also categorized as normal diploid when two copies were present in > 50% of tumor cells and aneuploid when 3-5 copies were seen.

**Results:** Deletion of the NF2 gene was identified in 10 tumors, including 2 out of 3 schwannomas, 5 out of 6 meningiomas, and 3 out 9 ependymomas. The remaining eight cases were diploid.

**Conclusions:** Our results show that schwannomas, meningiomas and to a lesser degree ependymomas express a high incidence of NF2 gene deletion, and support the hypothesis that NF2 gene plays an important role in the tumorigenesis of these tumors. We have used CISH as an efficient, economic and reliable method for routinely assessing NF2 gene deletion in sporadic schwannomas, meningiomas and ependymomas.

### 1320 1p/19q Loss in Gliomas: Microsatellite Amplification in Comparison with FISH

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**Background:** 1p/19q loss is a favorable indicator of chemosensitivity and prolonged survival in anaplastic oligodendroglioma. Determination of 1p/19q status may also help to subclassify oligoastrocytomas and to distinguish glioblastoma from anaplastic oligodendroglioma. Fluorescent in situ hybridization (FISH) and microsatellite amplification by polymerase chain reaction (PCR) are the two main techniques used for 1p/19q loss determination.

**Design:** To compare the results of FISH and PCR in 1p/19q loss determination, both techniques were carried out on 48 glioma tissue samples. These included 9 grade II oligodendrogliomas (ODs), 8 grade III oligodendrogliomas (AODs), 2 grade II oligoastrocytomas (OAs), 3 grade III oligoastrocytomas (AOAs), 4 grade III astrocytomas (AAs), and 22 glioblastomas (GBs). In regard to FISH, for each chromosome we used a probe specific for the potentially deleted arm and a probe for the opposite arm as control. A total of 300 cell nuclei were evaluated per slide. As for PCR, 4 microsatellites were amplified for each chromosome (D1S199, D1S214, D1S508 and D1S2734 for 1 p; and D19S112, D19S219, D19S412 and D19S596 for 19q). LOH was analyzed with GeneScan. Peripheral blood or normal tissue from the same patient was used as control.

**Results:** In 41 of the 48 cases, FISH and PCR results were completely coincidental. In 4 cases (3 ODs and 1 AOD) tissue was insufficient for FISH evaluation. In 1 case (AA) PCR showed 1p deletion undetected by FISH, and in 2 GBs FISH suggested 1p and 19q losses not shown by PCR. Combined 1p /19q losses were present in 4 ODs, 3 AODs, 1 OA and 2 GBs, by either FISH or PCR, or by both. Isolated 1p loss was seen in 1 AA and 4 GBs, and isolated 19q loss in 1 AOA and 5 GBs.

**Conclusions:** Our findings indicate that FISH and PCR provide virtually coincidental results in regard to determination of 1p/19q loss. It seems thus that the experience and facilities available should be the main considerations to be taken into account by each institution when deciding which method to implement for the study of 1p/19q status.

### 1321 Diagnostic Utility of Microtubule Associated Protein-2 in Separating Schwannoma from Meningioma Including the Fibrous Subtype

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**Background:** Both schwannomas and meningiomas can occur at the cerebello-pontine angle and histologically display a spindle cell morphology. Separating schwannomas from meningiomas is usually not difficult based on clinical, histologic and immunohistochemical studies. However, fibrous meningioma can show some histological and immunophenotypic features of schwannomas such as cellular and acellular areas, thickened hyalinized blood vessels, elonged and twisted nuclei, positive immunostaining for S-100 and negative immunostaining for EMA. Microtubule-associated proteins are a major component of cytoskeleton family proteins associated with microtubule assembly and is specifically expressed in the central and peripheral nervous systems. However, the expression of MAP-2 in schwannoma, as well as diagnostic utility of MAP-2 in separating schwannoma from meningioma including the fibrous subtype, has not been studied.

**Design:** A total of 40 cases, consisting of 20 schwannomas and 20 meningiomas including 3 fibrous meningiomas, were retrieved from the hospital database. Immunostaining with antibodies to MAP-2 were performed on paraffin-embedded tissue. Immunostains were performed on an automated immunostainer with appropriate positive and negative controls.

**Results:** Diffuse and strong MAP-2 immunoreactvity was demonstrated in 19/20 (95%) of schwannomas, while focal immunoreactivity was demonstrated in 1/20 (5%) meningiomas. None of the fibrous meningiomas exhibited immunoreactivity for MAP-2.

**Conclusions:** MAP-2 is expressed in schwannomas, but not in most meningiomas, including fibrous meningiomas. The expression of MAP-2 may be useful in distinguishing schwannomas from fibrous meningiomas especially when limited material is present in a brain biopsy.

### 1322 PTEN Loss by Gliomas Induces Endothelial Tissue Factor Expression

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**Background:** Glioblastoma (GBM) is a high grade, rapidly fatal infiltrative astrocytoma distinguished by pseudopalisades, which are hypoxic, densely cellular zones surrounding necrosis. Pseudopalisades are critical to the rapid biologic progression of GBM, yet initiating mechanisms are unknown. We have proposed that intravascular thrombosis promotes pseudopalisade formation following PTEN loss. The aim of this study is to define factors secreted by gliomas following PTEN loss that lead to endothelial tissue factor (TF) expression and thereby promote thrombosis.

**Design:** We used a PTEN null human GBM cell line (U87MG) with a stably transfected muristerone-inducible wt PTEN (23.11 cells) to model glioma progression. Conditioned media from 23.11 +/- PTEN was collected after 96 hours of hypoxia (1% $O_2$) or normoxia (21% $O_2$) and tested for its ability to induce TF expression by human dermal microvascular endothelial cells (HDMEC). Conditioned media was added to HDMEC for 24 hours and endothelial TF was analyzed by Western Blot. VEGF and