1263 Renal Pathology in Hematopoeitic Cell Transplantation Recipients

ML Troxell, M Pilapil, D Miklos, JP Higgins, N Kambham. OHSU, Portland, OR; Stanford Univ, Stanford, CA.

Background: Hematopoietic cell transplantation (HCT) associated acute and chronic renal toxicity can be due to cytotoxic conditioning agents, radiation, infection, immunosuppressive agents, ischemia, and graft versus host disease (GVHD). We have reviewed consecutive renal biopsy specimens in HCT patients from a single center.

Design: The files of Stanford University Medical Center Department of Pathology were searched for renal biopsy specimens in patients who received HCT (1995-2005); 11 cases were identified (post BMT time 0.7 to 14.5 years). The biopsies were processed using standard techniques, and the findings were correlated with clinical data derived from medical records.

Results: The most common indication for HCT was a hematopoietic malignancy while one patient had stage IV breast carcinoma; 5 patients had autologous HCT using GCSF mobilized peripheral blood stem cells (PBSC), and 6 received HLA identical allogeneic PBSC transplants. Indications for renal biopsy included severe proteinuria (n=4), increased serum creatinine (n=4), or both (n=3). Findings on renal biopsy were variable, with many patients having more than one type of lesion. Membranous glomerulonephritis (MGN) was the most common diagnostic category (n=5), including 2 patients with autologous HCT and 3 with evidence of chronic GVHD elsewhere. 3 MGN patients achieved sustained remission with Rituximab therapy. Acute tubular necrosis (ATN) and/or tubulointerstitial nephritis was seen in 3 patients. Features of thrombotic microangiopathy (TMA) were present in the remaining 3 biopsies, including one attributable to calcineurin inhibitor toxicity. Of 10 patients with followup (2-64 months, median 14 months), 6 had chronic renal insufficiency, 1 had ESRD, and 3 had essentially normal renal function; only 2 had relapse of primary disease (one with ATN, one TMA).

Conclusions: HCT patients exhibit variable biopsy findings, the most common being MGN. Although MGN is frequently observed in allogeneic HCT patients with chronic GVHD, its occurrence after autologous HCT suggests other etiologies. After both auto and allogeneic HCT, MGN may be treated successfully with Rituximab therapy.

1264 Time Dependent Effects of Peroxisome Proliferator-Activated Receptor-γ (PPARγ) Agonist on Acute Puromycin Aminonucleoside (PAN)-Induced Nephrotic Syndrome

Y Zuo, L Ma, AB Fogo. Vanderbilt University, Nashville, TN.

Background: We have previously observed that PPARγ agonists benefit injury in both acute and chronic PAN models, but not if given before PAN. We therefore investigated the mechanisms of time dependence of PPARγ agonist pioglitazone (Pio) effects in PAN nephrotic syndrome.

Design: Adult male SD rats received a single dose of PAN (100mg/kg Bwt, i.p.), and were divided into 4 groups: PAN without further treatment (PAN); Pio (10 mg/kg/d) starting 7 days before PAN (prePio), Pio starting the same day as PAN (Pio0) and Pio starting 4 days after PAN (Piopost). Pio was continued until sacrifice. Serial functional and structural analyses were performed and compared to saline injected rats (N).

Results: Proteinuria (Uprot) increased similarly in all PAN groups by day 4 (PAN 405.8±10.4, prePio 403.9±13.2, Pio0 331.4±69.7, vs N 154.4±12.8 mg/24hr, $P<0.05$). Treatment with Pio at the time of injury or 4 days after significantly reduced day 10 and 21 Uprot (Pio0 605.8±54.2, Piopost 483.3±27.8, vs PAN 758.2±71.6 mg/24hr and Pio0 458.0±78.1, Piopost 474.1±96.4, vs PAN 923.8±93.4 mg/24hr, respectively). By contrast, pretreatment with Pio failed to reduce Uprot either at day 10 or 21 (651.2±50.2 and 729.8±55.6mg/24hr, respectively). Body weight, creatinine clearance (a measure of glomerular filtration rate), plasma volume, and solute-free water clearance rate did not differ among the PAN groups. Pio remarkably decreased the urinary fractional excretion of sodium (Pio0 0.34±0.01, Piopost 0.39±0.05, prePio 0.52±0.02 vs PAN 1.03±0.23% FeNa, $P<0.05$). Potassium excretion also decreased in Pio0 or Piopost but not in prePio (Pio0 26.6±3.7, Piopost 29.7±4.6, prePio 44.9±6.0 vs PAN 44.5±6.2% FeK). Podocyte differentiation, assessed by synaptopodin, was preserved in Pio0 and Piopost, but was remarkably downregulated in prePio. Electron microscopic analysis at day 10 revealed prominent podocyte degeneration with complete foot process effacement (FPE), vacuoles, and microvillous transformation in prePio. In contrast, podocyte injury was less severe in postPio with only subtotal FPE.

Conclusions: Our study shows that PPARγ agonist given simultaneously or even after injury provides time-dependent protective effects against proteinuria in acute nephrotic syndrome without significantly impacting glomerular filtration and fluid excretion. These complex time dependent effects of PPARγ on proteinuria in acute nephrotic syndrome are dependent at least in part on effects on podocyte injury.

Liver & Pancreas

1265 Centrilobular Hepatitis in Pediatric Liver Transplants

SC Abraham, AM Krasinskas, TT Wu. Mayo Clinic, Rochester, MN; Univ of Pittsburgh, Pittsburgh, PA.

Background: Centrilobular hepatitis (CLH) encompasses dropout of zone 3 hepatocytes, red blood cell extravasation, and varying degrees of mononuclear inflammation in the pericentral regions. In the liver transplant (OLT) setting, CLH can occur in isolation or it can occur in association with portal-based disease such as acute rejection (AR). CLH is thought to represent one manifestation of chronic rejection, particularly when accompanied by zone 3 fibrosis. Prior studies of CLH in pediatric liver allografts have been hampered by lack of protocol biopsies and low rates of histologic follow-up.

Design: We studied 58 consecutive liver allografts from 53 pediatric patients (≤18 yrs) who underwent OLT from 1995-2006. All allograft biopsies were scored for the following features: 1) CLH (mild, moderate, severe), 2) portal AR (mild, moderate, severe), 3) zone 3 fibrosis (mild=perivenular or severe=bridging), and 4) ductopenia. Five explanted livers that were removed during the course of retransplantation for graft failure in this group were also reviewed.

Results: Mean age at OLT was 7 yrs (range 7 wks-18 yrs) with 29 boys and 24 girls. We reviewed a total of 417 allograft biopsies (mean 7.2 per allograft) obtained 2 days - 11 yrs post-OLT; 200 (48%) of these were protocol biopsies. Forty-six allografts (79%) had ≥1 yr of histologic follow-up, 29 (50%) had ≥3 yrs, and 21 (36%) ≥5 yrs. Overall, CLH was observed on at least one occasion in 38 (66%) allografts. Of 119 biopsies showing CLH, 70 had CLH + AR, 18 had CLH following an episode of AR within the prior month, 28 had isolated CLH, 3 had CLH + *de-novo* autoimmune hepatitis, and 1 had CLH + EBV infection. Isolated CLH was seen in only 15 (26%) liver allografts; in 2 cases it was due to ischemia or Budd-Chiari syndrome whereas in the other 13 (22%) it appeared to be an immunologic phenomenon. Twenty-four (63%) allografts with CLH developed zone 3 fibrosis (16 mild, 8 severe), 6 (16%) developed ductopenia, and 4 (11%) required retransplant for chronic rejection (n=4). In contrast, only 1 (5%) allograft without CLH developed zone 3 fibrosis (mild; $p<0.0001$) and none developed ductopenia ($p=0.08$) or required retransplant for chronic rejection ($p=0.29$).

Conclusions: CLH is frequent (66%) in pediatric OLT patients. It is most common in association with portal AR or following an episode of portal AR; isolated CLH occurs in only 26% of pediatric liver allografts. CLH is significantly associated with the development of zone 3 fibrosis and there is a trend toward development of ductopenia and need for retransplantation.

1266 Value of Glypican-3 Immunostaining in the Diagnosis of Hepatocellular Carcinoma on Needle Biopsy

F Anatelli, ST Chuang, XJ Yang, HL Wang. Washington University School of Medicine, St. Louis, MO; Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: Histologic diagnosis of hepatocellular carcinoma (HCC) on a needle biopsy can be challenging, particularly in a cirrhotic background. It has been recently reported that glypican-3 (GPC3), a membrane-bound heparan sulfate proteoglycan, is overexpressed in a large proportion of HCCs but undetectable in benign liver tissues. These observations suggest that GPC3 may serve as a very useful biomarker in the diagnosis of difficult HCC cases. However, the diagnostic value of GPC3 immunostaining on needle biopsies has not yet been assessed.

Design: A total of 108 liver needle biopsies were examined in this study. These included 46 cirrhotic livers and 62 HCCs with diagnoses confirmed by subsequent liver resections. Formalin-fixed, paraffin-embedded tissue sections were subjected to immunohistochemical staining using a monoclonal antibody specific for GPC3 (clone 1G12, Biomosaics, Burlington, VT). Positive staining was graded as focal (≤ 50 of the tumor cells stained) or diffuse (>50%).

Results: Strong cytoplasmic and membranous staining for GPC3 was observed in 28 HCCs (45.2%), among which 18 cases (64.3%) showed diffuse immunoreactivity. None of the 46 cirrhotic livers exhibited positive GPC3 immunostaining. The non-neoplastic liver tissues (cirrhotic or non-cirrhotic) that were present in the majority of the HCC cases were also completely negative for GPC3 expression.

Conclusions: GPC3 is a reliable immunohistochemical marker for the diagnosis of HCC on needle biopsies with a high specificity (100%). However, the sensitivity (45%) in our series appears to be lower than that reported in previous studies employing resection specimens as the studying materials. Our findings emphasize the fact that GPC3 immunoreactivity can be focal and that negative staining should not exclude the diagnosis of HCC in challenging needle biopsies.

1267 Reevaluation of "Cancerization of the Duct" by Pancreatic Cancers

S Ban, Y Shimizu, F Ogawa, M Shimizu. Saitama Medical University School of Medicine, Moroyama, Iruma, Saitama, Japan.

Background: Cancerization of the duct (CD) was introduced as one of the mimickers of PaIN in 2001 (Am J Surg Pathol 25:579-586). Afterwards, a consensus meeting of PanIN was held in 2003, and at that meeting two patterns of CD were illustrated by one of the authors of this abstract (MS). At that meeting, CD was defined as a process of secondary involvement of the duct by invasive pancreatic cancer. However, the concept of CD was not totally clarified, especially regarding CD vs. PanIN-3.

Design: Twenty cases of resected pancreatic cancer were evaluated using elastica Masson Goldner stain (EMG) to identify preexisting pancreatic ducts and/or ductules within the area of invasive cancer. Then, the following two patterns of CD were reevaluated. One of the patterns of CD, which is called ductal colonization, and is also known as intraductal spread or intraductal extension, is defined as a direct invasion by invasive cancer cells into the duct continuous to the main lesion. The other CD pattern is ductal invasion, defined as an invasion from the outside of the duct by invasive cancer cells.

Results: Intraductal components (either with papillary structure or with tubular structure) identified by using EMG were often found in the area of invasive cancer. Compared to ductal colonization, luminal obstruction or ductal destruction was more frequently observed in ductal invasion, which was clearly identified by using EMG. In most cases, either ductal colonization or ductal invasion showed similar microscopic findings of the cancer cells between the inside and the outside of the ducts in most cases. Detection of PanIN-3 was very difficult in areas far from the cancerous area.

Conclusions: Recognition of the degree of atypia and the presence of the papillary structure may be helpful to differentiate noninvasive components from invasive components when evaluating the duct by EMG. Luminal obstruction or ductal destruction may favor the diagnosis of ductal invasion. In addition, a mixed pattern comprised of the above-mentioned two patterns may be present.

1268 Development of Unbiased Quantitative Method for the Estimation of Liver Steatosis

M Bansal, A Ormsby, D Schultz, B Jones. Henry Ford Hospital, Detroit, MI.

Background: The degree of macrosteatosis is an important factor in determining whether or not a donor liver is acceptable for transplantation. Livers with >30% macrosteatosis have a 25% chance of developing primary graft failure. The degree of steatosis in liver biopsies is usually assessed by a morphological semiquantitative approach in which percentage of the tissue occupied by steatosis is estimated. This method has limitations as it is subjective and has considerable interobserver variation, particularly in frozen section material with the introduction of freezing artifact. Our aim was to assess the utility of stereological point counting as an objective measure of macrosteatosis using an eyepiece grid.

Design: A total of 52 liver biopsies stained with H and E, both with frozen and corresponding paraffin sections were selected from the files of the Department of Pathology at Henry Ford Hospital. Out of these 52, three biopsies were not evaluable as frozen section tissue was small and poorly prepared. The remaining 49 biopsies were independently scored by three observers (one resident, one transplant general pathologist and one gastrointestinal pathologist). Four representative fields from each biopsy were selected and a point grid lattice was superimposed. Numbers of hits on fat globules were counted, out of a total of 400 points assessed; this tally was divided by 4 to calculate an average score per field.

Results: On frozen section, for each observer the range of count of hits for each case (median) were: 0-42.5 (2), 0-35.5 (1), and 0-19 (1), respectively; on paraffin section, these were: 0-40.5 (1.5), 0-39 (1.25) and 0-21 (1.5), respectively. Overall (between all observers and tissue preparation types), Kendall's coefficient of concordance was 0.88, for frozen section, between the three observers it was 0.91; for permanent section, this was 0.97 (all $p<0.001$). Intra-observer concordance between frozen and permanent section counts was 0.91, 0.91 and 0.93, respectively (all $p<0.001$).

Conclusions: Stereological point counting for steatosis in allograft liver needle biopsies is feasible and highly reproducible among pathologists with differing proficiencies in gastroenterologic pathology. Further study will compare the relationship between this method of assessing steatosis to allograft survival, compared to traditional semiquantitative assessment.

1269 Overexpression of Ataxia Telengectasia Group D Complement Gene (ATDC) in Pancreatic Adenocarcinoma

O Basturk, DM Simeone, L Wang, JK Greenson, E Levi, D Thirabanjasak, NV Adsay. New York Univ, New York, NY; Univ of Michigan, Ann Arbor, MI; Wayne State Univ-Karmanos Cancer Inst, Detroit, MI.

Background: We have recently determined that human pancreatic adenocarcinomas specifically overexpress Ataxia-Telangectasia Group D Associated gene (ATDC) 20 fold higher than normal pancreas and chronic pancreatitis (Cancer Res 63: 2649-57,2003) and have data strongly implicating ATDC as a novel DNA damage response gene. We hypothesized that ATDC expression levels may correlate with treatment response or survival time.

Design: The expression level of ATDC was tested by immunohistochemical staining in a tissue array containing 112 ductal adenocarcinoma of the pancreas; 18 PanIns and control samples of normal pancreas and chronic pancreatitis. 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, and Fas ligand expression as well as clinical data including survival.

Results: ATDC was not expressed in samples of normal pancreatic tissue or chronic pancreatitis. There was a progressive increase in the expression of ATDC with advancing neoplastic transformation 0/4 of PanIN1, 0/7 of PanIn2 and 3/6 PanIN3, and 104/112 (94%) of carcinoma samples showed ATDC expression (score 1: 24%, score 2: 39%, score 3: 31%). ATDC expression showed a very strong correlation with p27 expression ($p=0.029$), and a weak inverse association with p53 expression in the carcinomas (rho=-0.202, $p=0.28$). No significant correlation was identified between ATDC expression and age, race, gender, location of the tumor, size of the tumor, LN status, margin status, or survival or the molecular markers analyzed.

Conclusions: This immunohistopathological study confirms that overexpression of ATDC, a putative novel DNA damage response gene, is very common in ductal adenocarcinoma and is not a feature of normal or inflamed pancreatic tissue. Further studies are needed to determine the role of this molecule in the aggressive behavior and therapy resistance of pancreatic cancer. ATDC may also have diagnostic value in tissue sections, since it is generally not expressed in normal tissue and almost uniformly present in carcinomas.

1270 Immunohistochemical Evidence of Different Progenitor Cell Types in Human Hepatocellular Carcinoma: What Is the Significance?

LK Campbell, S Takemoto, EM Brunt. Saint Louis University, St. Louis, MO.

Background: The multistep model of hepatocarcinogenesis includes chronic liver injury and regeneration, yet there is increasing evidence for a significant role of liver stem/progenitor cells in the progression of malignancy (Cell Prolif 2005;38:407) as well as in prognosis (Nat Med 2006;12:410). There have been case reports and small series of hepatocellular carcinoma (HCC) with unique phenotype illustrating various progenitor cell markers. We questioned the presence of these markers in cirrhosis (CN), dysplastic nodules (DN) and common HCC.

Design: 40 nodules from 15 specimens (7 biopsies of HCC, 7 explants with CN, DN and HCC, and 1 wedge resection for HCC) were evaluated by HE and immunohistochemistry (IHC): MIB-1 (Dako,1:800), p53 (Dako,1:200), CK7 (Dako,1:1000), and CD117 (Dako,1:200). HCCs were subdivided by pattern (trabecular or other). All IHCs were scored 0-3 by examining 5 200X fields. Only hepatocyte nuclear reactivity was considered for MIB-1 and p53 IHC; MIB-1: 0: < 5%; 1: 6-10%; 2: 11-50%; 3: >50%; p53: 0:0; 1: 1-10%; 2: 11-50%; 3: >50%. CK7 positive progenitor cells (HPC) were single cells with high N:C ratio, not associated with ductular reactions or septa; intermediate hepatocytes (IH) were medium-sized hepatocytes with membranous/submembranous CK7 reactivity, usually in clusters. CD117 positive progenitor cells were single ovoid cells (some had cytoplasmic extensions) with cytoplasmic reactivity, lower N:C ratio than CK7 HPC, and sinusoidal in location (not within septa or portal tracts). CK7 HPC and IH and CD117 scored: 0: 0, 1: <5, 2:6-20; 3:>20.

Results: 8 CN, 13 DN, 19 HCC were studied. MIB-1showed progression from CN to DN to HCC ($p=0.017$), but did not correlate with either progenitor cell marker. p53 was greater in HCC than in CN or DN ($p=0.021$). While CD117 showed a trend of increasing reactivity CN to DN to HCC ($p=0.074$), no differences among nodules were seen with CK7 HPC or IH. There was a strong correlation ($p=0.002$) between CK7 HPC and IH that was not seen between CD117 and HPC or IH. Features that correlated with the 58% of HCC with positive p53 were trabecular pattern ($p=0.013$), increasing MIB-1 ($p=0.003$) and lower CD117 ($p=0.027$).

Conclusions: Our results highlight significant differences between at least two populations of progenitor cells in CN, DN and common HCC. Both may be involved in the pathways of hepatocarcinogenesis.

1271 Inhibitiory Effect of Celecoxib, a Nonsteroidal Anti-Inflammatory Drug, on Spontaneously Developed Hepatocellular Carcinomas in AOX Null Mice

W Cao, W Wiseman, P Kashireddy, MS Rao. Northwestern University, Chicago, IL.

Background: Fatty acyl-CoA oxidase (AOX) is the first and rate-limiting enzyme that participates in the peroxisomal β-oxidation of fatty acids. Mice with disrupted AOX gene (AOX null mice) develop progressive changes in the liver characterized by steatohepatitis by 2-4 months, spontaneous peroxisome proliferation between 4-6 months, altered areas between 6-8 months and hepatocellular carcinomas (HCC) in 100% of the animals between 10-15 months. It is suggested that high levels of arachidonic acid and its metabolites in the livers of AOX null mice cause steatohepatitis, cell proliferation and peroxisome proliferation leading to the development of HCC. Non-steroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of prostaglandin synthesis by inhibiting the activity of cyclooxygenases (COX). In this study we have evaluated the anticarcinogenic potential of celecoxib, a specific COX-2 inhibitor, in AOX null mice that develop HCC spontaneously.

Design: Five to six week old male and female AOX null mice were fed normal chow or chow containing celecoxib (500 ppm) until the termination of experiment at 13 to 14 months. Livers were sliced at 2-3 mm intervals and analyzed for the number and size of grossly visible lesions. Multiple sections from lesional and nonlesional areas of the liver were evaluated histopathologically.

Results: Ten of 12 (83%) male and 7 of 11 (63%) female AOX null mice fed normal chow developed HCC. Whereas, 6 of 12 (50%) male and 2 of 11 (18%) female AOX null mice fed diet supplemented with celecoxib developed HCC. Interestingly, the incidence of adenomas was similar in all groups (82-92%).

Conclusions: The results of this study clearly demonstrate that celecoxib caused a significant reduction in the incidence of HCC in AOX null mice suggesting the important role of COX-2 in HCC development in these mice. Since the development of HCC in humans with hepatitis C infection and fatty liver disease is associated with inflammatory reaction and increase in COX-2 enzyme, NSAIDs may be useful in inhibiting the development of these tumors. (This research was supported by NIH Grant CA84472).

1272 Patchy Distribution of Pathologic Abnormalities in Autoimmune Pancreatitis

VS Chandan, C Iacobuzio-Donahue, TC Smyrk, SC Abraham. Mayo Clinic, Rochester, MN; Johns Hopkins University, Baltimore, MD.

Background: Autoimmune pancreatitis (AIP) is a distinctive form of chronic pancreatitis that can mimic pancreatic carcinoma. In the past, AIP accounted for up to 34% of Whipple resections performed for benign conditions. More recently, with increased awareness of AIP as a mimic of carcinoma and with reports of steroid-responsiveness in this condition, tru-cut needle biopsies are increasingly used as an aid in pre-operative diagnosis of AIP. We noticed a distinctive patchy distribution to the pathologic abnormalities in some cases of resected AIP which could potentially interfere with pre-operative diagnosis by needle biopsy. This phenomenon has not yet been systematically investigated.

Design: The study population included 39 benign pancreatic resections with AIP. AIP was defined by the following triad of features: 1) lymphoplasmacytic infiltrates around ducts, 2) acinar lymphoplasmacytic inflammation, atrophy, and fibrosis, and 3) obliterative phlebitis. In 17 (44%) cases, immunostaining for IgG4 had also been performed as a confirmatory test, demonstrating ≥15 IgG4+ plasma cells/high power field. Criteria for inclusion in the study included either submission of the entire resection specimen (n=21) or extensive histologic sampling (n=18) defined as submission of ≥10 sections. All H&E-stained sections were reviewed and areas of involvement by AIP and sparing were mapped on each section. To be included as an area of sparing, both duct and acinar parenchyma had to be free of lymphoplasmacytic inflammation, and the focus had to be at least 0.5 cm in diameter. Overall percentage of uninvolved pancreas and largest area of sparing for each specimen were calculated.

Results: Mean age of the study population was 57.8 yrs (range, 15-78 yrs), with 28 men and 11 women. In 32 (82%) cases there were areas of sparing by AIP, with a mean of 21.6% of the specimen spared (range, 0.8-80%). The largest focus of uninvolved pancreas varied from 0.5 cm^2 to 8.8 cm^2 (mean, 1.9 cm^2). In the remaining 7 (18%) cases the changes of AIP were diffuse, with involvement of the entire submitted specimen.

Conclusions: In a majority of cases, AIP shows a patchy distribution of disease. In pancreata with large areas of uninvolved parenchyma, this raises the potential for underdiagnosis or misdiagnosis by tru-cut biopsy. In patients with radiologic and serologic features (e.g., elevated serum IgG4 level) suspicious for AIP, this potential pitfall in pathologic diagnosis should be considered before proceding to surgery.

1273 Gene Expression Profiling Identifies Calcium-Binding Proteins and Periostin among Genes Overexpressed in Pancreatic Adenocarcinoma

SM Chung, RK Yantiss, H Tada, P Mukherjee, Y-T Chen. Weill Cornell Medical College, New York, NY; Temple University Hospital, Philadelphia, PA.

Background: Although various studies have analyzed pancreatic ductal adenocarcinoma (PDA) for differentially expressed genes and proteins, most have not identified any "cancer-specific" markers and many of the molecular mechanisms underlying the development of this highly aggressive disease are not known. The purpose of this study was to utilize DNA microarray technology to identify functionally related genes and novel tumor markers that are upregulated in PDA, in order to gain insight into its pathogenesis.

Design: Gene expression profiling was performed using Affymetrix gene chips and RNA obtained from 11 PDA and 6 samples of normal pancreas (NP). The array data was analyzed using GeneSpring Software. Functional category (GO) and molecular pathway (KEGG) databases were searched for statistically enriched gene clusters among the genes overexpressed in PDA. RT-PCR was performed to validate selected genes that were most highly expressed.

Results: There were 108 genes that showed >3-fold overexpression in PDA vs. NP. The most significantly enriched cluster of genes on this list were members of the collagen family, reflecting RNA derived from the desmoplastic stroma. Excluding this group, 20/108 (19%) genes were found to encode calcium-binding proteins, representing the second most significant cluster. The chance that these 20 genes would "randomly" cluster among 108 genes is exceedingly low (p=3.12 x 10-6). Of these, thrombospondin 2, versican, and S100P all showed >8-fold overexpression, and S100A11, S100A4, and osteonectin were increased >4-fold. In addition to these calcium-binding proteins, this list also contained other genes of biological interest, including periostin, lipocalin 2, cathepsin E, and TROP2, with periostin showing highest fold-change between PDA and NP (15.8 fold). Semiquantitative RT-PCR of these genes on 6 PDA and 6 NP showed overexpression of these genes in a proportion of PDA over NP, confirming the microarray findings.

Conclusions: Calcium-binding proteins represent the largest functional group of genes upregulated in PDA, suggesting that calcium signaling may play an important role in pancreatic cancer biology. Several members of this family, including S100P, S100A4 and osteonectin, have individually been associated with PDA previously. Confirmation of these data in larger series may aid the identification of novel diagnostic markers.

1274 HLA Class I Antigen Processing Machinery (APM) Is Altered in Short-Term Survivors (STS) of Pancreatic Adenocarcinoma (PAC) Compared to Long-Term Survivors (LTS)

SM Chung, CR Ferrone, S Ferrone, DS Klimstra. Weill Cornell Medical College, New York, NY; Massachusetts General Hospital, Boston, MA; Roswell Park Cancer Institute, Buffalo, NY; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Malignant tumors may escape the immune response of the host if the APM of tumor cells is altered. Previous studies have shown abnormalities in the APM in gastric, head and neck, ovarian and metastatic PAC. The APM includes proteosomes (LMP2, LMP7, LMP10) that cleave antigens and transport/chaperone proteins that support the HLA-I heavy chain/β2-microglobulin complex. The object of this study is to assess if components of the APM and/or HLA-I heavy chain/β2-microglobulin complex are altered in PAC of short-term survivors (STS) that died within 18 months of resection compared to those of long-term survivors (LTS) that lived beyond 5 years after resection of their tumors.

Design: Ten cases each of PAC from STS and LTS (matched for pathologic stage and post-operative therapy) were immunostained with the following antibodies against components of the APM and HLA-I; SY-1 (anti-LMP2), HB2 (anti-LMP7), NB1 (anti-LMP 10), TO-3 (anti-tapasin, a chaperone), HC-10 (anti-HLA heavy chains) and L368 (anti-β2-microglobulin). The extent of expression by tumor cells was scored as retained (>79%), reduced (20-79%) and negative (<20%).

Results: Expression of SY1 and was reduced and/or negative in 80% of STS versus 40% of LTS (p=0.0139). Expression of NB1 was reduced and/or negative in 90% of STS versus 50% of LTS (p=0.0116). Expression of HC-10, L368, and HB2 in both groups was similar, with no statistical difference. TO-3 expression was reduced and/or negative in 20% of STS versus 90% of LTS (p=0.0011).

Conclusions: PAC of patients who survived less than 18 months after cancer resection surgery showed decreased expression of SY1 and NB1, compared to those who survived more than 5 years. Both are antibodies against the proteosome complex that generates peptide fragments from antigens, including ones that may be generated by malignant tumors. Alterations in the proteosome complex may lead to abnormal tumor antigen presentation and decreased anti-tumoral immune response, resulting in the difference in survival times between STS versus LTS. The equivocal results seen with the 4 other markers imply that other components of the APM of the HLA-I may be intact in STS.

1275 Calponin Is Strongly Expressed in Serous Adenomas of the Pancreas but Not in Adenocarcinomas or Endocrine Tumors

J Colonna, MM Yearsley, WL Marsh, M Bloomston, WL Frankel. The Ohio State University Medical Center, Columbus, OH.

Background: Serous adenomas (SA) of the pancreas are almost always benign and thought to arise from centroacinar cells. When cystic they are relatively easy to identify, however, solid areas can be confused with other tumors. SA express cytokeratin and α-inhibin and are negative for synaptophysin, CEA, desmin, vimentin, and actin. We sought to identify additional immunostains useful to distinguish SA from other primary pancreatic and metastatic clear cell tumors and further delineate cell of origin.

Design: 27 SA of the pancreas were identified and slides were reviewed. The percentage of cystic and solid areas was estimated for each and tissue cores from formalin-fixed, paraffin embedded donor blocks (2 cores per block) were arrayed to create a tissue microarray of cores measuring 2.0 mm each. Additionally, tissue microarrays that had previously been constructed from 56 pancreatic adenocarcinomas (PA) and 64 pancreatic endocrine tumors (PET) were sectioned. The slides were stained with antibodies against calponin, chromogranin, CD10, α-inhibin and NSE. Staining was considered positive when >5% of tumor cells were immunoreactive.

Results: The solid component in SA varied from 1-10% with one tumor 95% solid. Strong calponin, NSE and α-inhibin immunoreactivity were seen in 85.2%, 96.2% and 96.2% of SA, respectively while CD10 and chromogranin were negative (table). In contrast, calponin was absent in PA and PET, and α-inhibin was negative in PA and only seen in 4.1% of PET. Chromogranin staining was seen in all PET and in 9.1% of PA, and NSE was positive in 73.7% of PET and 26.8% of PA.

Conclusions: We confirm the utility of α-inhibin, chromogranin and NSE; and report for the first time the usefulness of calponin to help differentiate SA from PA and PET. These stains may also be useful to distinguish SA from metastatic clear cell tumors. Since calponin is expressed in cells with smooth muscle or myoepithelial differentiation, further investigation as to the cell of origin of SA is warranted.

Immunohistochemical Staining in Pancreatic Tumors

Antibody	Serous adenoma	Adenocarcinoma	Endocrine Tumor	p Value
Calponin	23/27 (85.2%)	0/56 (0%)	0/62 (0%)	0.001
Chromogranin	0/26 (0%)	5/55 (9.1%)	64/64 (100%)	0.001
CD10	0/27 (0%)	2/55 (3.6%)	2/64 (3.1%)	1
Alpha-inhibin	26/27 (96.2%)	0/56 (0%)	2/49 (4.1%)	0.001
NSE	26/27 (96.2%)	15/56 (26.8%)	42/57 (73.7%)	0.001

1276 Expression of Oct3/4, Nanog, CD34, CD56, ER and PR in Solid Pseudopapillary Neoplasms of the Pancreas; a Tissue Microarray Study of 50 Cases

J Dancer, S Shen, J Jhai, M Kim, J Ro. Weill Medical College-The Methodist Hospital, Houston, TX; Asan Medical Center, Seoul, Republic of Korea.

Background: Solid pseudopapillary neoplasms (SPEN) of the pancreas are unusual indolent tumors that affect women almost exclusively. Many investigators favor the theory that SPENs originate from multipotent primordial cells while others suggest an extra-pancreatic origin from genital ridge angle-related cells. However, histogenesis is still unclear. OCT3/4 and NANOG are transcription factors required to maintain the pluripotency and self-renewal of embryonic stem cells. They have been noted as being specifically expressed in embryonic stem cells and in tumor cells, but not in cells of differentiated tissues.

Design: We evaluated expression of stem cell markers OCT3/4, NANOG in a tissue microarray (TMA) of SPEN of pancreas by immunohistochemical (IHC) stain in order to investigate it's histogenesis. We also studied alternate markers: CD34, CD56, ER, and PR by IHC. A TMA was constructed with 50 cases of SPENs. The cases are represented by two 1mm cores of in each SPENs, and two cores of normal pancreatic tissue, for a total of 102 cores on a TMA. Immunohistochemical staining of OCT3/4, NANOG, CD34, CD56, ER, and PR were performed on the paraffin sections of TMA. Results were graded as positive if more than 10 percent of the cells showed staining.

Results: None of the tumors showd positive nuclear OCT 3/4 or NANOG expression. Strong cytoplasmic expression of CD56 was demonstrated in 45 out of 50 (90%) tumors. Positive nuclear expression of PR showed in 24 of 50 (48%) tumors. No tumor showed cytoplasmic CD34 or nuclear ER expression. The patients data included 42 female and 8 male (F: M= 5.3:1). The tumors occurred in patients from 12-64 (mean age = 36.5) years of age. The tumor size ranged from 1.8 to 13.5 (mean 5.2) cm. Sixteen (32%) tumors showed invasion into the peripancreatic tissue. Two tumors showed metastasis.

Conclusions: On account of total negative expression for Oct3/4 and Nanog in all studied SPENs in this study, we conclude that this tumor appears to be not originating from stem cells that are expressing these transcription factors. Ninety percent of the cases revealed positivity for CD56. Forty-eight percent of studied cases showed PR positive, whereas no solid pseudopapillary neoplasm expressed CD34 or ER. Female predominance cannot be answered only by PR positivity alone with negative ER staining. The enigmatic phenotypic appearance of SPENs still remains to be further investigated.

1277 Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration Biopsy (FNAB) Is a Powerful Predictor of Malignancy in Intraductal Papillary Mucinous Neoplasms (IPMN) and Mucinous Cystic Neoplasms (MCN) of the Pancreas: A Retrospective Analysis of 110 Cases

V Deshpande, MB Pitman, GY Lauwers. Massachusetts General Hosptial, Boston, MA.

Background: EUS guided FNABs of pancreatic IPMNs and MCNs are diagnostically challenging because of frequent paucicellular aspirates and that gastrointestinal (GI) contaminating epithelium is virtually indistinguishable from neoplastic epithelium. Prior cytologic studies have been limited by small cohort sizes and diagnostic algorithms

in cystic pancreatic lesions have focused on cross sectional imaging. We evaluated 110 histologically confirmed IPMNs and MCNs to investigate the ability of cross sectional imaging studies and EUS guided FNAB to distinguish benign from malignant mucinous neoplasms.

Design: Clinical and imaging data were reviewed. Specifically, the presence of a mural nodule and main pancreatic duct dilatation (MPD), defined as a diameter of > 5mm, was noted. Cytological material was reviewed on all cases. 3-dimentional cellular aggregates, and any cell with atypia was deemed evidence of neoplastic epithelium. No attempt was made to distinguish benign epithelium from GI contaminating epithelium. Only the presence of moderate and abundant extracellular mucin was recorded. Nuclear membrane irregularities and tumor necrosis was evaluated.

Results: There were 91 IPMNs included 32 adenomas, 29 borderline neoplasms, 18 in-situ, and 12 with invasive carcinomas. The 19 MCNs included 14 adenomas, 3 borderline neoplasms, and 2 with invasive carcinomas. Prospectively, 52.2% of carcinomas were classified as suspicious/positive, while 4.8% of adenomas and borderline lesions were characterized as suspicious, and none as carcinoma. Abundant extracellular mucin was identified in 29 % (n=32) of all cases, 22.8% (n=25) of benign and borderline lesions, and 45% (n=14) of cases of carcinoma. The presence of atypical epithelium on cytology (p=0.001), radiological evidence of MPD dilatation (p=0.0002), and a mural nodule (p=0.002) were the only statistically significant predictors of malignancy.

Conclusions: The presence of any atypical mucinous epithelium on a EUS guided FNAB is a powerful predictor of malignancy in mucinous tumors of the pancreas. Cytology is a vital tool in the preoperative diagnostic assessment of pancreatic mucinous cystic tumors for whom a conservative management is contemplated.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Atypical epithelium	75	83.3	64.9	89
Mural nodule	40	89.6	63.2	76.9
MPD dilatation	68.9	81	64.5	85

1278 Lymphocyte Subpopulations in Human Fatty Liver Disease Related to Obestiy

TJ Eichelberger, GA Abrams, RH Clements, VB Reddy, NC Jhala, AJ Lazenby. University of Alabama at Birmingham, Birmingham, AL.

Background: As Americans struggle with an obesity epidemic, physicians confront an increased case load of non-alcoholic fatty liver disease (NAFLD) including steatosis and steatohepatitis. However, the inflammatory response which may lead to severe liver injury is incompletely characterized in man. In obese murine models, studies have shown reductions in NKT cells (CD57+), which are part of the innate immune system and regulate proinflammatory (Th-1) and anti-inflammatory (Th-2) immune responses. A reduction in NKT cells has been reported in ob/ob and overfed mice leading to Th-1 polarization of hepatic cytokines. In humans, the lymphocyte subpopulations, particularly NK and NKT cells, are not well studied in tissue.

Design: Immunohistochemical stains for CD3, CD8, CD56, and CD57 were performed on liver biopsies from 21 morbidly obese individuals with NAFLD, biopsied just prior to bariatric surgery (16F:5M, mean age 44). Ten of these had been diagnosed as steatohepatitis (ballooning), and 11 as moderate steatosis, and were compared to 10 histologically normal livers. Serial sections were stained with each antibody and the same selected central vein area was photographed. Prints were used to count the number of positive cells of each type in a defined 0.7mm by 0.6mm area (extrapolated to 1 mm^2). Statistical differences between the groups of patients were calculated using the Mann-Whitney U test.

Results: Table 1 shows the inflammatory cell distribution between the three groups. Statistically significant ($p = 0.04$) lower CD57 expression was noted in patients with NAFLD (steatosis and steatohepatitis) in comparison to the control group. However, no statistically significant ($p > 0.05$) difference in CD57 count was noted between steatohepatitis (mean, 19 CD57+ cells/mm^2) and steatosis (mean, 14 CD57+ cells/mm^2).

Table 1

	Number of cases	Mean (Range) CD3+ cells/mm^2	Mean (Range) CD8+ cells/mm^2	Mean (Range) CD57+ cells/mm^2
Steatohepatitis	10	136 (52 - 260)	113 (45 - 193)	19 (2 - 38)
Steatosis	11	91 (45 - 174)	80 (21 - 133)	14 (7 - 26)
Controls	10	117 (38 - 264)	108 (45 - 304)	34 (9 - 92)

Conclusions: 1) Compared to steatosis, steatohepatitis, as defined by ballooning, has more lymphocytic inflammation, which is primarily CD8+, with a minor component of NK and NKT cells. 2) In human tissue, decreased CD57+ cells in NAFLD results in increased proinflammatory cytokine production similar to the mouse model.

1279 Is Low Titer Autoantibodies Preferentially Produced in Fatty Liver Disease Patients Compared to Patients with No Steatosis?

M Fagih, OA Adeyi. University Health Network/University of Toronto, Toronto, ON, Canada.

Background: Observation by many practitioners and in the literature indicates presence of low titer autoantibodies, especially smooth muscle antibodies (ASMA) and anti-nuclear antibodies (ANA) in patients with fatty liver disease (FLD). These patients often have no other evidence of an active autoimmune disease. The frequency with which this association has been observed appears significant from personal experience and published literature. It is not clear however if this association is unique to FLD, in which case there may in fact be a causal relationship. This study has compared the incidence of autoantibodies in FLD biopsied patients with patients biopsied for viral hepatitis and in whose biopsies significant steatosis is not identified. Other variations such as alcohol, severity of steatosis, and stage of disease, among others are also analyzed with a view to detecting, if any, direct association with autoantibodies to disease severity and/or progression among FLD patients.

Design: Patients with liver biopsies at the University Health Network, Toronto over 5 years are reviewed, and data collected, including pathologic diagnosis, serologic findings, and other demographic information. Patients with incomplete serology for autoantibodies, as well as those with clinical or other evidence of autoimmune disease(s) are excluded. Patients are divided into three categories: non-alcoholic fatty liver disease, alcoholic fatty liver disease, and viral hepatitis without significant steatosis on biopsy. Data are analyzed to determine level of association of autoantibodies with the different categories. The titer of antibodies are also compared with disease severity.

Results: Autoantibodies in low titer is significantly associated with FLD, compared to patients with viral hepatitis without significant steatosis. The titer of antibodies do not appear to correlate with disease severity, and does not differ between alcohol and non-alcohol related FLD.

Conclusions: Autoantibody production in low titer is preferentially detected in FLD patients. This association may have a causal role in the development of this disease. Alternatively FLD, as a cause of liver injury, may lead to release of otherwise unidentified sequestered self-antigens, resulting in secondary production of autoantibodies, thereby implying outcome, rather than cause, of FLD.

1280 Clinical Significance of Microscopic Vascular Invasion in Resection Specimens with Hepatocellular Carcinoma

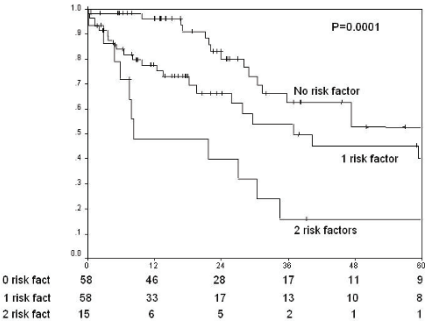
MI Fiel, G Levi, R Xu, M Guido, M Sayed, I Blume, S Roayaie, M Schwartz, SN Thung. Mount Sinai School of Medicine (MSSM), New York, NY; University of Padua, Padua, Italy; MSSM, New York, NY.

Background: Resection of hepatocellular carcinoma (HCC) is associated with recurrence rate in ~70% of cases at 5 years. Vascular invasion, either gross (GVI) or microscopic (MVI), has been identified as a risk factor. MVI represents a wide spectrum between no invasion and GVI and its clinical significance is unclear.

Design: We retrospectively reviewed all HCC resections at our institution between 1/90 and 3/06 to identify those with histology-proven MVI; cases with GVI were excluded. Each case was assessed for vessel number (single, 2-5, >5), size (< or >0.5mm), type (sinusoids, or with or without muscular wall), distance from tumor, presence of satellite nodules and manner of invasion (invading wall, free floating or adherent). Log rank test to determine correlation with recurrence and survival and Cox regression analysis to identify independent predictors of survival were used.

Results: MVI was present in 131/384 (34%) HCC resections. Median followup = 29 months with 68 recurrences and 54 deaths. On univariate analysis, invasion of a vessel with muscular wall (p=0.0181), invasion of a vessel > 1cm from the tumor (p=0.0378), and invasion of > 5 vessels (p=0.0491) were associated with recurrence; invasion of a vessel with muscular wall (p=0.002) and invasion of a vessel > 1cm from the tumor (p=0.001) were also associated with decreased survival. Both were also significant predictors of survival on multivariate analysis: Vessel w/ muscular wall p=0.018 Exp (B) = 2.2 95% CI 1.1-4.2; Vessel >1cm from tumor p=0.015 Exp (B) = 2.1 95% CI 1.2-3.7; A risk score assigning one point for the presence of each variable correlated significantly with both recurrence (p=0.0379) and survival (Figure1). MVI was also shown to be an independent prognostic factorfrom other known factors such as size, grade or clinical stage.

Conclusions: Patients undergoing resection for HCC with MVI are a heterogeneous group; a risk score based on invasion of a vessel with muscular wall and invasion of a vessel > 1cm from the tumor can stratify patients into more accurate prognostic subsets.



1281 Japanese Herbal Medicines in the Prevention of Hepatic Fibrosis in NAFLD

M Fujimoto, K Tsuneyama, M Kainuma, N Sekiya, Y Takano, Y Shimada. University of Toyama, Toyama, Japan; Iizuka Hospital, Iizuka, Fukuoka, Japan; Chiba University, Chiba, Japan.

Background: Non-alcoholic fatty liver disease (NAFLD)/ non-alcoholic steatohepatitis (NASH) is known to progress to cryptogenic cirrhosis at high frequency, so early diagnosis and suitable treatment are necessary. In this study we evaluated the effects of three Japanese herbal medicines (Kampo formulas) which have antioxidant activity, keishibukuryogan (KBG; TJ-25), orengedokuto (OGT; TJ-15) and shosaikoto (SST; TJ-9), on cholesterol-fed rabbits, a model of NAFLD/NASH. As control, the effects of vitamin E and pioglitazone were evaluated.

Design: Fifty six male Japanese rabbits (2 kg body weight) were divided into seven groups and were fed for 12 weeks. Group N was fed with standard rabbit chow (SRC) only (n=8). Group C was fed with SRC containing 1 % cholesterol (n=8). Group KBG was fed with SRC containing 1% cholesterol and 1% KBG (n=8). Group OGT was fed with SRC containing 1% cholesterol and 1% OGT (n=8). Group SST was fed with SRC containing 1 % cholesterol and 1 % SST (n=8). Group E was fed with SRC containing 1 % cholesterol and 0.045 % vitamin E (n=8). Group PG was fed with SRC containing 1 % cholesterol and 300 ppm pioglitazone (n=8).

Results: Groups KBG, OGT, SST, E and PG showed significantly lower level of plasma concentration of total cholesterol and urinary concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG). However, only group KBG showed significant lower level of liver triglyceride content and plasma lipid peroxide concentration when compared to group C. In the evaluation of fibrosis, groups KBG, OGT, SST, E and PG showed significantly lower level of α-smooth muscle actin positive lesions than those of group C. Notably, group KBG had the lowest level among the groups that were exposed to drugs.
Conclusions: Oxidative stress is favored as one of the candidate in the pathogenesis of NAFLD/NASH. Among the five drugs tested in this study, KBG has the strongest antioxidant activity and as such has a potential as a therapeutic agent against NAFLD/NASH.

1282 The IKB Family Member Bcl-3 and NF-KB Subunit p52 Are Frequently Co-Expressed in the Nucleus of Human Hepatocellular Carcinoma

WK Funkhouser, D Kashatus, H Kelly, AS Baldwin, BH O'Neil. University of North Carolina, Chapel Hill, NC; Duke, Durham, NC; University of North Carolina, Chapel Hill, NC.
Background: The anti-apoptotic transcription factor NF-KB has previously been shown to be abnormally activated in some human hepatocellular carcinomas (HCC), but most studies of NF-KB in patient samples have focused only on the p50 or p65 subunits of NF-KB. Recent information has implicated the atypical I-KB family member Bcl-3 as a possible mediator of NF-KB activation in other inflammation-associated cancers. We sought to determine whether Bcl-3 is overexpressed in HCC compared with adjacent non-neoplastic liver, and whether Bcl-3 expression is associated with particular NF-KB subunit activation by examining expression of several NF-KB and I-KB proteins by immunohistochemistry.
Design: Archived HCCs from 30 patients were evaluated by paraffin immunohistochemistry for the NF-KB proteins P50, P52, P65, and for the I-KB protein Bcl-3. DAB chromogen staining was scored for signal strength, percent positivity of carcinoma and normal cells, and nuclear vs cytoplasmic signal location. Frozen HCC tissue was available in 7 of these cases for Western Blotting.
Results: NF-KB p50 and p52 subunits were frequently localized to tumor cell nuclei (40% and 48%, respectively), indicative of activation, whereas p65 positivity was seen infrequently. Bcl-3 was found to be overexpressed in tumor cell nuclei in 90% of cases when compared with adjacent non-neoplastic liver. Western blots of frozen HCC tissue on 7 of the cases confirmed increased Bcl-3 protein expression. Little or no Bcl-3 expression was noted in adjacent normal or cirrhotic liver tissue. P52 expression was most strongly correlated with Bcl-3 expression.
Conclusions: Aberrant Bcl-3 nuclear expression occurs in the vast majority of HCCs, compared with little or no expression in adjacent normal or cirrhotic liver tissue. Bcl-3 is known to activate NF-KB p52 homodimers. Our observation of frequent Bcl-3 expression and p52 nuclear localization in HCC suggests a role for Bcl-3-induced p52 homodimer activation in HCC pathogenesis.

1283 Investigation of a Novel Oncogenic Pathway in Hepatocellular Carcinoma

MF Gayyed, J Dong, M Torbenson, A Klein, DJ Pan, RA Anders. Johns Hopkins School of Medicine, Baltimore, MD.
Background: Simultaneous evaluation of genomic DNA in hepatocellular carcinoma (HCC) arising in humans and tumor prone p53 null mice uncovered a low frequency chromosomal amplification. This amplification occurred on mouse chromosome 9qA1 and human chromosome 11q22. A novel oncogene, YAP, is located in this amplified region in both mice and humans. We determined the frequency of YAP over-expression in human HCC relative to the same patient's non-tumor tissue. In addition, we wished to examine whether inactive p53, which was critical in the tumor prone mice, correlated with the development of HCC in humans. Our findings were also correlated with tumor size, patient age, and cyclin D1 expression.
Design: HCC and non tumor tissue from 61 patients were evaluated through construction of a tissue microarray. Each patients non-tumor or tumor tissue was represented by 4 cores. YAP expression was determined with immunohistochemistry and the degree of staining was scored as 0 none; 1 focal; 2 <50%; 3 >50% for cytoplasmic staining. Positive staining was indicated by at least 3 cores showing >= 1 cytoplasmic staining. Inactive p53 and cyclin D1 expression was evaluated with immunohistochemical staining. Patient age and tumor size was also recorded. Test for significance and odds ratio were calculated with Wilcoxon signed rank test and logistic regression respectively.
Results: We found positive cytoplasmic staining for YAP in 18.9% of patients' non tumor hepatocytes compared to 42.6% ($p<0.01$) in these same patients' HCC. Inactivation of p53 was seen in 48% and over expression of cyclin D1 was seen in 12.6% of patients. The gain of YAP staining in tumor tissue compared to non-tumor tissue in the same patient showed an odds ratio of 1.6 for p53 inactivation, 1.7 for cyclin D1 expression, 1.0 for age and 0.9 for tumor size, however, none of these odds ratio were statistically significant.
Conclusions: YAP is a transcriptional co-activator that appears to be a newly recognized oncogene. Here we report a surprising high frequency of YAP over-expression in human HCC. The gain of YAP expression in a patients tumor does not significantly correlate inactive p53, expression of cyclin D1, patient age or tumor size. This study highlights the YAP pathway as a novel oncogenic pathway in HCC.

1284 Severity of Oxaliplatin Induced Hepatic Regeneration Is Dose Dependant

B Geramizadeh, R Salem, D Jain, A Iyer, J Lacy, B Burtness, M Robert. Yale University, New Haven, CT; U. Pennsylania, Philadelphia, PA.
Background: Neo-adjuvant chemotherapy followed by hepatectomy is the standard of care for patients with metastatic colon cancer in the liver. Oxaliplatin (OX) based therapies are associated with improved response rates in this setting. Recently, significant sinusoidal injury, including nodular regenerative hyperplasia, in adjacent benign liver has been reported following OX based therapy. However, data regarding liver function, dose response and effects of other therapies are scarce. The purpose of this study was to compare the pathology in hepatic resections following OX vs other therapies and to correlate these changes with clinical parameters.
Design: 31 patients with hepatic resections for metastatic colon cancer were identified. Two sections from the adjacent benign liver were examined on H&E, trichrome, and reticulin stains in each case. The following histological features were evaluated on a 0-3+ scale: sinusoidal dilatation, atrophy, regeneration, fibrosis and hepatocyte necrosis. Immunostains for smooth muscle actin (SMA) were performed and scored. Findings were correlated with type of chemotherapy, number of treatment cycles and length of post operative stay.
Results: Of 31 patients, 15 received OX based therapy, 8 other chemotherapy and 8 no chemotherapy. Severe regeneration, atrophy and sinuosoidal dilatation (scores of 3) were seen exclusively in patients who had received OX based therapy. SMA staining of hepatic stellate cells was also increased compared to other groups. In the OX group, severe scores were seen more frequently in patients who received > 9 chemotherapy cycles. Length of hospital stay did not correlate with histologic changes. All histologic parameters were focal and mild in patients receiving other agents or no chemotherapy.

Average Histologic Scores by Chemotherapy

	Oxaliplatin	Other Chemo	No Chemo
Regeneration	2.0	1.2	1.0
Atrophy	2.2	0.8	1.2
Sinusoidal Dilatation	1.40	0.28	1.12

Conclusions: We confirm an association between OX chemotherapy and hepatic injury, manifesting as atrophy and regeneration (sinusoidal obstruction syndrome). The degree of injury seen increases with the number of preoperative chemotherapy cycles given. The immediate post-operative course does not appear to be affected by hepatic injury. However, as the risk of long-term liver function abnormalities is unknown, careful documentation of the pathology in resection specimens is warranted.

1285 Expression of γ-H2AX Is an Excellent Diagnostic Biomarker of Hepatocellular Carcinoma: Test Performance for Distinguishing Hepatocellular Carcinoma from Hepatic Adenomas and Regenerative Cirrhotic Nodules

J Grau, KS Gustafson, T Pasha, EE Furth. University of Pennsylvania Medical Center, Philadelphia, PA; Johns Hopkins Hospital, Baltimore, MD.
Background: The evaluation of liver lesions poses frequent diagnostic challenges, particularly in the distinction of well-differentiated hepatocellular carcinomas (HCC) from adenomas and regenerative cirrhotic nodules. H2AX is a histone protein which becomes phosphorylated in response to DNA damage such as DNA double-strand breaks. While the role this protein plays in maintenance of DNA integrity has been explored in various cell types, its use as a diagnostic biomarker in malignancies such as HCC has not been investigated.
Design: Cases of HCC (n=14; 10 well, 1 moderately, 3 poorly differentiated), hepatic adenomas (n=16), regenerative cirrhotic nodules (n=16) secondary to Hepatitis C, and normal livers (n=8) were selected from the Hospital of the University of PA Pathology archives and stained with a polyclonal antibody against phosphorylated H2AX (γ-H2AX) using standard immunohistochemical techniques. For quantitative analysis, fifty high-power fields in hepatocyte-rich regions were assessed for the number of positive-staining heptocellular nuclei and the total number of hepatocellular cells; the percentage of γ-H2AX positive cells (%γ-H2AX) was calculated. The Mann-Whitney test was used to test for differences between groups. The area under the receiver operating characteristic (ROC) curve (AUC) was used as a measure of test performance for the ability of γ-H2AX expression to distinguish HCC from other liver lesions.
Results: The median age (years) and male:female ratio were as follows: HCC (57, 12:2), adenoma (41, 0:16), regenerative cirrhotic nodule (55, 13:3), normal (71, 4:4). HCC had significantly more frequent γ-H2AX nuclear expression compared to hepatic adenomas, regenerative cirrhotic nodules, and normal hepatocytes with median %γ-H2AX of 42.7%, 0.009%, 0.0025%, and 0%, respectively ($p<0.0001$). γ-H2AX expression was detected in all cases of HCC and showed little geographic staining heterogeneity. Using ROC analysis, the AUC for γ-H2AX expression as a test for HCC was 1 indicating a perfect test.
Conclusions: Expression of γ-H2AX was detected in all HCC with perfect test performance by ROC analysis making this antibody an excellent diagnostic tool to distinguish even well-differentiated HCC from hepatic adenomas, regenerative cirrhotic nodules, and normal hepatocytes.

1286 The "Lipopeliosis" Lesion Can Occur in Native Livers Too, Not in Transplanted Ones Only

M Guindi. University Health Network, University of Toronto, Toronto, ON, Canada.
Background: The lipopeliosis lesion is characterized by apparent distension of sinusoids by large fat globules. It typically occurs in newly transplanted steatotic donor livers. Presumably fat is released from hepatocytes undergoing preservation injury. Recent evidence shows the fat to accumulate mainly outside sinusoids. Eight cases of lipopeliosis, 7 in native livers, are studied.

Design: Liver biopsies and liver explants are examined prospectively between October 2005 and July 2006 for the presence of the lipopeliosis lesion. Biopsies and explants are studied with a liver panel of special stains. Immunostains for CD68, type 4 collagen and CD34 are performed.
Results: Six cases occurred in native livers; one in an unused steatotic donor liver lobe; and one occurred late in an engrafted, originally nonsteatotic liver, several months post transplant. The first case is an alcoholic patient with daily acetaminophen intake. Needle biopsy showed steatohepatitis, zone 3 necrosis and lipopeliosis in zone 3. Three cases are encountered in explants from patients transplanted for alcoholic and nonalcoholic steatohepatitis. One patient had a recent febrile illness and recent portal vein thrombosis; one patient developed intractable ascites, hepatorenal syndrome, and received diuresis; and one patient had developed post paracentesis sepsis. The fifth case is seen in an explant of chronic hepatitis C (HCV) with steatosis. The sixth case occurred in a steatotic donor left lobe unused due to prolonged cold ischemic time. The seventh case is liver biopsy with steatosis and ischemic zone 3 necrosis from cardiogenic shock. The eighth case is a late biopsy of engrafted liver obtained during sepsis from meningitis in a patient transplanted for cryptogenic cirrhosis, who had become diabetic post transplant. In each case there was a recent insult prior to encountering the lesion, which would have contributed to liver cell injury and release of fat globules e.g. simultaneous ingestion of alcohol and acetaminophen, ischemia from portal vein thrombosis or shock, and intravascular volume contraction associated with severe ascites and diuresis.
Conclusions: The demonstration of lipopeliosis type lesion in native livers with steatosis or steatohepatitis expands the morphologic spectrum of fatty liver disease, demonstrates this lesion to possess similar morphologic features in the nontransplant setting, and could contribute to our understanding of fibrosis development in steatohepatitis and slow recovery in some patients with fatty livers pending fat removal.

1287 Histopathologic Features of Radiologically Underdiagnosed Hepatocellular Carcinoma

BS Henninger, EW Johnson, PE Swanson, MP Upton, MM Yeh. University of Washington, Seattle, WA.
Background: Hepatocellular carcinoma (HCC) is a major health problem, resulting in up to 1 million deaths globally per year. The incidence is also rising in the US. While non-invasive, the sensitivity and specificity of image studies are not optimal. We examined the histopathologic features of HCCs that were underdiagnosed radiologically.
Design: HCC from liver explants from 1999-2005 were correlated with pretransplant image studies. CT impressions were categorized as follows: (1) Detected HCC: if reported as "highly suspicious for HCC" "worrisome for HCC" "consistent with HCC" or "HCC vs high grade dysplatic nodule"; (2) Underdiagnosed HCC: if reported as "regenerative nodule" "cirrhotic liver with no suspicious lesions", or as another benign conditions. The histopathology of HCCs were examined and the following features were quantitatively or simiquantitatively assessed: (1) size (2) differentiation (well, moderate, or poor) (3) Edmonson-Steiner grade (4) morphology of HCC (5) number of unpaired arteries per 5 10X fields (6) CD34 expression by sinusoidal endothelial cells, scored according to standard protocol (7) bile (8)Mallory hyaline (9) intratumoral fat (10) mitoses (11) necrosis. Statistical analysis using the Pearson Chi-Square test was performed when appropriate.
Results: 79 HCCs were included. 45 were detected and 34 were missed by CT. Of the histopathologic features evaluated, HCCs smaller than 1 cm tended to be missed, whereas HCCs larger than 2 cm were usually detected (Table: $p<0.05$). Only 35% of the well-differentiated HCCs were detected, while 66% and 50% of the moderately and poorly differentiated HCCs were detected, respectively (Table: $p<0.05$). In blocks available for staining, there was increased CD34 expression in sinusoidal endothelial cells in detected HCCs when compared to missed HCCs ($p=0.001$). Other features did not reach statistical significance.
Conclusions: Size, differentiation and the expression of CD34 in sinusoidal endothelial cells appear to be associated with accurate radiologic detection of HCC. The latter suggests that capillarization in hepatocarcinogenesis reflects the radiologic "washout". A larger prospective cohort study may be needed to substantiate these observations.

HCCs detected vs missed in differentiation and size (cm)

	Well Diff	Moderate Diff	Poor Diff	<1	1 to <2	2 to <4	≥4
N	20	57	2	18	31	22	8
% Detected	35.0	66.7	50.0	27.8	41.9	95.5	87.5
% Missed	65.0	33.3	50.0	72.2	58.1	4.5	12.5

1288 Evaluation of the Diagnostic Value of Glypican 3 for Hepatocellular Carcinoma in Liver Needle Biopsies

M Hishinuma, N Fukushima, J Shibahara, S Ota, T Kodama, H Aburatani, M Fukayama. Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan; Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Meguro-ku, Tokyo, Japan.
Background: We have recently reported that Glypican3 (GPC3), a heparan sulfate proteoglycan anchored to cell surface, is overexpressed in majority of hepatocellular carcinoma (HCC). Several studies have reported that CD34 is expressed in the endothelial cells of sinusoid-like tumor vessels in the majority of HCC. We evaluated validation of these two markers in diagnosing HCC in liver biopsies.
Design: Immunohistochemical expressions of GPC3 and CD34 were investigated in liver biopsy specimens to distinguish well-differentiated HCC from non-cancerous liver tissues. Ninety-two liver biopsy specimens including 42 well-differentiated HCC (Edmondson Grade 1 and Grade 2), 7 dysplastic nodules and 43 non-neoplastic liver tissues were studied.
Results: The positive staining rate of GPC3 was significantly higher in cancerous tissues than in non-cancerous tissues ($p<0.001$). GPC3 immunohistochemistry was highly specific for detecting HCC in biopsy specimens (86.0%). Strong diffuse membranous and cytoplasmic stainings were observed in GPC3-positive HCC. GPC3 expression was undetectable in majority of non-cancerous liver tissues, and all of the 6 positive cases showed weak immunoreactivity. CD34 positive rate in HCC was 50.0%, while the expression was undetectable in non-cancerous tissues. Complementary use of GPC3 and CD34 yielded high sensitivity (69.0%) and specificity (86.0%) for the diagnosis of well-differentiated HCC.
Conclusions: Combined use of GPC3 and CD34 immunohistochemistry is useful for the biopsy diagnosis of well-differentiated HCC.

1289 Diabetic Microangiopathy in the Liver: An Autopsy Study of Incidence and Association with Other Diabetic Complications

RM Hudacko, B Fyfe-Kirschner. Robert Wood Johnson Medical School, New Brunswick, NJ.
Background: Diabetic Hepatosclerosis (DH) is a recently described form of diabetic microangiopathy with extensive hepatic sinusoidal fibrosis and basement membrane deposition without non-alcoholic steatohepatitis (NASH). The overall incidence of DH and its association with pathologic evidence of other vascular diabetic complications has not been previously studied. The purpose of this study is to investigate the frequency of DH and its correlation with other microangiopathies in an autopsy series of diabetic patients.
Design: Complete autopsies from 42 adult diabetic patients were reviewed. Evaluation of livers for DH was done using the criteria of Harrison, et al. Other liver changes were noted. Incidence of diabetic diffuse/nodular glomerulosclerosis, hyaline arteriosclerosis (HAS), and other diabetic complications were noted after chart/autopsy report review. Hepatic HAS was graded as absent (0), mild HAS in few portals tracts (+1), or moderate HAS in many portal tracts (+2).
Results: Seven cases of hepatic sinusoidal fibrosis (HSF) were identified. Only one case fit the definition of DH. This case occurred in a 68 year old African-American woman with Type 1 diabetes for 58 years. Other end-organ damage included severe nodular diabetic glomerulosclerosis (the most severe case in the study) and coronary artery disease. Hepatic HAS was noted in 43% (3/7) of the HSF cases. Diabetic glomerulosclerosis was seen in 50% (3/6) of cardiac sclerosis, 40% (2/5) of simple steatosis, and 25% (2/8) of NASH cases. Coronary artery disease was present in 100% of cardiac sclerosis, 60% (3/5) of simple steatosis, and 37.5% (3/8) of NASH cases. Retinopathy and peripheral neuropathy were rarely clinically noted. One third of all cases had peripheral vascular disease/systemic atherosclerosis.
Conclusions: DH is an uncommon pattern of liver disease in diabetic patients, and in our limited sample, is associated with severe end-organ damage. Hepatic HAS is not infrequent in diabetic livers and was the most severe in DH. This study supports the presumed vascular etiology for DH, confirms the rarity of the lesion, and establishes conclusive evidence for other diabetic end-organ damage when present. However, since the lesion is so rare, a review of additional cases will be necessary to better define the overall incidence of DH and its associated complications.

1290 A Centrovenular Form of Acute Graft Versus Host Disease Is Rare in the Liver in the Bone Marrow Transplant Population

SD Hurtt, EE Furth. Hospital of the University of Pennsylvania, Philadelphia, PA.
Background: Graft versus host disease (GVHD) is an etiology of hepatic dysfunction in patients following allogeneic bone marrow transplantation (BMT). In the liver, acute GVHD is defined by lymphocytic infiltration and damage of small bile ducts, a pattern similar to that seen in acute cellular rejection in hepatic allografts. While acute rejection in the liver allograft was initially thought to be confined to portal tracts, it is now recognized that a distinct "centrovenular" form of acute cellular rejection, characterized by lymphocytic infiltration and damage of central veins (central venulitis [CV]), may occur either alone or in combination with classic portal rejection in 16% and 34% of liver allografts, respectively. We hypothesized that an analogous "centrovenular" form of acute GVHD may also exist in the bone marrow transplant population.
Design: All liver biopsies performed at the Hospital of the University of Pennsylvania from 1997 to 2006 obtained from BMT patients were reviewed. Biopsies were examined for the presence of acute and/or chronic GVHD and CV. Acute GVHD was defined as lymphocytic infiltration of small bile ducts with associated bile duct damage. Chronic GVHD was defined as loss of 50% of bile ducts. CV was defined as centrovenular damage by lymphocytes with surrounding hepatocyte dropout, the same diagnostic criteria as that reported in the literature for CV in liver allografts.
Results: A total of 96 biopsies were reviewed, 85 of which were performed to rule out GVHD and 11 of which were performed for other indications. Of the biopsies performed to rule out GVHD, 35/85 (41%) exhibited histologic features consistent with acute GVHD, 6/85 (7%) chronic GVHD, and 1/85 (1%) with both acute and chronic components. CV was identified in only one case and was found in combination with classic portal tract acute GVHD. The remaining biopsies exhibited various abnormalities, including viral hepatitis, cholestasis, and steatosis. None of the 11 biopsies from patients without clinical suspicion of GVHD displayed acute GVHD or CV. Thus, only 1/35 (2.8%) cases with pure acute GVHD showed CV. No cases of isolated CV were seen and, thus, its prevalence is <2%.
Conclusions: The central vein is not a target of immunologic damage in acute GVHD in the bone marrow transplant population, with CV occurring with a 10-fold less frequency compared to that in the liver allograft patients. These differences raise interesting issues regarding the biology of rejection in each of these populations.

1291 SNP Analysis of Fibrolamellar Carcinoma

K Johnson, P Vivek, M Torbenson. Johns Hopkins University School of Medicine, Baltimore, MD.
Background: Fibrolamellar carcinomas (FLC) are primary hepatic tumors with unique clinical and morphological characteristics. Little is known about specific genetic abnormalities in FLC. Previous cytogenetic studies have demonstrated chromosomal stability in most cases of FLC, except for frequent gains of chromosome 1q. Typical hepatocellular carcinomas also show frequent gains of 1q and an important hepatocellular oncogene is suspected to lie within this region.

Design: SNP analysis was used to study DNA changes in three primary FLCs, each with paired normal liver tissues. All tumors were obtained at the time of surgery and confirmed by routine histological analysis. Affymetrix 250K NspI chips were used, providing a SNP density of approximately 250,000 unique dimorphic positions. Both LOH analysis and DNA copy number analysis were performed.
Results: Copy number analysis confirmed an overall high frequency of DNA gains on chromosome 1q, particularly in regions 1q21.3 (5 potential genes) and 1q25.3 to 1q31.2 (9 potential genes). Of note, these same regions have been identified in previous studies using comparative genomic hybridization as well as gene expression profiling to be potential areas of DNA gain. No other significant regions of DNA copy gain were identified, confirming chromosomal stability of these tumors. In addition, LOH analysis also showed the tumors to be chromosomally stable, with LOH in 1/3 FLC. The region of LOH was located to a small region of chromosome 3p corresponding to the collagen type VIII, alpha 1 gene (COL8A1). This gene is important in cell adhesion, phosphate transport, protein binding, and extracellular matrix. Its precise role in FLC is unclear, but given the unique extracellular matrix findings characteristic of FLC, this gene warrants further studies.
Conclusions: SNP analysis confirms the chromosomal stability of FLC and further narrows the potential regions of interest on chromosome 1q. In addition, LOH analysis reveals a potential role for the COL8A1 gene in FLC tumorgenesis.

1292 Indoleamine 2,3-Dioxygenase Expression in Liver Biopsy Samples from Hepatitis C Infected Patients

TS Johnson, R Shahidzadeh, RR Schade, JR Lee, AL Mellor, DH Munn. Medical College of Georgia, Augusta, GA; MCG, Augusta, GA; VAMC, Augusta, GA.
Background: Dysregulation of T cell responses may set the stage for chronic Hepatitis C infection. Hep C infection is commonly treated with Interferon-α and ribavirin, often with poor clinical response. In mice, interferon-α is a potent inducer of indoleamine 2,3-dioxygenase (IDO), which is expressed by specific dendritic cell subsets that have been shown to suppress T cell proliferation cells in an antigen-specific manner (J Immunol 175:5601). IDO-inhibitor drugs are in pre-clinical development for treatment of IDO-induced immunosuppression in cancer and chronic infection.
Design: Immunohistochemical staining for IDO was performed on 107 archival liver biopsy specimens, obtained prior to treatment, from patients with Hep C infection at the Medical College of Georgia. Biopsy grading was based on the regional involvement of portal and parenchymal areas (1+ - 4+). Biopsies from Hep C negative transplant donor candidates were used as negative controls, while tumor draining lymph nodes with IDO+ cells were used as positive controls.
Results: Three distinct patterns of IDO positivity were observed in patients with Hep C: 1) IDO+ cells in portal triads and fibrotic septae; 2) IDO+ cells marginated within sinusoids; and 3) IDO+ cells in parenchyma. Biopsies from liver transplant donors (n=10) showed 40% negative and 60% 1+. Therefore, we considered only grades 2-4+ to represent abnormal IDO enhancement. Of 107 biopsies from patients with confirmed Hep C, 61% (65/107) had abnormally elevated IDO+ cells. Within our sample, a total of 44 patients received treatment (IFNa and/or ribavirin) and had evaluable pre- and post-therapy viral load measurements. Response to therapy was defined as a 2-log reduction in viral load, or reduction below detection. 15 of 44 patients were classed as non-responders; of these, 80% showed abnormally elevated IDO+ cells (12/15). Of the patients classified as responders, 58% (17/29) showed abnormally elevated IDO.
Conclusions: IDO positive cells were increased in a majority of patients with chronic Hep C infection. We speculate that the presence of IDO+ cells may be immunosuppressive in the liver, as has been shown in models of malignancy (J Clin Invest 114:280). We further speculate that adjuvant therapy with IDO-inhibitor drugs (in preclinical development) may improve response to conventional therapy.

1293 Chromosomal Abnormalities Determined by Comparative Genomic Hybridization Are Helpful in the Diagnosis of Atypical Hepatocellular Lesions

S Kakar, X Chen, LJ Burgart, D Jain, L Ferrell. UCSF, San Francisco, CA; Abbott NW Hospital, Minneapolis, MN; Yale University, New Haven, CT.
Background: Hepatic adenomas(HA)can be difficult to distinguish from well-differentiated hepatocellular carcinoma(HCC)on morphologic grounds.HCC shows typical chromosomal aberrations like gains at 1,7,8,20,X and losses at 8,13 and 16.Since these changes are not seen in HA,it has been suggested that chromosomal abnormalities can help in this distinction.However,the HA and HCC cases studied have largely been morphologically typical examples,where diagnostic problems are less likely. In this study,we focus on the chromosomal abnormalities in atypical hepatocellular lesions(AHL)that pose a serious diagnostic challenge and explore whether cytogenetic studies are helpful.
Design: Chromosomal abnormalities were determined by comparative genomic hybridization(CGH) in 30 resected hepatocellular neoplasms(HA=10,AHL=10,HCC=10).The designation of AHL was used in two situations:(1)HA-like morphology in atypical clinical setting(HA-like, n=4)including males(any age)and females>50 yrs and <15 yrs(2)HA-like morphology with focal atypical areas showing small cell change, abnormal trabeculae and/or loss of reticulin(HA-like with focal atypia, n=6). For CGH,human 1.14 arrays with 2433 bacterial artificial chromosome clones covering the genome at 1.5 Mb resolution were used.Tumor and gender-matched reference DNA (lymphocytes) was labeled by random priming using Cy3-dCTP and Cy5-dCTP respectively.Following denaturation,hybridization and mounting in DAPI containing solution,3 single-color intensity images (DAPI, Cy3 and Cy5) were collected using a charge coupled device camera.
Results: CGH abnormalities were seen in none of the HA(0/8), 8(80%)conventional HCC and 8 (80%)AHL.Of the 4 HA-like AHL, 2 were males>70 yrs, 1 was 65/F and 1 was 3/F with glycogen storage disease.All 4(100%)showed chromosomal gains/losses typical of HCC.One(72/M)recurred after 2 years and another(65/F)developed metastatic disease after 4 years.Of the 6 HA-like cases with focal atypia,4 (67%) showed CGH changes typical of HCC(27/F,50/F, 50/M, 72/M),while 2 were normal(33/M, 57/M).
Conclusions: Majority of the hepatic adenoma-like neoplasms with atypical clinical setting or with focal atypical features may represent well-differentiated hepatocellular carcinoma.Even though these tumors are well-differentiated and morphologically mimic adenoma,they can potentially recur and metastasize.Array-based CGH analysis can be useful in the differential diagnosis of these atypical hepatocellular lesions.

1294 The Potential Relation of Bax/Bcl-2 Ratio and Caspase-3 Expression, with Apoptosis, in an Experimental Model of Acute Liver Failure

C Kalogeropoulou, I Tsota, P Zabakis, V Tzelepi, A Liava, D Kardamakis, T Petsas, AC Tsamandas. University of Patras School of Medicine, Patras, Greece.
Background: Apoptosis has been linked to liver cell depletion and ensuing liver fibrosis. Bax/Bcl-2 ratio has been considered as the best regulator of apoptosis. This study investigates the relation of bax/bcl-2 ratio and expression of the apoptosis co-ordination enzyme, caspase-3 with the degree of apoptosis 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. Liver tissues were evaluated for a) bax, bcl-2 and caspase-3 mRNA (real time RT-PCR), b) bax, bcl-2 and caspase-3 protein levels (Western blot), and distribution (immunohistochemistry), c) immunohistochemical expression of antigens CK7, HEPAR, CD68, αSMA and d) apoptosis (TUNEL method). Results were expressed following image analysis and the bax/bcl-2 ratio was calculated.
Results: The death rate of animals was 80% within 48 hrs after chemical institution. Liver sections developed combined (periportal+pericentral) parenchymal necrosis, which 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+. BaxmRNA was significantly increased and bcl-2mRNA decreased towards 48hr (+252% and -61%). Similar results were recorded for bax and bcl-2 proteins (+276% and -27% by 48hr). Bax/bcl-2 ratio reached the peak at 48hr (+201%) and thereafter decreased. Apoptosis reached the peak at 48 hr. Apoptotic bodies were sequestrated in the adjacent hepatocytes and sinusoidal cells. Double stain with TUNEL and antigen αSMA or CD68, revealed that apoptotic bodies were incorporated to stellate cells or phagocytosed by Kupffer cells. Bax/bcl-2 ratios were correlated with caspase-3 expression (r=0.63 and 0.74 p=0.0038 and 0.0024), apoptosis (r =0.58 and r=0.64, p=0.004 and 0.0034) and SGPT values (r=0.581 and 0.612, p=0.0042 and 0.0038).
Conclusions: This study demonstrates that changes of the bax/bcl-2 ratio may contribute to caspase-3 activation and increase of liver apoptosis in an experimental model of acute liver failure. These results may have prognostic and therapeutic implications in acute liver failure.

1295 Oval Cell Activation and Oxidative Stress in Experimental Liver Fibrosis

C Kalogeropoulou, I Tsota, P Zabakis, A Liava, V Tzelepi, T Petsas, D Kardamakis, AC Tsamandas. University of Patras School of Medicine, Patras, Greece.
Background: Oval cells are liver stem cells involved in the progress of liver disease and hepatocellular carcinoma development, in experimental models. In animals, the combination of oxidative liver damage and inhibition of mature hepatocyte proliferation increases the numbers of oval cells. This study investigates whether oval cells increase in experimental liver fibrosis and tries to clarify the mechanisms for this response.
Design: The study comprised 66 male Wistar rats divided in 2 groups: A (n=6): controls and B (n=60): CCl4 injection (intraperitoneally 2ml/Kg BW, 1:1 vol in corn oil twice weekly). Rats were sacrificed at 4, 8 and 12 weeks. Liver tissues were evaluated for: a) H_2O_2 production (known inducer of cell cycle inhibitors), b) content of reduced glutathione (GSH-regulator of reactive oxygen species), c) degree of fibrosis (Masson's trichrome), d) expression of cytokeratin19 (CK19), cytokeratin 7 (CK7) and AFP mRNA (in-situ hybridization) for oval cell phenotype, and e) cell proliferation (evaluation of %Ki67$^+$ oval cells). H_2O_2 production was expressed as integrated counts x10^6 and GSH content as μg/mg protein. Cells with morphologic features of oval cells that expressed cytokeratins 19 and 7 and AFPmRNA were scored.
Results: The table shows the results. Group B showed higher hepatic mitochondrial production of H_2O_2 and lower GSH content compared to group A (controls). Furtrthermore group B developed higher oval cell accumulation. In subgroup B3, with the greatest H_2O_2 production, the highest number of oval cells was recorded, implying that there was the maximal degree of inhibition of mature hepatocyte proliferation.
Conclusions: This study demonstrates that in experimental liver fibrosis, oval cell accumulation evolves due to inhibition of mature hepatocyte proliferation. Oval cells differentiate into intermediate hepatocyte-like cells after a regenerative challenge. However, cirrhosis is not required for oval cell accumulation. Activation of oval cells during experimental liver fibrosis may increase the risk for hepatocellular cancer, similar to that observed in the Solt-Farber model of hepatocarcinogenesis in rats.

Groups	H_2O_2	GSH	CK19	CK7	AFPmRNA	Ki67*
A	23	16	2.3±0.2	1.8±0.07	2.4±0.3	1.8±0.1
B1	39[a]	10[b]	14.2±4.1[c]	11.1±2.7[d]	16.3±6.2[e]	13.8±4.2f
B2	48[a]	8[b]	35.3±6.1[c]	31.4±4.3[d]	37.4±8.5[e]	34.2±6.6[f]
B3	64[a]	7[b]	55.6±7.2[c]	50.7±5.1[d]	57.3±8.6[e]	48.3±7.3[f]

[a, c, d, e, f] : p<0.001 and [b] : p<0.05 when compared to controls. *: Ki67$^+$ oval cells

1296 Over-Expression of Wnt Signaling Pathway Member Pygo in Hepatocellular Carcinoma

R Kannangai, P Vivekanandan, M Torbenson. Johns Hopkins University School of Medicine, Baltimore, MD.

Background: The Wnt signaling pathway is known to be activated in a subset of all primary hepatic neoplasms including approximately 25% of hepatocellular carcinomas. Beta-catenin plays a key role in this pathway, with signaling dependent on the translocation of beta-catenin from the cytoplasm to the nucleus. Pygopus is a recently described nuclear protein that stabilizes and is critical for beta-catenin's interaction with TCF and subsequent activation of down stream targets of Wnt signaling. Pygopus was originally identified in drosophila, but two human homologs have been found: PYGO1 and PYGO2. Expression of these genes has not been studied to our knowledge in neoplastic tissues. We focused on PYGOP2 because it is located on chromosome 1q21, a region strongly suspected to contain a key oncogene in liver carcinogenesis.

Design: Using real time PCR, we quantitated the mRNA expression levels of PYGOP2 in 17 hepatocellular carcinomas by examining two potential gene transcripts, Pygo P and Pygo N. The coding region of the genes was also sequenced. Results were correlated with beta-catenin expression levels, beta catenin mutations, and beta catenin nuclear accumulation detected by immunohistochemistry.

Results: No PYGO2 mutations were found. Pygo P was at least 2 fold over expressed in 13/18 (72%) HCCs, while Pygo N was over expressed in 8/17 (44%), and beta-catenin in 8/18 (44%) cases. Both Pygo P and N were over expressed in the same tumor in 6/18 cases (33%). No association was found between over-expression of Pygo P, Pygo N, and beta catenin, all $p > 0.05$. Beta catenin mutations were detected in 3/18 cases (16%) and beta catenin nuclear accumulation in 5/18 (27%). All of the five cases with beta catenin mutations showed nuclear beta catenin mutations (2/5) or over-expression of both Pygo P and N (3/5).

Conclusions: All HCCs with beta-catenin nuclear accumulation by immunohistochemistry had either beta-catenin mutations or over-expression of pygopus2. Over-expression of the PYGOP2 gene may be important in dysregulation of the Wnt signaling pathway and may explain cases of HCC where nuclear beta-catenin accumulation is detectable despite the lack of beta-catenin mutations.

1297 The Prevalance of Biliary Intraeptihelial Neoplasia in the Extrahepatic Bile Ducts with and without Cholangiocarcinoma

U Kapur, G Aranha, S Yong, K Oshima. Loyola University Mecial Center, Maywood, IL; Loyola University Medical Center, Maywood, IL.

Background: Atypical / proliferative intraepithelial lesion in biliary tract is often associated with cholangiocarcinoma (CCA), and tumorgenesis of CCA is hypothesized to develop in a stepwise fashion from low-, high- grade dysplasia to in-situ and invasive carcinoma. To know the prevalence of these lesion has been difficult, because histological criteria for grading system has not been well defined. Zen et al have proposed the criteria for low-, high-grade biliary intraepithelial neoplasia and carcinoma in situ (BilIN 1,2,and 3 respectively) in 2005 based on the studies of intrahepatic CCA associated with hepatolithiasis. However, the prevalence of BilIN has not been studied in the extrahepatic biliary tracts with CCA.

Design: 28 extrahepatic CCA including 4 papillary carcinoma were retrieved from our pathology files (1992 - 2006). All available sections from non-neoplastic extrahepatic bile ducts (mean 4.9, range 2-14 sections) were examined for the presence of metaplasia and BilIN 1-3. Each slide was graded by the highest BilIN, when multiple foci were identified. The non-cholangiocarcinoma (non-CCA) group consists of 26 pancreaticoduodenectomy cases including pancreatitis(6), duodenal carcinoma (14) and pancreatic neuroendocrine tumor (6). The mean number of bile ducts sections examined per case was 2.6, range 1-8. The mean age of CCA and non-CCA patients was 69.0 and 62.7 years respectively.

Results: BilIN was identified in 26 out of 28 cases of CCA (92.9%), compared to 4 out of 26 cases of non-CCA (15.4%). The frequency of higher grade BilIN (BilIN 2 and 3) in the bile ducts with CCA was 71.4%, whereas no higher grade BilIN was seen in non-CCA group. BilIN 1 was present in 4 out of 26 cases of non-CCA group. Metaplasia was present in normal as well as dysplastic mucosa.

The prevalance of metaplasia and BilIN in the non-neoplasitc extrahepatic bile ducts

	Cholangiocarcinoma N=28	Non-cholangiocarcinoma N=26
	Number (%)	Number (%)
Metaplasia	19 (67.9%)	10 (38.5%)
BilIN1	6 (21.4%)	4 (15.4%)
BilIN2	9 (32.1%)	0(0%)
BilIN3	11 (39.3%)	0 (0%)
BilIN1-3	26(92.9%)	4 (15.4%)

Conclusions: 1.) The high frequency of BilIN in CCA support the precancerous role of BilIN in the extrahepatic biliary tract. 2)Our study suggests that many but not all CCA developed in a stepwise fashion. 3) Zen's criteria is applicable for extrahepatic CCA; however, it needs to be refined especially to distinguish between BilIN 1 and reactive changes.

1298 New Markers of Pancreatic Cancer Identified through Differential Gene Expression Analyses: Claudin 18 and Annexin A8

ZE Karanjawala, PB Illei, R Ashfaq, J Infante, K Murphy, A Maitra, M Goggins, RH Hruban. The Johns Hopkins Medical Institutions, Baltimore, MD; University of Texas Southwestern Medical Center, Dallas, TX.

Background: New markers to distinguish reactive glands from infiltrating carcinoma of the pancreas are needed.

Design: Gene expression patterns of 24 surgically resected primary pancreatic adenocarcinomas (CA) were compared to 18 non-neoplastic samples (NL)(14 duodenal mucosa and 4 chronic pancreatitis) using the Affymetrix U133-plus chips and the GeneExpress® system (Gene Logic Inc.). Gene fragments from four genes (Annexin A8[present in 92% of CA, 11% of NL; mean intensity 393 in CA, 79 in NL], Claudin 18[present in 100% of CA, 6% of NL; mean intensity 743 in CA, 19 in NL], CXCL5[present in 100% of CA, 39% of NL; mean intensity 1096 in CA, 52 in NL]and S100 A2[present in 71% of CA, 11% of NL; mean intensity 829 in CA, 27 in NL]) were selected from the fragments highly expressed in CA. Protein expression was examined by immunohistochemical labeling of TMAs.

Results: Claudin 18 labeled carcinomas in a membranous pattern, consistent with its role in cellular adhesion. When compared to normal and reactive ducts, Claudin 18 was overexpressed, at least focally, in 159 of 165 evaluable carcinomas (96%). Of these 159 cancers, 52% expressed Claudin 18 strongly and diffusely. Strong and diffuse claudin 18 overexpression was most often seen in well-differentiated carcinomas, while more poorly differentiated carcinomas often labeled focally or did not label. Annexin A8 is a member of a family of proteins that are calcium- and lipid-binding proteins that have proposed roles in membrane structure and transport. Annexin A8 was at least focally overexpressed in 149 of 154 evaluable carcinomas (97%). Of these 149 cancers, 69% expressed Annexin A8 strongly and diffusely. S100 A2, a calcium binding protein, was at least focally overexpressed in 118 of 154 evaluable carcinomas (77%). Of these 118 cancers, 34% demonstrated strong and diffuse overexpression of S100 A2. Non-neoplastic glands also frequently expressed S100 A2 diminishing its potential diagnostic utility. No significant differences in CXCL5 expression were observed between adenocarcinomas and normal ducts.

Conclusions: Claudin 18 and Annexin A8 are frequently overexpressed in infiltrating ductal adenocarcinomas when compared to normal reactive ducts, suggesting a role for these molecules in pancreatic ductal adenocarcinomas. Furthermore, these may serve as diagnostic markers, as screening tests and as therapeutic targets.

1299 Synergistic Effect of Ciprofibrate, a PPARα Agonist, and Choline Deficient Diet in Hepatocarcinogenesis in Rats

P Kashireddy, WQ Cao, MS Rao. Feinberg School of Medicine, NU, Chicago, IL.

Background: Nonalcohoic fatty liver disease (NAFLD) which encompasses fatty liver and steatohepatitis, is closely associated with metabolic syndrome and is becoming a significant public health problem in western countries. Although, fatty liver is considered innocuous and easily reversible, if the inciting cause persists it progresses to steatohepatitis, cirrhosis and hepatocellular carcinoma (HCC). To prevent complications of fatty liver, it is suggested to treat not only the comorbid conditions but also NAFLD with various pharmacological agents, including peroxisome proliferator-activated receptor (PPAR) agonists. In rodent models of nonalcoholic steatohepatitis (NASH) induced by feeding choline deficient diet (CDD) PPAR agonists were shown to prevent or inhibit steatohepatitis and also reverse fibrosis following short-term treatment. However, the long-term effects of combined administration of CDD and ciprofibrate, a PPARα agonist, have not been investigated. Accordingly the present study was conducted to determine the long-term effect of ciprofibrate in rats maintained on CDD for 1 year.

Design: Male F-344 rats weighing 80-90 grams are divided into 4 equal groups of 13 animals and maintained on CDD or control diet with or without ciprofibrate (0.025%). At the end of 52 weeks rats were sacrificed and livers were analyzed by light microscopy for steatosis, steatohepatitis, fibrosis and hepatocellular neoplasms.

Results: In rats fed only CDD the livers showed fatty change (100%), septal fibrosis (54%) and HCC (31%). Rats fed normal diet containing ciprofibrate showed no fatty change or fibrosis. However, neoplastic nodules and HCC developed in 100% and 31% of the animals, respectively. Interestingly, 100% of rats fed CDD with ciprofibrate developed HCC. None of these animals had fatty change or fibrosis.

Conclusions: Although, short-term administration of ciprofibrate protected against CDD-induced steatohepatitis and fibrosis, as shown in this study long-term administration resulted in accelerated development of HCC. In humans PPAR agonists are suggested as useful agents in prevention of complications of NAFLD. Based on this study, we suggest that long-term administration of these pharmacologic agents may be associated with more serious complications. (This study was supported by NIH Grant CA84472).

1300 CD24 Expression Predicts Poor Outcome in Cholangiocarcinoma

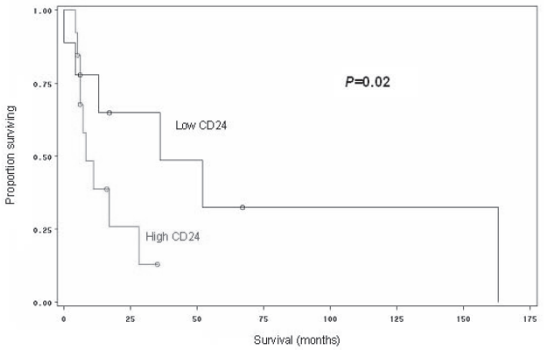
T Khoury, S Argawal, J Yu, JF Gibbs, MG Brattain, MM Javle, B Kuvshinoff, Y Zakharia, DG Hicks. Roswell Park Cancer Institute, Buffalo, NY.

Background: CD24 has been described as an adverse prognostic marker in several malignancies. This study evaluates CD24 expression in cholangiocarcinoma and correlates the findings with clinico-pathologic data and patient survival.

Design: Between 1996 and 2002, 23 consecutive patients with cholangiocarcinoma were treated at Roswell Park Cancer Institute. Demographic data, SEER stage, pathologic data, treatment, expression of CD24, MAPK, phosphorylated-MAPK and survival were analyzed.

Results: Majority of the tumors demonstrated CD24 (81.8%) and p-MAPK (87%) expression. A negative association was noted between CD24 and p-MAPK ($p = 0.0501$). Median survival for low CD24 expression was 36 months and high expression 8 months. On multivariate analysis, use of chemotherapy (Hazard Ratio = 0.069, $p = 0.0014$) and CD24 over-expression (Hazard Ratio = 7.528, $p = 0.0253$) were identified as independent predictors of survival.

Conclusions: CD24 is commonly expressed in cholangiocarcinoma and over-expression is predictive of poor survival. Mutimodality treatment should be considered in these patients with emphasis on development of targeted therapy.



1301 Clear Cell Carcinoma of the Pancreas: A Study of Its Pathologic Features and a Unique Biomarker-Hepatocyte Nuclear Factor-1 Beta

L Kim, J Liao, M Zhang, M Talamonti, D Bentrem, S Rao, GY Yang. Feinberg School of Medicine, Northwestern University, Chicago, IL.

Background: Clear cell carcinoma as a variant of ductal carcinoma of the pancreas is not well recognized and little is known about its pathologic features. Hepatocyte nuclear factor-1 beta (HNF-1β) is a transcription factor that regulates tissue-specific gene expression including pancreas and its expression has been identified as a specific marker to confirm the diagnosis of clear cell tumro of female genital tracts. However, there is no study on HNF-1β as a marker of clear cell carcinoma of the pancreas.

Design: 89 cases of pancreatic adenocarcinoma (diagnosed and archived at Northwestern University) were reviewed from 2002-2006. All cases were analyzed using an immunohistochemical approach with HNF-1β antibody and proper positive and negative controls. The identified clear cell carcinomas were further studied by PAS, DPAS, and mucicarmine stains. Pathologic features and clinical follow-up were documented.

Results: 17 of 89 cases (11.2%) of pancreatic carcinoma were identified with clear cell change which were further subdivided into 10 clear cell carcinomas (defined as over 75% of tumor showing clear cell change) and 7 ductal carcinoma with clear cell features (defined as less than 75% of tumor with clear cell change). Pathologically, the 10 clear cell carcinomas exhibited architecture that was moderately to poorly differentiated;4 of these 10 cases showed distant metastasis to lymph nodes. Cytologically, clear cell carcinomas exhibited clear cytoplasm with centrally located and mildly atypical nuclei. PAS, DPAS, and mucicarmine stains confirmed that the clear cytoplasm was not due to accumulation of glycogen or mucin. Among the 6 of 10 patients with clear cell carcinoma that had died, the survival range was 112-950 days (median 442.5 days). The results of HNF-1β immunostaining showed that HNF-1β is overexpressed in all 10 clear cell carcinomas and in the clear cell components of 7 ductal carcinoma with clear cell change. In contrast, in usual ductal adenocarcinoma, HNF-1β exhibited overall weak or focally positive staining and only 7 cases were strongly positive (17%; consisting of 5 poorly differentiated and 2 moderately differentiated carcinomas).

Conclusions: Clear cell carcinoma in the pancreas is not an uncommon variant of pancreatic ductal adenocarcinoma. HNF-1β is a useful marker to identify this clear cell carcinomas. This result warrants further investigation to elucidate the pathologic and clinical behavior of this variant.

1302 Overexpression of HMG-CoA Reductase, a Potential Therapeutic Target for Pancreatic Adenocarcinoma

L Kim, J Liao, L Yan, M Zhang, S Rao, GY Yang. Feinberg School of Medicine, Northwestern University, Chicago, IL.

Background: Over 95% of pancreatic cancers carry the *K-ras* gene mutation. The Ras protein requires prenylation by both farnesylation and geranylation for its cell membrane localization and activity. HMG-CoA reductase is a key enzyme mainly involved in cholesterol synthesis and production of farnesyl pyrophosphate and geranyl pyrophosphate. However, the expression of HMG-CoA reductase has not been studied in pancreatic carcinogenesis. We systematically analyzed the expression of HMG-CoA reductase immunohistochemically in pancreatic adenocarcinoma and a cell line and tested the therapeutic target of HMG-CoA reductase inhibitor, Lipitor, on the inhibition of pancreatic cancer cell growth and its related mechanism.

Design: Thirty cases of pancreatic adenocarcinoma were analyzed immunohistochemically with anti-HMG-CoA Reductase antibody (Upstate, New York) using heat-treated antigen retrieval with appropriate positive and negative controls. The avidin-biotin-peroxidase method was used. Cases were scored positive if cytoplasmic labeling was seen. The *in vitro* colony formation and Western blot assays were performed to examine the growth inhibitory effects of Lipitor and related mechanism using a human pancreatic cancer cell line CRL-2547 carrying the *k-ras* gene mutation.

Results: All 30 cases of pancreatic adenocarcinoma showed moderate to intense immunolabeling of HMG-CoA reductase (including 5 well-differentiated, 14 moderately-differentiated and 8 poorly-differentiated carcinoma, and 3 mucinous carcinoma). Staining signals were confined to the cytoplasm of cancer cells. Adjacent PanIN lesions (including PanIN I, II, and III) displayed moderate staining intensity of this enzyme while morphologically normal acinar tissue and ducts exhibited mild intensity of staining. With 10 and 20 uM of Lipitor treatment for 24 hours, CRL-2547 cells grew in a spindled-shaped fashion and displayed a significant decrease in cytologic atypia with loss of cell mitosis. There was a dose-dependent inhibition of pancreatic cancer cell growth with an IC50 dose of 15 uM. Using the Western blot approach, membranous binding of p21-kras protein and nuclear protein farnesylation (HDJ-2) were further inhibited after Lipitor treatment.

Conclusions: We have first demonstrated overexpression of HMG-CoA reductase in pancreatic adenocarcinoma and PanIN lesions. Targeting of this enzyme by its specific inhibitor, Lipitor, suggests its potential as a therapeutic and chemopreventive agent.

1303 β-Catenin and E-Cadherin Are the Most Sensitive and Specific Markers for Solid-Psuedopapillary Neoplasm of the Pancreas

M-J Kim, S-J Jang, E Yu. University of Ulsan College of Medicine Asan Medical Center, Seoul, Republic of Korea.

Background: Histologic differentiation of solid-pseudopapillary neoplasm (SPN) from pancreatic endocrine neoplasm (PEN) or even from adenocarcinoma is not always easy, because these tumors share common morphologic features and immunohistochemical phenotypes. Thus, it is necessary to find out more reliable ancillary markers which are useful for the differential diagnosis among various pancreatic neoplasms. Recently, altered APC/β-catenin pathway that leads to nuclear accumulation of β-catenin was identified as a genetic event contributing to the development of SPNs, however, its diagnostic utility in SPNs has been rarely described.

Design: In the present study, we examined expressions of β-catenin and E-cadherin in addition to the known epithelial and neuroendocrine markers and CD10 in the primary pancreatic tumors to assess the diagnostic unitility of β-catenin and E-cadherin for the diagnosis of SPN. We constructed tissue microarray (TMA) using a total of 504 formalin-fixed paraffin-embedded pancreatic tumors that were resected from July 1995 to March 2006. Histologically unequivocal cases of 302 adenocarcinomas, 56 PENs, and 50 SPNs were evaluated for β-catenin, E-cadherin, cytokeratin (CK), CD10, CD56, synaptophysin and chromogranin by the immunohistochemical staining method. All markers were accessed as negative or positive. Positive cases were defined when 5% or more of tumor cells were immunostained.

Results: In cases of adenocarcinoma, all were positive for CK (100%), 300 cases for E-cadherin (99.3%), 30 cases for CD10 (9.9%), two cases for synaptopysin (0.7%), one case for CD56 (0.3%), and none for chromogranin and β-catenin. In cases of SPN, 47 cases were positive for β-catenin (94%), 41 cases for CD10 (82%), 26 cases for CD56 (52%), 13 cases for synaptophysin (26%), one case for CK (2%), and none for E-cadherin and chromogranin. In cases of PEN, 54 cases were positive for synaptophysin (96.4%) and E-cadherin (96.4%), 50 cases for chromogranin (89.3%), 26 cases for CK (46.4%), 15 cases for CD56 (26.8%), six cases for CD10 (10.7%), and none for β-catenin.

Conclusions: Loss of E-cadherin and nuclear expression of β-catenin are the most important immunoprofile of SPN. Thus, immunostainings for β-catenin and E-cadherin should be applied for a definite diagnosis of SPN. Furthermore, CD10 immunopositivity supports the diagnosis of SPN, however, it should be carefully interpreted in cases of pancreatic adenocarcinoma or PEN.

1304 Up-Regulation of Cellular Prion Protein Expression in Pancreatic Adenocarcinoma Carcinogenesis and the Prognostic Implications

SJ Kim, J Xu, C Li, MS Sy, W Liu, W Xin. Case Western Reserve University, Cleveland, OH; University Hospitals of Cleveland, Cleveland, OH.

Background: The normal cellular prion protein (PrP^c) is a glycosyl-phosphatidylinositol anchored membrane protein present on many cell types. PrP^c may function as a metal transporter. It also has antioxidant, cellular adhesion and signaling, and apoptotic regulatory activities. The functions of PrP^c may be cell type dependent. Because of the pro- and anti-apoptotic properties of PrP^c, we studied the PrP^c expression pattern in pancreatic adenocarcinoma (ADC) and explored the role of PrP^c in pancreatic carcinogenesis.

Design: Sixty-four consecutive cases of primary pancreatic ADC were selected. Forty eight of these 64 ADC cases had lymph node metastasis. For our control group, normal and pre-cancerous conditions were selected including normal pancreatic tissue and pancreatic intraepithelial neoplasia (PanIN-1, 2, 3; n=28, 40, and 30 cases respectively). Immunohistochemical studies were performed on tissue microarray slides using a high affinity monoclonal antibody specific for an epitope at the C-terminal domain of PrP^c. Clinical follow-up was up to 5 years in 39 patients.

Results: Spotty and weak immunostaining for PrP^c protein was detected in normal acinar and islet cells, but not in normal ductal epithelial cells. No detectable PrP^c was identified in any PanIN-1(28) or PanIN-2 (40) cases. Of the 30 PanIN-3 cases, 4 cases (13%) were weakly positive. Of the 64 ADC cases, focal or diffuse positive staining for PrP^c protein was found in 16 cases (25%). The PrP^c positive patients had a similar rate of lymph node metastasis, 75% (12/16), to that of PrP^c negative patients, 77% (37/48). Additionally, patients (n=8) with PrP^c protein expression had a shorter median survival time of 435 days, while 31 patients with no expression had a longer median survival time of 722 days. The difference of survival is statistically significant ($P<0.01$).

Diagnosis	No. of Cases	PrPc Positive
PanIN-1	28	None
PanIN-2	40	None
PanIN-3	30	4 (13%)
ADC	64	16 (25%)

Conclusions: Our data indicates that the up-regulation of PrP^c protein expression is present in a subset of pancreatic ADCs. Since PrP^c is an adhesion protein, apoptosis regulator, and matrix receptor, it may play an important role in the carcinogenesis and metastasis of pancreatic ADCs. As up-regulation of PrP^c protein expression in a tumor is related to a shorter survival period, we propose PrP^c as a prognostic marker and a potential treatment target.

1305 Intraductal Tubular Carcinoma of the Pancreas: Clinicopathologic and Immunohistochemical Analysis of 18 Cases

DS Klimstra, NV Adsay, D Dhall, M Shimizu, K Cymes, O Basturk, G Kloppel. Memorial Sloan-Kettering Cancer Ctr, New York, NY; Wayne State Univ-Karmanos Cancer Inst, Detroit, MI; Saitama Medical School, Saitama, Japan; New York Univ, New York, NY; Univ of Kiel, Kiel, Germany.

Design: 18 examples of intraductal pancreatic neoplasms with a predominantly tubular growth pattern were analyzed.

Results: 10 patients were female and 7 male. Mean age 53 yrs (25-72). Mean tm size = 6,9 cm (2.5-15). More than half occurred in the head, and a third showed diffuse involvement. The tumors were characterized by cellular intraductal nodules with tightly packed small acinar glands lined by predominantly cuboidal cells with modest amounts of cytoplasm which did not contain any apparent mucin. The nuclei were round to oval and atypical. Mitotic figures were readily identifiable. Necrosis was noted in some cases, focally showing comedo pattern. Eight cases had foci of invasion ranging from microscopic (n=5) to larger foci (n=3). All of the tumors labeled for CK7 and CK19, most expressed CA19.9, e-cadherin, MUC1 and MUC6, while MUC2, CDX2 and MUC5AC were negative. Loss of DPC4 was identified in only 1. p53 was overexpressed in 18% and p16 expression was lost in nearly half. In a mean follow-up of 69 mos, nine patients were alive and free of tumor after. Five patients had recurrence of disease, including local recurrences in the pancreas as well as lymph node and liver metastases, after 5 and 36 mos. Two died of tumor at 24 and 34 mos, respectively; two were alive with disease at 12 and 18 mos, respectively, and one died of unrelated causes at 48 mos.

Conclusions: Intraductal tubular carcinoma is a distinct clinicopathologic entity in the pancreas. By its intraductal nature, it resembles IPMNs, and by acinar pattern, mimics acinar cell carcinoma; however, it has several distinguishing characteristics. Common expression of MUC6, in the absence of MUC5AC, raises the possibility of pyloric differentiation. Despite the histologic indicators of a high-grade malignancy (atypia, mitosis and necrosis), overall outcome appears relatively favorable.

1306 FHIT Expression Is Decreased in Pancreatic Adenocarcinoma but Does Not Correlate with Survival

JR Kneile, M Bloomston, WL Marsh, K Huebner, WL Frankel. Ohio State University, Columbus, OH.

Background: Pancreatic adenocarcinoma is an aggressive disease with a poor prognosis. Recent advances have contributed to the understanding of molecular alterations in the pathogenesis of pancreatic cancer. The fragile histidine triad (FHIT) gene is a putative tumor suppressor gene located at chromosome 3p14.2 spanning the FRA3B fragile site. Alteration of FHIT expression has been implicated in the carcinogenesis of a variety of human carcinomas, including ductal carcinoma of the pancreas. Our purpose was to evaluate the expression of FHIT and other clinicopathologic findings in pancreatic adenocarcinoma and determine whether any correlated with survival.

Design: Cases of pancreatic adenocarcinoma with resection (n=98) were identified from the archival files and clinicopathologic information including patient age, gender, tumor size, grade, stage, lymph node status and outcome were noted. Control tissue was obtained from 34 cases of chronic pancreatitis and 11 normal pancreas. Tissue microarrays from formalin-fixed, paraffin embedded blocks were constructed, stained with an antibody against FHIT and staining was graded as strongly positive, weakly positive or absent. Tumor size, stage, grade, lymph node status were assessed for association with survival, and FHIT expression was evaluated for correlation with any of these features.

Results: The mean survival for adenocarcinoma patients was 14.6 months. The mean patient age was 64.5 years and the male to female ratio 1.2:1. The average age for patients with chronic pancreatitis and normal pancreas was 45 and 62, respectively, with a male to female ratio of 3.9:1 and 0.6:1, respectively. Tumor stage but not size, grade or lymph node status correlated with survival. Expression of FHIT was observed in benign ducts in 33/34 (97%) chronic pancreatitis and 11/11 (100%) normal pancreas. In adenocarcinoma, FHIT was absent in 50 (51%) cases, weak in 26 (27%), and strong in the remaining 22 (22%). There was no association between FHIT expression and tumor size, stage, grade, lymph node metastasis, or survival.

Conclusions: Of the clinicopathologic data evaluated, only tumor stage correlated with patient survival. There was no correlation between FHIT expression and patient survival, however, expression of FHIT was lost in half of the pancreatic adenocarcinomas studied. The results suggest that FHIT may play a role in the progression of pancreatic adenocarcinoma and indicate a potential role for FHIT-based gene therapy in a substantial portion of these patients.

1307 Immunohistochemical Stains for FHIT, CDX2, p53, Maspin and Ki-67 Are Altered in Gallbladder Adenocarcinoma but Only Tumor Size Correlates with Survival

JR Kneile, M Bloomston, M Butterfield, K Huebner, WL Frankel. Ohio State University, Columbus, OH.

Background: Gallbladder adenocarcinoma (GA) is an aggressive disease associated with a poor prognosis despite improvements in diagnostic imaging and surgical techniques. Recent studies have attempted to identify molecular markers to predict the behavior of GA and possibly influence post-surgical adjuvant therapy. The fragile histidine triad (FHIT) gene is a putative tumor suppressor gene altered in several carcinomas. We evaluated FHIT expression in GA and assessed the relationship between clinicopathologic features and immunostains for FHIT, CDX2, p53, maspin, and Ki-67 with survival in GA to determine if any features were useful to help predict outcome.

Design: Primary GA cases (n=23) were retrieved from archival files; and patient age, gender, tumor grade and stage and survival were noted. Cholecystectomies with benign gallbladders (n=15) were identified as controls. Tissue microarrays were created from formalin-fixed, paraffin-embedded blocks and stained with antibodies against FHIT, CDX2, p53, maspin, and Ki-67. Expression of FHIT was graded as positive, weak or absent; while >5% staining was considered positive for CDX2, p53, and maspin; and the proliferation index was determined using Ki-67 staining.

Results: 23 patients with GA were identified and the mean age at diagnosis was 65 years with male to female ratio of 0.4:1. The medium survival was 8.9 months, and the 5-year survival as 22%. Tumor size varied from 0.6 to 6.0 cm with a median of 2.4 cm. Tumor size but not grade or stage correlated with survival (univariate p=0.04 and multivariate p=0.05). In the GAs, immunoreactivity for CDX2, p53, and maspin were observed in 5/23 (22%), 9/23 (39%), and 16/23 (70%), respectively. In the benign gallbladders, all cases were negative for CDX2 and p53 and only 1/15 (7%) was positive for maspin. The average proliferative index in GAs was 36% and in the benign controls was 2.5% (p<0.001). Loss of expression for FHIT was seen in 10/23 (43%) GA cases, but was present in all benign controls. None of the immunohistochemical markers correlated with survival.

Conclusions: Immunostains for FHIT, CDX2, p53, maspin, and Ki-67 are useful to distinguish GAs from benign gallbladders. Tumor size but not grade, stage or immunostains for FHIT, CDX2, p53, maspin, or Ki-67 correlated with survival in GA. Loss of FHIT expression was observed in nearly half of the GA, suggesting a potential role for FHIT-based gene therapy in these patients.

1308 Unexplained Morphologic Hepatitis and Fibrosis in Baseline Donor Liver Biopsies: Follow-Up Histologic Findings and Clinical Outcome

M Krishna, MS Ghabril, RC Dickson, RE Nakhleh. Mayo Clinic, Jacksonville, FL.

Background: Abnormalities in donor liver may influence outcome after transplantation(LT). We investigated whether unexplained morphologic hepatitis with/without fibrosis(H/F) in baseline donor wedge biopsies(BLB) influenced pathologic findings and outcome after LT.

Design: Pathology and clinical databases were reviewed for all primary LTs at our institution between 1998-2004. Patients(pts) undergoing LT for disease other than viral or autoimmune hepatitis, PBC or PSC who received graft from a donor without known liver disease were considered. Study group(SG) consisted of pts whose BLBs had H/F and control group(CG) consisted of pts without these findings. Both groups were matched for age and gender. BLBs (1hr recirculation) and core biopsies(bxs) at 7days(d), 4mo and 1yr were reviewed by a pathologist(MK) for hepatitis, fibrosis and rejection. Activity and stage were scored on 0-4 scale. Pts' records were reviewed for outcome.

Results: Each group had 27 pts. Indications for LT were (SG/CG): cryptogenic cirrhosis(12/7), endstage fatty liver disease(11/13), hepatocellular carcinoma(3/1), A1AT deficiency(1/1), hemochromatosis(0/2), Caroli's disease(0/1), acetaminophen toxicity(0/1) and Budd Chiari syndrome(0/1). Mean follow-up was 735d (223-1520) for SG and 760d (557-920) for CG. Outcomes for both groups were similar for biliary/ vascular complications, recurrent disease, graft loss and death. Bx findings are summarized below:

* p<0.1 ** p<0.05	Baseline n=27		Day 7 n=27		4 mo n=27			1 yr SG n=22 CG n=27			delta stage
	**Hep	**Fib	Rej	*Fib	Rej	Hep	Fib	*Rej	Hep	*Fib	4m-1yr
Study (%)	100	48	52	19	30	37	30	32	55	45	0.32
Control (%)	0	0	52	0	19	30	11	11	52	19	0.11

Prevalence of hepatitis and mean grade at 4mo(0.41 vs 0.26) and 1yr(0.55 vs 0.48) were similar in both SG and CG respectively. Prevalence of acute rejection(AR) at 1yr was higher in SG, but not significant(p<0.1). Mean stage in SG vs CG was 0.44 vs 0.15 at 4mo(p<0.1), and 0.77 vs 0.26 at 1yr(p<0.05). 27% of SG vs 4% of CG had 1yr stage≥2, but fibrosis progression was similar in both groups.

Conclusions: 1) Fibrosis in donor livers is overestimated in wedge bxs (48% in BLB vs 19% at 7d in SG) 2) Pts with H/F in BLBs had similar grade and fibrosis progression by 1yr vs CG, but showed a trend towards more frequent AR at 1yr 3) Baseline fibrosis may account for increased fibrosis at 1yr. 4) Short term outcomes for both groups are similar.

1309 Patterns of Hepatic Injury Following Chemotherapy for Pancreatic Cancer

T Lee, R Anders, CA Iacobuzio-Donahue. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Most patients with pancreatic cancer will develop hepatic dysfunction that is believed due to progressive biliary obstruction and/or increasing metastatic burden in the liver. However, the effects to the liver of aggressive chemotherapy in these patients in unknown. Our goal was to characterize the histopathologic changes of the uninvolved liver in patients with advanced pancreatic cancer, and to correlate these changes with the tumor burden at autopsy and the treatment history to determine those factors that most contribute to the hepatic failure common among these patients.

Design: All patients with known pancreatic carcinoma who underwent a research autopsy as part of the Gastrointestinal Cancer Rapid Medical Donation Program were identified, and their clinicopathologic information including complete treatment history was reviewed. Histopathology of the hepatic parenchyma uninvolved by metastatic carcinoma was scored for the degree of bile plugging, periportal/ lobular inflammation and fibrosis using the criteria of Knodell, and steatosis and/or steatohepatitis using the criteria of Kleiner. Frequencies were compared using the Chi-squared text, or for sample sizes <5 the Fisher exact test. p-values ≤0.05 were considered significant.

Results: Thirty-one patients were identified for which paraffin-embedded sections of liver were available for review and the complete treatment history was available. The mean age was 61.3±10.5 yrs (range 43-85 yrs), the M:F ratio was 19:12, and all patients were Caucasian. Eleven of 31 patients' primary carcinoma was surgically resected but recurred. Four patients received no treatment and 27 patients received one or more

chemotherapy regimens, most commonly a flouropyrimidine based regimen (5-FU, 15 patients) or a nucleoside analog (Gemcitabine, 17 patients). Overall, the liver of all 31 patients showed at least one feature of hepatic injury (periportal inflammation, steatosis ≥30%, or bile plugging). No relationship was found among the presence or absence of hepatic injury and treatment (any or none), or type of treatment regimen, nor was an association found among bile plugging and the size and/or presence of the primary pancreatic cancer.

Conclusions: Hepatic injury is common in the liver of patients with advanced stage pancreatic cancer, but the histopathologic patterns of injury are heterogeneous. The lack of an association among hepatic injury and treatment history or mechanical obstruction supports metastatic tumor burden as a major contributing factor to hepatic dysfunction.

1310 Early Detection of Post-Transplant Recurrent Hepatitis C (RHC) as a Predictor of Accelerated Progression to Fibrosis and Liver Failure – A 56 Case Study

X Li, M Facciuto, G Rmaswamy. Westchester Medical Center, Valhalla, NY.

Background: Orthotopic liver transplant (OLT) becomes a life saving procedure for patients with end-stage liver disease. Among them, hepatitis C is the most common indication, acounts for 40-50% of cases. However, viral recurrence often causes graft loss and the need for retransplantation. The clinical course of RHC is variable - some experience indolent diseae, whereas others progress rapidly to cirrhosis. The aim of our study is to investigate the ocurrence of RHC and its progression to fibrosis and cirrhosis in post OLT patients.

Design: Total of 56 patients with hepatitis C cirrhosis who underwent OLT between 1999 and 2005 in Westchester Medical Center (WMC) and diagnosed with RHC in follow ups were selected, 44 males and 12 females with an average age of 55 years. Liver biopsies were done following a standard protocol at 6, 12, 24, 36 and 48 months, with additional biopsies when clinically indicated. H&E, trichrome, reticulin and iron stains on each biopsy were reviewed by pathologist based on accepted criteria.

Results: Result I: Histological RHC occurs as early as 1 month post OLT. 1. 40 patiens (71.4%) showed RHC within 6 months, mean time 3.3±1.5 month. 2. 9 patiens (16.1%) showed RHC between 6 to 12 months, mean time 8.6±1.1 months. 3. 7 patiens (12.5%) showed RHC after 12 months, mean time 24.8±9.6 months. Result II: Progression of RHC to liver fibrosis or cirrhosis1. 27 patients (48.2%) had progressed to stage 2 fibrosis, mean time 13.6±7.7 months. 2. 13 patients (23.2%) had progressed to stage 3-4 fibrosis, mean time 35.3±19.0 months. Result III: Relationship between detection time of RHC and disease progression.

Relationship between detection time of RHC and progression of liver fibrosis

Mean Time	RHC ≤6 (months)	RHC 6-12 (months)	RHC≥12 (months)
RHC	3.3±1.5 (n=18)	7.3±1.1 (n=2)	24.8±9.6 (n=7)
Stage 2 fibrosis	11.8±8.7 (n=18)	N/A (n=2)	22.5±6.7 (n=7)
Stage 3-4 fibrosis	31.6±20.0 (n=2)	38.8±25.8 (n=2)	35.5±11.3 (n=3)
RHC with no fibrosis		9.2±1.5 (n=9)	

*n=case number

Conclusions: 1. Majority of RHC (71.4%) occurs within the first 6 months post OLT. Histological evidence of RHC can be detected as early as 1 month post OLT. 2. Individuals with early onset RHC (≤ 6 months) progress to fibrosis much faster (mean 11.8±8.7 months) than the ones with late onset (> 6 months) (mean 22.8±9.6 months), indicating that the onset of RHC may be used as a prognostic predictor in post OLT patient.

1311 Expression of MAP4K4 Is Associated with Worse Prognosis in Patients with Pancreatic Ductal Adenocarcinoma

JJ Liang, H Wang, A Rashid, T-T Wu, KR Cleary, SR Hamilton, DB Evans, RF Hwang, HF Gomez, TH Tan, GS Zhou, JL Abbruzzese, H Wang. The University of Texas M.D. Anderson Cancer, Houston; Baylor College of Medicine, Houston, TX.

Background: MAP4K4 is a serine/threonine kinase and belongs to the mammalian STE20/mitogen-activated protein kinase kinase kinase kinase (MAP4K) family. Recent studies have shown that MAP4K4 is overexpressed in many types of human cancer cell lines and tumors compared to normal tissue. MAP4K4 plays an important role in cell transformation, invasiveness, adhesion, and cell migration. The expression of MAP4K4 and its significance in pancreatic ductal adenocarcinoma (PDA) has not been studied. In this study, we examined the expression of MAP4K4 in 58 PDAs and correlated expression with clinicopathologic features.

Design: We constructed tissue microarrays using formalin-fixed, paraffin-embedded archival tissue from 58 Whipple's specimens for PDA (2 stage I, 53 stage II, and 3 stage IV). Each tumor and its paired benign pancreas were sampled in duplicate with a 1-mm punch core. The expression levels of MAP4K4 were evaluated by immunohistochemistry with a rabbit polyclonal antibody (1:250 dilution). The staining results were categorized independently by two gastrointestinal pathologists as positive (strong cytoplasmic and nuclear staining of ≥10% of tumor cells) or negative (no staining or staining of <10% of tumor cells). Statistical analyses were performed using SPSS software (version 12 for Windows; SPSS, Chicago, IL) and survival was evaluated by Kaplan-Meier log-rank test.

Results: MAP4K4 was overexpressed in PDAs (30/58, 52%) compared to benign pancreatic ducts (12/54, 15%, p=0.0001). The median overall survival of patients with MAP4K4 positive PDAs was 26.1 months compared to 82.9 months for patients with MAP4K4-negative tumor (log-rank test, p=0.046). Recurrence was present in 92% (23 of 25) MAP4K4-positive tumors compared to 58% (14 of 24) in MAP4K4-negative PDAs (p=0.008). Expression of MAP4K4 was also associated with reduces disease-free survival in PDAs (log-rank test, p=0.001). No association was found between MAP4K4 expression and other clinicopathologic features.

Conclusions: MAP4K4 is overexpressed in PDA and is associated with worse prognosis and higher frequency of recurrence/metastasis. Our study suggests that expression of MAP4K4 may serve as a useful prognostic marker for PDA.

1312 Nucleolar Expression of Catalytic Subunit of Human Telomerase Reverse Transcriptase (hTERT) Is Associated with Better Prognosis in Patients with Pancreatic Ductal Adenocarcinoma

JJ Liang, A Rashid, T-T Wu, H Wang, KR Cleary, SR Hamilton, DB Evans, RF Hwang, HF Gomez, JL Abbruzzese, H Wang. The University of Texas M.D. Anderson Cancer, Houston; The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Background: Telomerase, a ribonucleoprotein that maintains telomers, is composed of the catalytic protein subunit (hTERT) and a telomerase RNA template. Recent studies have shown that hTERT is a prognostic marker for several malignancies including gastrointestinal cancers. The expression of hTERT and its significance in pancreatic ductal adenocarcinoma (PDA) has not been studied in detail. In this study, we examined the expression of hTERT in 69 PDAs and correlated expression with clinicopathologic features.

Design: We constructed tissue microarrays using formalin-fixed, paraffin-embedded archival tissue from 69 Whipple's specimens for PDA (1 stage I, 65 stage II, and 3 stage IV). Each tumor was sampled in duplicate with a 1-mm punch core. The expression levels of hTERT were evaluated by immunohistochemistry with a mouse anti-hTERT monoclonal antibody (Clone 44F12, 1:25, Novacastra, Novwel, MA). The staining results were scored semiquantitatively using Ariol Image Analysis System (San Jose, CA). The tumor cells were selected and marked with a handdraw tool and the hTERT labeling index in tumor cells was measured as a ratio between the stained nuclear area and the total nuclear area. The computerized images and results were reviewed by two gastrointestinal pathologists. Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL) and survival was evaluated by Kaplan-Meier log-rank test.

Results: Nucleolar expression of hTERT-high (defined by labeling index of >10% in tumor nuclei) was present in 35 of 69 (51%) tumors. The median overall survival of patients whose tumors had hTERT-high was 67.8 compared to 18.0 months in patients whose tumors had hTERT-low (defined by labeling index of < 10% in tumor nuclei, log-rank test, p=0.013). Distant metastasis was present in 11 of 35 (31%) hTERT-high tumors compared to 23 of 34 (68%) in hTERT-low tumors (p=0.004). No association was present between hTERT expression and other clinicopathological features.

Conclusions: Nucleolar expression of hTERT in PCA is associated with better prognosis and lower frequency of distant metastasis. Our study suggests that nucleolar expression of hTERT may be a useful prognostic marker for PDA.

1313 Expression of S100A4, S100A6, and S100P in Pancreatic and Ampullary Adenocarcinomas

F Lin, ME Hull, J Shi, JW Prichard, RE Brown, HL Wang, C Schuerch. Geisinger Medical Center, Danville, PA; Washington University School of Medicine, St. Louis, MO; University of Texas at Houston, Houston, TX.

Background: Several S100 family proteins, including S100A4, S100A6 and S100P, have recently been identified as potential markers for pancreatic ductal adenocarcinoma (PDAD) and may be associated with poor disease survival. Even though ampullary adenocarcinoma (AAD) often shows similar histologic features to PDAD, it carries a much more favorable prognosis. In this study, we compared the diagnostic value of S100A4, S100A6, and S100P in the detection of PDAD and compared the expression of those 3 markers in PDAD and AAD.

Design: Forty-four cases of PDAD and 17 cases of AAD were included in this study. Immunohistochemical stains were performed with monoclonal antibodies against S100A4, S100A6, and S100P. The staining intensity was graded into weak, moderate or strong. The distribution was recorded as negative (no staining), 1+ (<25% staining), 2+ (26-50% staining), 3+ (51-75% staining), or 4+ (>75% staining). Only nuclear staining or nuclear and cytoplasmic staining patterns were recorded as positive.

Results: Of the 44 PDAD cases, all were positive for S100P and S100A6, with strong and diffuse staining (>3+) in 38 and 37 cases, respectively. In contrast, S100A4 was positive in 32 cases, with strong and diffuse staining (>3+) in only 14 cases. Importantly, non-neoplastic ductal epithelium was negative for S100P in all cases; whereas weak staining for S100A6 and S100A4 was seen in benign ductal epithelium in 24 and 12 cases, respectively. For AAD, positive staining for S100P, S100A4, and S100A6 was observed in 16 of 17, 17 of 17, and 15 of 17 cases, respectively. In contrast, focal and weak positivity for S100P, S100A4, and S100A6 was seen in normal small intestinal epithelium in 3, 6, and 5 cases, respectively.

Conclusions: Our data suggest that 1) S100P is a better marker for identifying PDAD than S100A4 and S100A6, since the expression of S100P in normal/reactive pancreatic ductal cells is not identified; 2) S100A4, S100A6 and S100P are expressed in both PDAD and AAD; therefore, they have no value in differentiating these two types of tumors; 3) in addition, these 3 markers probably do not play a key role in determining the poor prognostic outcome of PDAD since they are also expressed in AAD.

1314 Immunohistochemical Expression of Vitamin D Receptor Correlates with Decreased Immunohistochemical Activity of Peroxisome Proliferator-Activated Receptor-α (PPARα) in Steatohepatitis

Q Liu, LK Campbell, M Tretiakova, C Gong, J Hart. The University of Chicago Hospitals, Chicago, IL.

Background: PPARα is a key transcriptional regulator of genes involved in hepatic β-oxidation of fatty acids. Its expression is reportedly reduced in experimental and human steatohepatitis. Vitamin D receptor (VDR) is known to inhibit PPARα transcriptional activity and our previous study revealed increased expression of VDR in hepatocytes in patients with NASH. This study was done to evaluate PPARα expression and its relationship to the expression of VDR in steatohepatitis.

Design: 53 biopsies (36 steatohepatitis, including 30 NASH and 6 alcoholic steatohepatitis (ASH), 9 steatosis, and 8 normal liver) were included. The grade of steatohepatitis was evaluated by the degree of hepatocytic ballooning degeneration (0-3+ scale). Immunohistochemistry was performed on formalin fixed tissue using polyclonal

antibodies to VDR and PPARα (ABR, 1:125). Similar staining patterns and intensity were obtained with two VDR antibodies (Santa Cruz, 1:250; ABR, 1:600). VDR staining was graded on a 0-3+ scale. PPARα expression in hepatocytes was graded as either nuclear staining with cytoplasmic coarse granules or nuclear staining alone.
Results: Reactivity for VDR was evident only in bile ducts in normal liver. Hepatocyte reactivity for VDR was higher ($p<0.01$) in steatohepatitis (24/36(67%) overall, 18/30 NASH and 6/6 ASH) than in steatosis (33%, 3/9). VDR expression was limited to foci of hepatocytic ballooning degeneration, largely in centrilobular regions, and there was a correlation ($p<0.05$) between intensity of VDR expression and severity of ballooning degeneration in steatohepatitis. Nuclear and cytoplasmic staining for PPARα, located predominantly in centrilobular hepatocytes, was found in all steatosis (9/9) and normal liver biopsies (8/8). In contrast, cytoplasmic staining for PPARα was evident in only 33% (12/36) of the cases of steatohepatitis ($p<0.01$). There was an inverse correlation of VDR to PPARα staining in two third of the steatohepatitis cases, ether positive VDR without coarse cytoplasmic granules for PPARα (33%, 12/36) or negative VDR with coarse cytoplasmic granules for PPARα (33%, 12/36).
Conclusions: VDR expression appears to significantly correlate with the degree of hepatocyte injury in steatihepatitis. The finding of decreased expression of cytoplasmic PPARα in VDR positive steatohepatitis supports the concept that VDR inhibits PPARα transcriptional activity and may play a role in the pathogenesis of steatohepatitis.

1315 Improvement of Nonalcoholic Fatty Liver Disease after Laparoscopic Roux-en-Y Gastric Bypass Surgery for Morbid Obesity

X Liu, GA Abrams, R Clements, N Jhala, AJ Lazenby. University of Alabama at Birmingham, Birmingham, AL.
Background: Obesity-associated nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disorder. Previous studies have been variable regarding the histological outcomes after the rapid weight loss from bariatric surgery. The aim of this study was to characterize the histopathologic changes in NAFLD following laparoscopic Roux-en-Y Gastric Bypass surgery (LRNY).
Design: We retrospectively analyzed paired needle liver biopsies in 39 morbidly obese subjects following LRNY according to the recent NIH-based histological scoring system for NAFLD: steatosis (0-3), hepatocellular ballooning (0-2), lobular inflammation (0-3), stage of fibrosis (0-4) and centrilobular fibrosis scored (0-3) according to the relative length of fibrosis extending from the central veins. Results were analyzed by student *t*-tests and Wilcoxon matched-paired signed rank test using SPSS (V13) for windows.
Results: The study population included 33 females and 6 males with a mean age of 44 years. Twenty three of the 39 cases (58.9%) were classified as steatohepatitis on their initial liver biopsies using ballooning as the criteria, 12 fatty liver (30.7%), and 4 normal (10.2%). Follow-up needle liver biopsies were performed at a mean interval of 18 months. Table 1 summarizes the histopathologic changes in NAFLD. Steatosis, ballooning, lobular inflammation, and centrilobular fibrosis were present in 35 (89.7%), 23 (58.9%), 39 (100%), and 18 (46.1%) of the initial liver biopsies, respectively. Follow-up biopsies showed resolution of steatosis and ballooning in all cases and a decrease in lobular inflammation and centrilobular fibrosis in 33.3% and 88.9% patients, respectively.

Table 1

	BMI (kg/m^2)	ALT	Steatosis	Ballooning	Lobular inflammation	Centrilobular fibrosis	Portal fibrosis
Pre-surgery	47.0±6.2	34.5±19.0	2.03±1.1	0.62±0.54	2.23±0.63	0.86±1.02	0.44±0.50
Post-LRNY	29.5±5.6	23.7±13.2	0.03±0.16	0.00	1.95±0.56	0.33±0.63	0.39±0.49
P value	0.000	0.014	<0.0001	<0.0001	0.01	<0.0001	0.50

Conclusions: Weight loss secondary to LRNY significantly improves steatosis, ballooning, lobular inflammation, and centrilobular fibrosis. In comparison, no alterations in portal inflammation and portal fibrosis were noted. Long-term studies are warranted to assess for total regression of fibrosis following LRNY.

1316 Liver Fibrosis Is Plastic: Regression and Remodeling of Terminal Venular Fibrosis Associated with Central Venulitis in Liver Allografts

S Lu, KS Gustafson, EE Furth. University of PA Medical Center, Phila, PA.
Background: Liver fibrosis is initiated by a variety of injuries which may cause marked liver dysfunction. The plasticity of fibrosis once injury has been withdrawn is not known. Acute rejection of the central venular (CV) type is often associated with fibrosis. Unlike viral hepatitis, acute rejection may be readily eliminated in a timely manner by therapy. Hence, this situation offers an ideal setting to study the dynamics of liver fibrosis. The goal of our study was to quantitatively determine the extent to which central venular fibrosis progresses and/or regresses in association with this form of injury.
Design: Liver biopsies (n = 176) from 31 allografts from 30 patients (15 males and 15 females, mean age 52 years) transplanted for viral hepatitis (19), PBC (4), PSC (2), and other (5) with central venulular type rejection at any time point were scored for the severity of central venulitis (0-3), central venular fibrosis (0[none]-4[central to portal bridging]), and portal fibrosis (0-4). Complete resolution was defined as a fibrotic score of 0 and unresolved fibrosis was defined as a fibrotic score >0 at the time of the last biopsy.
Results: Central venulitis and CV fibrosis paralleled each other in all cases. Resolution at some time point occurred in 52% of cases with complete resolution of CV fibrosis in 39% of cases. The distribution of CV fibrotic scores for those with complete resolution of fibrosis tended to be lower than those with unresolved fibrosis, but did not reach statistical significance ($p=0.0683$); 33% with complete resolution and 42% with unresolved fibrosis had maximum CV fibrotic scores of 4. The median number of episodes of central venulitis was significantly greater in cases with unresolved versus completely resolved CV fibrosis (3 vs 1; $p=0.0112$), although the severity of central venulitis was not different. The median length of engraftment and time to the first episode of central venultitis were not significantly different between cases with completely resolved and unresolved CV fibrosis. In contrast to the reversibility of CV fibrosis, portal tract fibrosis was progressive with no cases of complete resolution ($p=0.0001$).
Conclusions: Central venular fibrosis is associated with central venular cellular rejection. Even with central to portal bridging fibrosis, remodeling and resolution of fibrosis may occur. This remodeling is independent from the progressive portal fibrosis usually attributable to continued hepatitis. Thus, even advanced liver fibrosis is plastic if the injury can be ameliorated.

1317 Fibrolamellar Carcinomas but Not Typical Hepatocellular Carcinomas Are Positive for Anterior Gradient-2

STL Micchelli, M Torbenson. The Johns Hopkins School of Medicine, Baltimore, MD.
Background: Anterior Gradient-2 is critical in forming the anterior-posterior gradient in embryonic development. Interestingly, gene expression studies have suggested it may also play a role in hepatocellular carcinogenesis.
Design: Immunohistochemistry was performed on tissue microarrays that included typical hepatocellular carcinomas (HCC), fibrolamellar carcinomas and hepatic adenomas. Staining distribution was scored on a scale of 1-4: 1 (from 5-25%), 2 (26-50%), 3 (51-75%) and 4 (>76%). Intensity was graded on a scale of 1-3. Benign colon served as a positive control.
Results: Only 2/40 (5%) typical HCC were positive for Anterior Gradient-2. In contrast, 6/8 (75%) of fibrolamellar and 3/4 (75%) metastatic fibrolamellar carcinomas were positive. The median staining distribution was 2, and the median staining intensity was 2. All 9 of the hepatic adenomas were negative. In the non-neoplastic livers, septal-sized bile ducts were positive, while smaller ducts were negative. In addition, 11/57 (19%) cases with background non-neoplastic liver showed weak immunostaining, limited to zone 3 hepatocytes. In the non-neoplastic liver, staining did not correlate with the underlying liver disease or with fibrosis stage.
Conclusions: Anterior Gradient-2 is normally expressed in septal-sized bile duct, and in non-neoplastic zone 3 hepatocytes. The majority of fibrolamellar carcinomas (both primary and metastatic) are positive for Anterior Gradient-2, while most HCC and all hepatic adenomas are negative.

1318 Differentiation of Serous Cystic Pancreatic Neoplasms and Metastatic Renal Cell Carcinoma by Immunohistochemistry

CP Molina, M Kim, J Zhai, AG Ayala, JY Ro. The Methodist Hospital, Houston, TX; Asan Medical Center, Seoul, South Korea.
Background: Distinguishing primary serous cystic pancreatic neoplasms (SCPN) from metastatic renal cell carcinoma (RCC) is important for both therapeutic and prognostic reasons. Even after thorough histological examination, metastatic RCC can still be mistaken for primary SCPN. Although some tumor markers have been advocated and are helpful in most cases, the distinction remains a problem.
Design: Seventy two (72) cases of SCPN were obtained from the surgical pathology files of the Departments of Pathology at The Methodist Hospital in Houston, Texas; and from Asan Medical Center in Seoul, South Korea (August 1996 to June 2006). We developed tissue microarrays that were used to survey several antibodies in a parallel single experiment. Ten cases of RCC were the control group. We evaluated the expression of pancytokeratin (panCK), vimentin, alpha inhibin, CD56, Pax-2 protein (transcription factor expressed in early kidney organogenesis), and RCCma or RCC marker (monoclonal antibody against normal human proximal tubular brush border). This panel was designed to test its diagnostic utility in the differential diagnosis of SCPN and RCC. The expression of RCCma and CD10 in renal cell carcinomas is well documented and its expression in SCPN has not been previously reported in the literature. The immunostaining was observed under light microscopy at ×100 and ×400. The grade and the distribution of the signal were scored semiquantitatively from absent (-) to strong (+++) and from focal to diffuse.
Results: There were 48 females and 24 males (F:M ratio 2:1) with an average age of 51.3 years (range from 23 to 81 years). The size of the lesions ranged from 1.0 to 10.0 cm (average 4.1 cm). All 72 cases stained strongly for PanCK (100%) and all were negative for RCCma, vimentin, and Pax-2 (100%). Alpha-inhibin was positive in 42% of SCPN (30 out of 72); CD56 was positive in only 4.2% (3 out of 72).
Conclusions: Our results suggest that the expression of RCCma, Pax-2, and vimentin are specific for RCC. Therefore, these markers are helpful in differentiating primary SCPN from metastatic RCC.

1319 Histologic Predictors for Recurrence of Primary Sclerosing Cholangitis in Liver Allografts

EM Naar, J Alexander, K Kowdley, PE Swanson, MP Upton, MM Yeh. University of Washington, Seattle, WA.
Background: Primary sclerosing cholangitis (PSC) typically affects adult men causing bile duct sclerosis, progressive ductopenia, cholestatic disease, and cirrhosis. The ultimate treatment for end-stage PSC is orthotopic liver transplantation (OLT); however, a significant number of allografts develop recurrent PSC after OLT. Our aim was to identify histologic features in explanted livers and post-OLT biopsies that predict recurrent PSC.
Design: 60 patients who underwent OLT for PSC at our institution were included in this study. Explanted native livers and serial post-OLT biopsies spanning from 1990 to 2005 were retrospectively reviewed. Recurrent PSC was confirmed in 10 patients based on the combination of histologic, clinical, and radiological findings. Slides of the liver explants and post-OLT biopsies of these 10 recurrent PSC patients and 10 patients without recurrence were reviewed blindly. Histologic features evaluated included periductal fibrosis, fibro-obliterative lesions, portal inflammation, periportal injury, parenchymal injury, fibrosis, bile duct injury, acute cellular rejection (ACR) and ductular reaction. Statistical analysis using chi-square and Mann-Whitney tests were performed where appropriate.

Results: Eight of 10 (80%) patients with recurrent PSC had at least one post-OLT biopsy showing marked bile ductular reaction in that 8 or more ductules were present in at least one portal tract, while only 3 of 10 (30%) patients without recurrence showed marked ductular reaction. ACR was significantly more common in allografts which subsequently developed recurrent PSC. The presence of fibro-obliterative lesions was diagnositic of recurrent PSC. Post-OLT biopsies showing ductopenia were present at least once in 9 of 10 recurrent patients, while only 2 of 10 non-recurrent patients had biopsies showing ductopenia (p=0.01). There was no significant difference in bile duct injury between the two groups. Inflammation and fibrosis in explants were not significantly different comparing the two groups.

Conclusions: In recurrent PSC there appears to be a phase of marked ductular reaction similar to what has been observed in the natural course of PSC. Episodes of ACR increase risk for recurrence. The appearance of marked ductular reaction or ductopenia in post-OLT biopsies should alert the pathologists and clinicians for impending recurrent PSC, although these changes must be distinguished from biliary obstruction and chronic rejection.

1320 Clinical Pathologic Review of 106 Failed Liver Grafts Treated with Retransplantion

RE Nakhleh, M Krishna, PV Genco, C Panjala, AP Keaveny. Mayo Clinic Jacksonville, Jacksonville, FL.

Background: We reviewed all failed liver transplant grafts occurring in our program through 2005 to better understand the pathology and associated clinical conditions and to determine their subsequent outcomes.

Design: Cases were identified by search of a surgical pathology database to identify failed liver allografts treated by retransplantation. All available pathologic material of the failed grafts was reviewed. Clinical charts were reviewed to determine the timing of failure, associated clinical conditions and outcomes.

Results: A total of 106 failed liver grafts were identified from 101 patients (64m, 37f). Original reason for transplantation included HCV(42), PSC(18), cryptogenic(10), ETOH(10), HBV(7), PBC(4), A1AT(4), NASH(4), tumor(3), biliary atresia(2) and one each of secondary sclerosing cholangitis and congenital hepatic fibrosis. Some patients had more than one disease entity. 82 patients had undergone one liver retransplant and 19 had two or more liver retransplants. Not all explants were available for review. Pathologic findings in failed grafts included ischemic cholangiopathy(ISC Chol)(40), pericentral necrosis suggestive of diffuse ischemia(17), chronic rejection(CR)(14), recurrent disease (9; 5PSC, 4HCV), confluent necrosis suggestive of localized infarction(9), cholestasis of unknown cause(7), cholangitis of unkown cause(5), cholangitis lenta(6), and veno-occlusive disease(3). The table below shows the time to failure and f/u after retransplantation.

Finding (n; alive, dead)	ISC Chol (40; 33,7)	Ischemia (17; 8,9)	CR (14; 9,5)	Recurrent disease (9; 8,1)	Cholestasis or cholangitis (18; 6,12)	Infarction (9; 7,2)	Others (5; 3,2)
Failtime, mo (median)	0-206 (2)	0-14 (2)	1-96 (10)	6-206 (96)	1-156 (2)	0-4 (1)	1-39 (4)
Alive f/u, mo (median)	9-92 (34)	14-94 (34)	11-101 (30)	9-88 (38)	27-101 (81)	30-73 (72)	12-38 (18)
Dead f/u, mo (median)	0-11 (2)	0-62 (4)	0-20 (5)	20	1-44 (5)	1d, 5d	0,4

Overall, 30 patients died and 71 were alive at last f/u. Survival was below 50% for patients whose explants showed cholestasis, cholangitis or ischemia. Survival was best for patients with recurrent disease (88%).

Conclusions: 1) Retransplantation is a reasonable option for failed liver allografts. 2) Some pathologic findings (ischemia, cholestasis and cholangitis) were associated with poor survival. 3) Patients with recurrent disease on the other hand had excellent patient survival. 4) Further studies are needed for exploring associated immunologic and clinical conditions to better define pathogenesis and prognosis.

1321 Islets, the King of Antigens? Non-Specific Reactivity of (Especially the) Islets Is a Common Pitfall in Immunohistochemical Evaluation of the Pancreas

I Nalbantoglu, O Basturk, D Thirabanjasak, F Sarkar, JD Cheng, E Levi, NV Adsay. St. John Hosp and Medical Center, Detroit, MI; New York Univ, New York, NY; Wayne State Univ-Karmanos Cancer Inst, Detroit, MI.

Background: As the role of immunohistochemistry (IHC) is the main and most practical tool to investigate the differential expression of proteins in different cellular compartments, it regains its importance in cancer research, some of the pitfalls are also becoming an important source of controversy and misinterpretation.

Design: The labeling profiles of 22 consecutive antibodies performed in the authors' research laboratory by the request of cancer researchers were analyzed. These antibodies were KGF, ADM, ATDC, BMI, FAK, GLUT1, RAP-1, CDX2, SP-1, P53, MIB-1, P21, VEGF, GP520, PAK, COX2, PR, AAT, GFAP, osteonectin, H3K27me3, and nestin. Many of these are commercially available but some are investigator-derived.

Results: 15 of these 22 antibodies showed labeling in the islets (KGF, ADM, BMI, FAK, GLUT1, RAP-1, CDX2, MIB-1, P21, VEGF, GP250, PAK, COX2, H3K27me3 and PR). While in some (progesteron, PAK, and GLUT-1) this labeling might have had some biologic explanation, in the others, this was an unexpected finding. In most (10 antibodies) the labeling was faint, homogenous and smudgy, while in 4; it was diffuse granular cytoplasmic and 1 weak nuclear. This labeling did not appear to have any preferential distribution pattern of the isletic hormones, and it was also evenly distributed in the positive cells, suggesting that it was a cross-reaction with a cytosolic component present diffusely in the cell. 18 of these antibodies (KGF, ADM, ATDC, BMI, FAK, RAP-1, CDX2, SP1, p53, MIB-1, p21, VEGF, GP520, PAK, COX2, osteonectin, H3K27me3 and nestin) also showed unexpected labeling in the cytoplasm of the acini. The common feature of this labeling was that it was predominantly paranuclear, multiple, and composed of relatively evenly-sized mini-globules, possibly corresponding to a ribosome-associated organelle. For a given antibody, this labeling was noted only in a percentage of cases.

Conclusions: Antigenic cross reactivity is an important pitfall in immunohistochemical evaluation of pancreatic tissue. Islets often show a diffuse but weak smudgy possible non-specific labeling with an undetermined cytosolic component. Some antibodies appear to cross react with acini in a paranuclear dot like pattern. If these staining patterns are encountered, its specificity ought to be verified with other confirmatory techniques.

1322 One-Year Interim Report on the HCV-3 Trial: Favorable Histologic ACR Rates in Steroid-Free Immunosuppression Regimen

GJ Netto, D Watkins, W Niklinsk, F Sharkey, N Jhala, S Sharma, C Coreless, T Colby, G Klintmalm, The HCV3 Trial Group. Johns Hopkins Univ, Baltimore; Baylor Univ. Med. Ctr, Dallas; UT San Antonio; Emory Univ; Univ. of Cincinnati; Univ. of Alabama at Birmingham; Oregon Health & Science Univ; Mayo Clinic, Scottsdale.

Background: Chronic hepatitis C (HCV) is currently the most common indication for liver transplantation. The primary objectives of the "HCV 3 trial" are to compare the rates of HCV recurrence, acute cellular rejection (ACR), and treatment failure among three immunosuppressive regimens.

Design: Patients (pts) receiving OLTX for end stage HCV are prospectively randomized to three Rx arms : arm 1: tacrolimus (TAC) and steroids (Pred); arm 2: TAC, mycophenolate mofetil (MMF) and Pred; and arm 3: TAC, MMF and daclizumab (steroid free arm). Total of 312 pts from 18 centers were recruited. Liver biopsies are obtained at days 1, 90, 365 and 730. All biopsies are originally reviewed by local pathologists. A central pathologist review is ongoing for the final study analysis. HCV recurrence and ACR are evaluated according to Batts and Ludwig and the 1997 Banff schema respectively. Primary composite end points are defined as a recurrent HCV of stage 2 during the first year or grade 3 at any point and or an ACR of Banff global grade 2 with RAI score 4. Using local pathologist readings, the current report compares histologic findings in the 3 Rx arms in 290 pts (827 biopsies) who completed one year F/U.

Results: ACR: Through one year post OLTX, significantly greater proportion of pts in Arm 3 (steroid free arm) remained free of histologic ACR, as defined above, compared to those in Arm 1, (81.9±4.4% vs. 93/0±2.2%; *P*=0.011) and to those in Arms 1 and 2 combined (*P*=0.033). No severe rejection episodes were recorded in treatment Arm 3, compared to 10.5% and 25% of ACR episodes in Arms 1 and 2 respectively. **HCV recurrence:** Kaplan-Meier time-to-event analysis revealed no significant difference in freedom from HCV recurrence across treatment arms. At one-year, freedom from recurrence HCV was 61.8±6.2%, 60.1±6.1% and 67.0±4.3% in arms 1, 2, and 3, respectively (*P*=NS). On Cox proportional hazards model, ACR emerged as a highly significant risk factors for HCV recurrence (hazard ratio 2.69).

Conclusions: At one year post OLTX, pts in the steroid-free immunosuppressive regimen arm had a significantly reduced incidence of ACR while maintaining a similar rate and time to onset of HCV recurrence compared to the other Rx arms.

1323 Overexpression of Human Carcinoma-Associated Antigen (HCA) in Pancreatic Adenocarcinoma

P Nigwekar, TR Lester, Q Yang, PA Bourne, JL Yao, PA di Sant'Agnese, J Zhai, H Wang, J Huang. University of Rochester Medical Center, Rochester, NY; Methodist Hospital, Houston, TX; University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Human carcinoma-associated antigen (HCA) was originally identified by a monoclonal antibody (HAE3) against epiglycanin, a mucin protein secreted by a mouse mammary carcinoma (CA) cell line. The level of serum HCA increases in patients with a variety of malignancies and is being explored as a potential biomarker for cancer screening. Over-expression of HCA has been shown in tumor cells of bladder, prostate and esophagus by immunohistochemistry. Expression of HCA in pancreatic tumors has not been studied.

Design: Thirty-four cases of pancreatic tumors (28 cases of pancreatic adenocarcinomas and 6 cases of pancreatic endocrine tumors) were stained with anti-HCA monoclonal antibody HAE3 (Egenix Inc, NY, used at 1:2000). Benign pancreatic tissue was present in all cases. Staining intensity (0 to 3+) and percentage of stained cells were recorded. Positive staining was defined as 2+ or 3+ staining in 5% or more of the cells. Statistical analysis was performed using Fisher Exact Test.

Results: Only rare benign pancreatic ductal epithelium showed positive staining and islet cells were always negative. All pancreatic endocrine tumors were negative for HCA while the majority of ductal carcinomas were positive (Table 1). The differences in HCA expression between adenocarcinoma and benign pancreatic ductal epithelium was statistically significant (p<0.001), as was that between adenocarcinoma and pancreatic endocrine tumors (P<0.001).

Conclusions: 1. The mucin protein HCA is selectively overexpressed in adenocarcinomas of the pancreas in comparison to benign pancreatic ductal epithelium. 2. HCA is not expressed in benign islet cells or pancreatic endocrine neoplasms. 3. The function of HCA in carcinogenesis of the exocrine system of the pancreas merits further study.

Immunohistochemical Staining of Benign and neoplastic Pancreatic Tissue with HAE3

	Positive	Negative
Benign Pancreatic Ductal Epithelium	8% (3/34)	92% (31/34)
Benign Islet Cells	0% (0/34)	100% (34/34)
Pancreatic Adenocarcinoma	61% (17/28)	39% (11/28)
Pancreatic Endocrine Tumors	0% (0/6)	100% (6/6)

1324 Pancreatic Ductal Carcinoma-Associated Changes May Histologically Simulate Autoimmune Pancreatitis

K Notohara, M Kojima, S Ban, H Hamano, T Uehara, M Fujisawa, S Nose, K Omonishi, Y Wani, C Tsukayama, M Shimizu. Kurashiki Central Hospital, Kurashiki, Japan; National Cancer Center Hospital East, Kashiwa, Japan; Saitama Medical University School of Medicine, Saitama, Japan; Shinshu University School of Medicine, Matsumoto, Japan; Himeji Red Cross Hospital, Himeji, Japan; Okayama Saiseikai General Hospital, Okayama, Japan; Fukuyama City Hospital, Fukuyama, Japan.

Background: Clinical distinction of autoimmune pancreatitis (AIP) from pancreatic ductal carcinoma (PDC) is difficult, and pancreatic biopsy is attracting attention as a diagnostic tool. However, pancreatic biopsies obtained from pancreata with PDC may be erroneously diagnosed as "pancreatitis" due to the sampling error or deceptive benign morphology of PDC, and pancreatitis in cases with PDC may histologically resemble AIP. For the accurate biopsy diagnosis of AIP, we analyzed histological features of PDC-associated changes in comparison with AIP.

Design: Resected pancreatic specimens were searched, and 15 cases with AIP and 52 with PDC were collected. All the cases with AIP were histologically classified as lymphoplasmacytic sclerosing pancreatitis. All the H&E-stained slides were reviewed, and histological features were evaluated on the following histological variables: type and degree of inflammatory cell infiltration, lymphoid follicles, fibrosis, proliferation of plump fibroblasts, and inflammation in lobules. In cases with PDC, both the inside and outside of carcinomas were evaluated. Immunostaining for IgG1 and IgG4 was done in selected cases.

Results: Histological distinction of AIP from PDC-associated changes was not difficult in most cases. Lymphoplasmacytic infiltration in fibrosis was more conspicuous in AIP. Plasma cells were numerous in AIP, while lymphocytes predominated in PDC. Proliferation of plump fibroblasts was specifically observed in PDC. However, 2 cases with PDC revealed numerous plasma cells in fibrosis, and one of them further contained numerous IgG4-positive plasma cells that were equivalent in number to IgG1-positive cells. Aggregates of small lymphocytes and lobular inflammation with edema and inflammatory cells were observed in the both groups.

Conclusions: Although overall histological features are different between AIP and PDC-associated changes, the distinction might be difficult in occasional cases. Aggregates of small lymphocytes and inflammatory changes of lobules should not be considered to be specific for AIP. In a biopsy with plump fibroblasts, the possibility of PDC should be carefully ruled out.

1325 Differential Expression of BAFF in Autoimmune Hepatitis, Chronic HCV Hepatitis, and Primary Biliary Cirrhosis

RK Pai, M Tretiakova, C Gong, J Hart. University of Chicago, Chicago, IL.

Background: The liver is commonly involved by autoimmune diseases; however, the mechanism by which autoimmunity develops and persists is unclear. Recent advances in immunology have led to a renewed appreciation for the role of the B-cell compartment in various autoimmune diseases. Indeed, autoimmune liver disease is commonly associated with various autoantibodies. Recently, BAFF (B-cell activating factor belonging to the tumor necrosis factor family) has been implicated in autoimmune diseases such as Sjogren's disease, systemic lupus erythematosus, and rheumatoid arthritis. BAFF is expressed by activated T-cells, dendritic cells, and certain epithelial cells and has been shown to promote the survival of autoreactive B-cells and support the development of plasma cells. Autoimmune hepatitis (AIH) and primary biliary cirrhosis are characterized by abundant plasma cells and autoantibodies. Patients with hepatitis C also develop cryoglobulinemia, and recently, elevated expression of BAFF has been implicated as a possible mechanism. In this study we characterized the expression of BAFF in liver biopsies from patients with HCV, AIH and PBC.

Design: We selected 17 AIH (treated and untreated), 12 chronic HCV hepatits, 6 PBC, and 4 HCV/AIH overlap paraffin embedded liver biopsies for immunohistochemical study with anti-BAFF rat monoclonal antibody (Alexa Biosystems, 1:200). Expression of BAFF in portal and lobular inflammatory cell populations were graded on a four tier scale (0 to +++).

Results:

Portal				Lobular			
None	+	++	+++	None	+	++	+++
AIH / 25%	67%	8%	0%	25%	0%	42%	33%
tAIH / 40 %	60 %	0%	0%	100%	0%	0%	0%
HCV / 0%	42%	42%	16%	25%	75%	0%	0%
PBC / 33%	0%	50%	17%	100%	0%	0%	0%
HCV+AIH / 0%	25%	25%	50%	25%	25%	25%	25%

In hepatitis C and PBC, BAFF expression was particularly strong within lymphoid aggregates. In contrast BAFF expression was primarily lobular in AIH and disappeared completely after treatment. No reactivity was evident in hepatocytes or bile ducts.

Conclusions: The expression of BAFF in the liver of patients with AIH, HCV and PBC could help explain the presence of autoantibodies and plasma cells (AIH and PBC) and the development of cryoglobulinemia (HCV). Portal BAFF expression may explain the presence of lymphoid follicles commonly seen in HCV and PBC liver biopsies. Lobular expression in AIH could be a result of expression in autoreactive T-cells, and may explain the abundance of plasma cells.

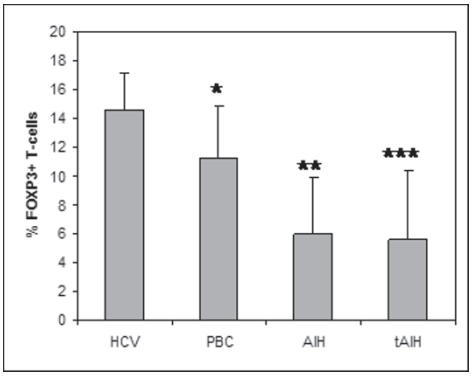
1326 Regulatory T-Cells in Autoimmune Liver Diseases and Chronic HCV Hepatitis

RK Pai, M Tretiakova, C Gong, J Hart. University of Chicago, Chicago, IL.

Background: Autoimmune liver diseases are characterized by the presence of autoreactive T-cells against hepatocyte antigens (autoimmune hepatitis [AIH]) and biliary antigens (primary biliary cirrhosis [PBC]). The mechanism by which autoimmunity develops and persists is unclear. Regulatory CD4+CD25+Foxp3+ T-cells (Tregs) have recently been shown to play a critical role in regulating effector T-cell responses. Indeed, mutations in Foxp3 have been implicated in the pathogenesis of IPEX, a human systemic autoimmune disorder. Moreover, various murine models of autoimmunity demonstrate the critical role of Tregs in the prevention of disease. Recently, circulating Tregs were shown to be decreased in patients with AIH compared with normal subjects; however, the number of Tregs in the liver of these patients was not analyzed. In this study we rigorously quantitated the number of Foxp3+ T-cells in liver biopsies from patients with AIH, treated AIH, PBC, and chronic HCV infection.

Design: Biopsies from 11 AIH, 5 treated AIH, 5 PBC, and 9 chronic HCV patients were analyzed by immunohistochemical double stain with anti-CD3 (1:250, NeoM) and anti-Foxp3 (1:40, Abcam). 500 CD3+ cells were manually counted and the percentage of double positive cells was determined.

Results:



The percentage of Foxp3+ T-cells in PBC, AIH, and treated AIH were compared to patients with chronic HCV hepatitis with statistically significant differences between HCV vs. PBC (* $p < 0.04$), HCV vs. AIH (** $p < 1 \times 10^{-5}$), and HCV vs treated AIH (*** $p < 0.0004$). No difference in the percentage of Foxp3+ T-cells was found between patients with active AIH and treated AIH.

Conclusions: This study clearly demonstrates that there are significantly fewer Tregs in autoimmune liver diseases (PBC and AIH) when compared to chronic HCV hepatitis. The deficit in Tregs in PBC and AIH may be a key component of the mechanism of hepatocyte and biliary destruction in these diseases. The deficit in Tregs appears no to be rescued by immunosuppressive therapy in AIH. Novel drug treatments targeted specifically at increasing Treg number may be beneficial in inducing durable remission in autoimmune liver diseases.

1327 Expression of CXCR3 and IP-10 in Recurrent Hepatitis C Following Liver Transplantation

LM Petrovic, L Chiriboga, M Zeremski, Q Liu, AH Talal. New York University School of Medicine, New York, NY; Cornell University, New York, NY.

Background: Acute allograft rejection (AAR) and recurrent hepatitis C virus (rHCV) infection following liver transplantation (LT) are associated with lymphocytic infiltrate. The factors influencing lymphocyte trafficking to the liver, however, are not well defined. Chemokines have been shown to play a crucial role in lymphocyte recruitment during inflammation in the liver parenchyma. The aim of this study was to evaluate the expression of CXCR3 chemokine receptor and chemokines IP-10, RANTES and I-TAC in AAR and rHCV.

Design: Fourteen liver biopsies from patients who underwent LT were evaluated. AAR was diagnosed in 6 patients and rHCV in 8, with two patients having features of both. Immunohistochemical analysis for CD4, CD8, CXCR3, IP-10, RANTES and I-TAC was performed. The number of positive lymphocytes was counted in 5 portal tracts and in the 5 representative fields in the liver parenchyma in each biopsy. In addition, the expression of IP-10 was correlated with the inflammatory changes.

Results: More CD4 and CD8 lymphocytes are present in the portal tracts in AAR compared to the patients with rHCV. The number of CXCR3 positive lymphocytes is significantly higher in portal tracts in AAR compared to rHCV (58 vs. 17, p=0.004). IP-10 was expressed in the hepatocytes, and correlated with the grade of inflammation and liver cell necrosis in rHCV. In AAR, there was no significant hepatocyte expression of IP-10, but there was focal expression in the bile duct epithelium. There was no significant expression of RANTES and I-TAC in either groups.

Conclusions: Our results demonstrate an increased expression of CXCR3 in AAR, suggesting its role in the recruitment of intrahepatic lymphocytes during the rejection process. Additionally, in contrast to its role in AAR our findings suggest that IP-10 is a predominant chemokine in rHCV infection in the post-transplant setting. IP-10 may potentially be a prognostic marker of disease progression.

1328 Sinusoidal Dilatation in Human Liver Biopsies Rarely Results from Hepatic Venous Outflow Obstruction

CR Price, JM Crawford. University of Florida, Gainesville, FL.

Background: Sinusoidal dilatation with thinned hepatic plates in the perivenular zone of liver biopsies is usually attributed to hepatic venous outflow obstruction, and often prompts Doppler flow examination of the hepatic venous circulation. Presumed causes include Budd-Chiari syndrome, congestive cardiac causes, and in the case of hepatic transplantation, compromise to the hepatic venous anastomosis. We tested the hypothesis that perivenular hepatocyte atrophy and sinusoidal dilatation is not a reliable indicator of hepatic venous outflow obstruction.

Design: The 2001-2006 surgical pathology archives were searched for liver biopsies with sinusoidal 'congestion' or 'dilatation', identifying 69 cases. Data was collected on clinical features and diagnosis, histologic features, and Doppler findings (if performed). Morphometric comparisons were made between this study group and a control set of 19 histologically normal liver case specimens.

Results: In the study group, the average maximal sinusoidal width was 40±4 μm (±SEM) versus the control group 24±3 μm (p<1E-07). The average minimum hepatocyte plate thickness was less than in the control group (9±2 μm vs. 12±3 μm, ±SD, p<0.0005). The average maximum plate thickness was greater than in the control group (64±14 μm vs. 54±6 μm, ±SD, p<0.0005). There was a definite radial location in the lobule in which an abrupt change to hepatocyte atrophy occurred, on average 431±17 μm (±SEM) from the portal tract, vs. a full lobular radius of 567±13 μm (p<0.0001). Only four cases in the study group had abnormal Doppler studies: hepatic artery stenosis; acute allograft rejection involving the hepatic artery; portal venous clots; and congestive heart failure.
Conclusions: Perivenular sinusoidal dilatation with hepatocyte atrophy presumably implicates hepatic venous outflow. We now demonstrate that most cases are due to other causes, including insufficiency of hepatic vascular inflow (portal vein or hepatic artery). An abrupt transition from thickened periportal hepatocyte plates to atrophied/thinned hepatocyte plates can be identified, on average ¾ of the lobular radius from portal tract to terminal hepatic vein. Correct identification and interpretation of this histologic finding may prevent inappropriate attribution of sinusoidal dilatation to obstructed hepatic venous outflow, and may direct clinical attention towards evaluation of hepatic vascular inflow.

1329 Recurrent Steatosis Following Liver Transplant for Non-Alcoholic Steatohepatitis (NASH) or Cryptogenic Cirrhosis (CC); Histomorphologic and Clinical Follow-Up in 231 Patients

JN Punia, VV Shah, MA Sherbondy. Henry Ford Hospital, Detroit, MI.
Background: NASH and CC are widely recognized as significant causes of cirrhosis. Frequently, these patients need liver transplant. Unfortunately, NASH may recur after liver transplant and the factors contributing to the recurrence of NASH and CC have not been clearly identified. Suspected risk factors include type II diabetes, hyperlipidemia, obesity and increase in patient body mass index. **Aim**: To determine which risk factors may contribute to recurrence of NASH and CC, and whether these factors predict severity of disease.
Design: We retrospectively evaluated all the patients who underwent liver transplantation between Jan.2002 and Dec.2004 at our institute. Mean followup period was 38 months. The recurrence of NASH or CC was histologically diagnosed by post-transplant biopsies and assessed for degree of steatosis, as mild (<33%), moderate (33-66%) and severe (>66%), for portal inflammation as absent or present, for portal fibrosis as none, mild and bridging, for lobular neutrophilic infiltration as none or focal, and for ballooning degeneration as none and moderate. The clinical parameters evaluated were corticosteroid use, weight change, diabetes, hyperlipidemia, body mass index, age and sex. Correlation of the histomorphologic recurrence with the clinical predictors was assessed.
Results: Over the period of three years, 231 liver transplants were performed. Of these, 28 (12.1%) patients had a diagnosis of NASH or CC. Followup over a period of 20 to 56 months showed a recurrence of disease in 7 patients (25%). Post transplant biopsies showed: severe steatosis in 3/7 and mild in 3/7 patients, portal inflammation in 6/7 patients, and bridging fibrosis in only 1/7 patients. The clinical parameters evaluated showed a significant correlation only with type II diabetes (86% vs. 33%, *p*value 0.046).
Conclusions: It is established that there is a recurrence of NASH or CC in a subset of patients. Currently, the recurrence can be established by histology of post-transplant biopsies. The need to identify clinical predictors is an important aspect for patient followup as this may help pinpoint patients at risk for disease recurrence. In our study, we found that coexistent type II diabetes mellitus is a significant factor for recurrence of NASH or CC. However, this study is limited by lack of protocol biopsies and small numbers. Continued monitoring of the post-transplant patients is essential to confirm these findings.

1330 MGMT Deficiency Distinguishes Pancreatic Endocrine Tumors from Gastrointestinal and Pulmonary Carcinoid Tumors: Diagnostic and Therapeutic Implications

M Redston, JL Hornick. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.
Background: O^6-methylguanine-DNA-methyltransferase (MGMT) removes O^6-alkyl adducts from DNA and thereby protects tumor cells from alkylating agents such as temozolomide. In some tumors, such as glioblastoma multiforme, MGMT deficiency has been shown to predict heightened sensitivity to temozolomide. In a recent phase II study, therapeutic responses to temozolomide were observed in some patients with metastatic endocrine tumors, particularly pancreatic endocrine tumors (PET). However, the basis for this chemosensitivity is unknown. We hypothesized that the response to temozolomide may be attributable to MGMT deficiency in a subset of these tumors. Therefore, the purpose of this study was to evaluate the expression of MGMT in PET and carcinoid tumors of other sites.
Design: 97 neuroendocrine tumors were evaluated: 37 PET, 20 ileal carcinoid tumors, and 40 pulmonary carcinoid tumors (including 20 typical and 20 atypical). Immunohistochemistry was performed following microwave heat-induced epitope retrieval using monoclonal antibody MT 3.1 (1:25; NeoMarkers/Lab Vision). Nuclear staining was scored as intact or absent. MGMT status in PET was correlated with clinical and pathologic features. Fisher exact test was used for comparisons between groups.
Results: 19 of 37 (51%) PET showed complete absence of staining for MGMT. In contrast, all ileal and pulmonary carcinoid tumors showed intact MGMT expression (p<0.0001). Interestingly, only 3 (16%) MGMT-deficient PET were insulinomas, compared to 7 (39%) insulinomas among PET that expressed MGMT (p=0.11). In addition, MGMT-deficient PET more commonly presented with lymph node or distant metastases (58%) than did PET with intact MGMT staining (28%; p=0.06).
Conclusions: Approximately 50% of PET are MGMT deficient, whereas all ileal and pulmonary carcinoid tumors express MGMT. Immunostaining for MGMT can thus be used to support pancreatic origin in the differential diagnosis of metastatic well or moderately differentiated neuroendocrine carcinomas. Absence of MGMT expression in PET appears to be associated with more aggressive behavior. These findings suggest that testing for MGMT deficiency in PET may identify patients for whom alkylating agents such as temozolomide may be efficacious.

1331 IgG4-Related Sclerosing Disease: Clinicopathological Correlations of 12 Cases of Retroperitoneal Fibrosis (RPF) without Autoimmune Pancreatitis

M Rodriguez-Justo, GH Neild, JO Connolly, C Wall, P Munson. Royal Free & University College Medical School, London, United Kingdom; Royal Free Hospital, London, United Kingdom; Middlesex Hospital, London, United Kingdom.
Background: Hyper-IgG4 disease, or IgG4-related sclerosing disease, is a multisystemic disease of presumed autoimmune aetiology. Histologically it is characterized by fibrosis, a dense lymphoplasmacytic inflammatory cell infiltrate and significant increase in IgG4+ve plasma cells. Its pathogenesis is unclear but data suggest a Th2-mediated pathology.A few cases of retroperitoneal involvement in patients with AIP have been reported. The aim of this study is to present the clincipathological features of RPF patients without associated AIP and to investigate the utility of IgG4 staining.
Design: All patient were seen in our centre from 1987 to 2006 and all of them presented with clinical and radiological features of RPF. Biopsy specimens (retroperitoneum, colon, liver, kidney) from patients and controls were immunostained with mouse anti human monoclonal IgG4 antibody. The degree of fibrosis and lymphoplasmacytic infiltrate was assessed. The number of IgG4 plasma cells was counted in 10HPFs. Biopsies were considered as positive if there was >10 IgG4+ve plasma cells/HPF.
Results: Clinically all patients (9 males and 3 females, 45-73 age) presented with variable degree of renal failure (due to ureteric obstruction) and raised ESR, CPR and IgG. Abdominal pain, weight loss and fever were also present in 6 patients. Extensive fibrosis, a lymphoplasmacytic inflammatory cell infiltrate and raised IgG4+ve plasma cell numbers were consistently elevated in peritoneal biopsies and in the renal biopsy. Control tissues showed less than 2 IgG4+ve plasma cells/HPF. The outcome of all the patients treated with steroid therapy was good.
Conclusions: We have proved that all cases of RPF so far examined by us, are part of the IgG4-related autoimmune disease. It is very important to recognise this entity as the diagnosis can be made on laboratory and histology backgrounds and the outcome with steroid treatment is good.

1332 Extrapancreatic Involvement in Autoimmune Pancreatitis (AIP): 11 Patients Seen at a Tertiary Referral UK Hospital

M Rodriguez-Justo, MG Deheragoda, NI Church, P Munson, N Sandanayake, EW Seward, MR Novelli, SP Pereira, ARW Hatfield, GJM Webster. Royal Free & University College Medical School, London, United Kingdom; University College London Hospital, United Kingdom; University College London, United Kingdom.
Background: IgG4-related sclerosing disease is widely recognised as a multisystemic disorder, which has been described mainly in Japan with small series of patients also reported in Europe and the USA. The recognition of this disease is important because these patients respond to steroid based therapy and a correct diagnosis avoids unnecessary surgery. The histological diagnosis of this condition has so far depended on histopathological examination and IgG4 immunostaining of pancreato-biliary tissue. However a new HISORTt system (Histology, imaging, serology, other organ involvement and response to steroid therapy) allows the assessment of extra-pancreatic tissues to be used in the diagnosis of AIP. The aim of this study is to evaluate the role of IgG4 immunostaining in extrapancreatic tissues in patients diagnosed with AIP in a western population.
Design: We reviewed 16 biopsies of extra-pancreatic tissues (liver, bone marrow, duodenum, stomach, gallbladder, colon and kidney) from 11 patients diagnosed with AIP and clinical and radiological evidence of extrapancreatic involvement. All biopsy specimens and control tissues were stained with CD138, CD38 and IgG4 (mouse anti-human monoclonal antibody). A mean number of IgG4+ve plasma cells/hpf (high-power field, x400) was calculated counting IgG4+ve cells in 10hpfs.
Results: The number of IgG4+ve plasma cells was significantly increased in extra-pancreatic biopsy specimens in all patients (11 males, 28-77 age). In 90% of the specimens we found >10 IgG4+ve plasma cells/hpf compared with <3 /hpf in 176 control tissues. In addition all specimens showed a lymphoplasmacytic infiltrate. Interestingly increased number of IgG4+ve plasma cells was seen despite normal serum IgG4 levels in four patients.
Conclusions: Using IgG4 immunostaining in extrapancreatic biopsies we were able to diagnose IgG4-related sclerosisng disease in patients with pancreatobiliary stricture/pancreatic mass. Routine use of IgG4 immunostaining in endoscopic biopsies may have a role in the diagnosis of AIP when pancreatic biopsies are not feasible or not considered clinically justifiable.

1333 Importance of Histological Tumor Response Assessment in Predicting the Outcome in Patients with Colorectal Liver Metastases Treated with Neoadjuvant Chemotherapy Followed by Liver Surgery

L Rubbia-Brandt, E Giostra, C Brezault, AD Roth, A Andres, V Audard, P Sartoretti, B Dousset, PE Majno, O Soubrane, S Chaussade, G Mentha, B Terris. University Hospital Geneva, Geneva, Switzerland; Hopital Cochin, Paris, France.
Background: Characterize histological response to chemotherapy of hepatic colorectal metastases (HCRM). Evaluate efficacy of different chemotherapies on histological response. Determine if tumor regression grading (TRG) of HCRM predicts clinical outcome.

Design: TRG was evaluated on 525 HCRM surgically resected from 181 patients, 112 pretreated with chemotherapy. Disease-free survival (DFS) and overall survival (OS) were correlated to TRG.
Results: Tumor regression was characterized by fibrosis overgrowing on tumor cells, decreased necrosis, and tumor glands (if present) at the periphery of HCRM. With irinotecan/5-FU, major (MjHR), partial (PHR) and no (NHR) regression were observed in 17%, 13% and 70% of patients, respectively. With oxaliplatin/5FU, MjHR, PHR and NHR were observed in 37%, 45% and 18% of patients, respectively. Five patients, treated with oxaliplatin, had complete response in all their metastases. MjHR was associated with an improved 3-year DFS compared with PHR or NHR. MjHR and PHR were associated with an improved 5-year OS compared with NHR.
Conclusions: Histological tumor regression of HCRM to chemotherapy corresponds to fibrosis overgrowth and not to increase of necrosis. TRG should be considered when evaluating efficacy of chemotherapy for HCRM. Histological tumor regression was most common among oxaliplatin treated patients and associated with better clinical outcome.

1334 Array Comparative Genomic Hybridization (aCGH) Analysis of Solid Pseudopapillary Tumors of the Pancreas

CR Rund, K Lee, H Zeh, K Cieply, S Dacic, AM Krasinskas. University of Pittsburgh, Pittsburgh, PA.

Background: Solid pseudopapillary tumors (SPTs) of the pancreas are considered to be low grade malignancies, but their aggressive potential cannot be reliably assessed on histopathologic criteria alone. Currently, aside from mutations in the APC/beta-catenin pathway and lack of involvement of genes commonly seen in pancreatic ductal adenocarcinomas, little known about the genetic alterations in SPT. To gain a better understanding of the molecular events that occur in SPTs, we applied aCGH to a series of eight SPTs.
Design: aCGH was performed on DNA that was extracted from microdissected, formalin-fixed, paraffin-embedded sections from resected tumors. Gene gains and losses were detected by the commercially available GenoSensor™ Array 300 kit. The cut-off ratio between normal and aberrant DNA copy numbers was the mean +/- 2 STD. Ratios ≥1.2 or ≤0.80 were considered as copy number gains or losses, respectively. The gains at 13q14 were validated by FISH on sequential sections of the same tumor blocks.
Results: All 8 SPTs were from women; the mean age was 33 years (range 15-52). 5 were found incidentally; 3 were found on CT prompted by abdominal pain. Additional histopathologic findings and the aCGH results are shown in Table 1.

Table 1

Case	Size (cm)	Panc Inv	Peri Inv	PN Inv	LV Inv	DNA Loss	DNA Gain
1	3	Y	N	Y	Y	11q22	18q21.3, 1q31.1, 8p22-q21.3, 13q14, 13q14.2, 15q11-q13, 17q23
2	2.2	Y	N	Y	Y		18q21.3, 1q31.1, 8p22-q21.3, 13q14, 13q14.2, 15q11-q13, 17q23
3	4.7	Y	Y	N	N	11q13, 11q22	
4	8	N	N	N	N		18q21.3
5	4.2	Y	Y	N	N	1p36.33, 11q13	
6	2	N	N	N	N		
7	5	Y	N	N	N	1p36.33, 11q13, 17q21	
8	9	N	N	N	N	1p36.33, 17q21	

Panc, Pancreatic; Inv, Invasion; Peri, Peripancreatic; PN, perineural; LV, lymphovascular

The gains at 13q14 were confirmed by FISH: ≥2 copies were present in 16.4% (case 1) and 30.5% (case 2) of the analyzed cells.
Conclusions: Utilizing the sensitive aCGH method, we were able to identify, for the first time, multiple chromosomal alterations in SPT. Chromosomal losses were identified at 11q (n=4), 1p (n=3) and 17q (n=2); chromosomal gains were identified at 18q (n=3) and, in 2 cases, at 1q, 8p, 13q, 15q, and 17q. The two tumors that had the most adverse histologic findings (pancreatic invasion, lymphovascular invasion and perineural invasion) were the only two tumors to show multiple chromosomal abnormalities. Further investigation into the genetic alterations seen in SPTs may lead to molecular prognostication in SPT.

1335 Hep Par 1 Expression in Intraductal Papillary Mucinous Neoplasms (IPMN) and Mucinous Cystic Neoplasms (MCN) of the Pancreas with and without Dysplasia and Invasive Carcinoma

AF Safo, EB Stelow, J Jessurun, SE Pambuccian. University of Minnesota, Minneapolis, MN; University of Virginia, Charlottesville, VA.

Background: IPMN and MCN of the pancreas are rare tumors with a heterogenous microscopic appearance. Four histologic variants are recognized: gastric (G), intestinal (I), pancreatobiliary (PB) and oncocytic. I and PB subtypes are more frequently associated with invasive adenocarcinomas of colloid (I) and tubular (I and PB) type. Immunohistochemically, the I type is MUC2, MUC5AC and CDX2+ and MUC1-. HepPar-1 has recently been identified as a reliable marker of intestinal differentiation, but has not yet been studied in pacreatic cystic neoplasms. The aim of this study was to investigate the expression of HepPar-1 in IPMN and MCN.
Design: Cases of IPMN and MCN diagnosed over 15 years were identified from the files of two university hospitals. The predominant type of IPMN and MCN, grade of dysplasia, presence and type of invasive carcinoma was recorded independently by two pathologists. HepPar 1 (OCH1E5, DAKO) and CDX2 (CDX2-88, BioGenex) stains were performed on the Ventana NexES and scored 0-3+.
Results: A total of 66 cases were studied, 40 IPMN (4 with invasive carcinoma) and 22 MCN. Of the former, 15 were PB, 12 G and 13 I. Within the MCN 16 were G and 6 I. Dual differentiation (PB + I), (I + G) was frequently observed.

IMPN (n=40), MCN (n=22)	HepPar1+	HepPar1-	CDX2+	Total
IPMN-PB (n=15)	4	11	4	11
IPMN-G (n=12)	0	12	2	10
IPMN-I (n=13)	12	1	12	1
MCN-G (n=16)	1	15	0	16
MCN-I (n=6)	2	4	6	0

HepPar 1 immunoreactivity was more prominent in areas of high-grade dysplasia in both IPMN and MCN. All cases without dysplasia were negative. Of interest, all invasive tumors were negative for Hep Par 1. The two colloid carcinomas preserved the immunoreactivity for CDX2.
Conclusions: 1. As CDX2, HepPar 1 is expressed in most cases of IPMN and MCN with intestinal phenotype. 2. Some cases with a PB phenotype may express HepPar 1 and CDX2, due to their frequent dual differentiation.3. Hep Par 1 is expressed in all cases with high-grade dysplasia but its expression is lost in the associated invasive colloid carcinomas while the immunoreactivity for CDX2 is preserved. 4. These results suggest that Hep Par 1 may be useful in confirming an intestinal phenotype and supporting the presence of dysplasia. When strongly expressed in the intraductal component, absence of expression in glandular clusters suggests the presence of invasive carcinoma.

1336 Adenylate Cyclase-Associated Protein 1 Overexpressed in Pancreatic Cancers Is Involved in Cancer Cell Motility

M Sakamoto, K Yamazaki. Keio University School of Medicine, Tokyo, Japan.

Background: Pancreatic cancer has the worst prognosis among cancers due to the difficulty of early diagnosis and its aggressive behavior.
Design: In order to characterize the aggressiveness of pancreatic cancers on gene expression, pancreatic cancer xenografts transplanted into severe combined immunodeficient mice served as a panel for gene-expression profiling. Among them, we further evaluated expression and function of adenylate cyclase-associated protein 1 (CAP1) in pancreatic cancer tissues and cell lines.
Results: As a result of profiling, the CAP1 gene was shown to be overexpressed in all of the xenografts. CAP1 was originally identified as a bifunctional protein involved in adenylate cyclase activation and actin reorganization in yeast; however, its expression and functional roles in cancer have not been reported. The expression of CAP1 protein in all 63 cases of pancreatic cancer was recognized by immunohistochemical analyses. Immunocytochemical analyses in pancreatic cancer cells demonstrated that CAP1 co-localized to membrane ruffles at the leading edge of lamellipodia with actin. Knockdown of CAP1 by RNA interference resulted in the reduction of lamellipodium formation, motility and invasion of pancreatic cancer cells.
Conclusions: This is the first report demonstrating the overexpression of CAP1 in pancreatic cancers and suggesting the involvement of CAP1 in the aggressive behavior of pancreatic cancer cells.

1337 C4d as a Marker for Acute Rejection Following Liver Transplantation – A TMA Application

DF Schaeffer, SW Chung, CH Scudamore, UP Steinbrecher, DA Owen. University of British Columbia, Vancouver, BC, Canada.

Background: Acute rejection represents a major complication following orthotopic liver transplantation (OLTx) and is thought to be mediated mainly by T-cell related mechanisms. Histological examination of a liver biopsy is regarded as the gold standard for diagnosis. Nevertheless, the diagnosis can be difficult especially in hepatitis-C reinfected patients. C4d, a split product of the activated complement cascade has become a useful marker in the diagnosis of acute rejection after kidney transplantation, where humoral mechanisms are postulated to play an important contributory role. In the present study we investigated whether C4d is present in acute rejection following OLTx and if it might serve as a specific marker in the differential diagnosis of hepatitis-C reinfection cases.
Design: Using a tissue microarray (TMA) we performed a retrospective analysis of 82 liver biopsies from patients who had received an OLTx at our institution between 2001 and 2004. 28 of 82 patients had been transplanted for HCV-induced cirrhosis.
Results: In 21 of 36 patients with histologically proven acute rejection we detected immunostaining for C4d in the specimen (58%). 7 of 18 patients with HCV-reinfection displayed C4d positive staining (38%), whereas in the control group (no rejection, no HCV) 2 of 28 biopsies showed C4D positivity (7%). Differences in the detection of C4d were statistical significant when comparing rejection cases to controls ($p<0,01$) as well as between rejection- and HCV-cases ($p<0,05$).
Conclusions: This study suggests that humoral components, represented by C4d staining, play a role in acute rejection following OLTx. Our data show detectable differences in the C4d expression between rejection biopsies and normal controls. Concerning the difficult task of distinguishing between acute rejection and HCV-reinfection in conventional histological examination we were able to find statistically significant differences in C4d expression. These findings are in keeping with recent reports, who, however, had lower patient numbers. Elucidating the humoral mechanism of acute allograft rejection might not only be valuable to improve the long-term outcome of OLTx, but might also have major implications for the histological grading of acute cellular rejection in a clinical setting, as C4d staining could be used as an objective marker, to complement the conventional histopathologic grading.

1338 An IgG4+ to IgG+ Plasma Cells Ratio in Ampullary Tissue as a Marker To Differentiate Autoimmune Pancreatitis from Pancreatic Cancer

A Sepehr, V Deshpande, GY Lauwers, M Mino-Kenudson. Massachusetts General Hospital, Boston, MA.

Background: The increased recognition of autoimmune pancreatitis (AIP) has led to a decrease in unnecessary operations. However, pre-operative differentiation of AIP from pancreatic cancer (PC) is a challenging task, due to the mass forming nature of both conditions. Previously, we have identified an increased number of IgG4+ plasma cells in the resected pancreas and extrapancreatic sites in AIP. Nevertheless, IgG4+ plasma cells were also identified in about 50% of PC and pancreatitis-NOS. Thus, the objective of this study was to evaluate a value of IgG4+ to IgG+ plasma cells ratio (IgG4/IgG ratio) in the endoscopically accessible ampullary tissue to diagnose AIP.

Design: A study group consisted of 14 AIP, 14 PC, and 14 pancreatitis-NOS cases who underwent a Whipple procedure. In each case, immunohistochemistry for IgG and IgG4 was performed in a section containing both major ampulla and periampullary duodenum. The number of IgG4+ and IgG+ plasma cells were counted in 10 sequential mucosal high-power fields in both regions. An IgG4/IgG ratio was recorded and correlated with the final diagnosis.

Results: IgG4+ plasma cells in the ampulla, and in the duodenum were significantly increased in AIP compared to pancreatitis-NOS and PC ($p < 0.01$ for ampulla; $p < 0.05$ for duodenum). However, there was an overlap in numbers of IgG4+ plasma cells, especially between AIP and pancreatitis-NOS. An IgG4/IgG ratio was significantly greater in AIP compared to pancreatitis-NOS and PC both in the ampulla ($p < 0.05$) and the duodenum ($p < 0.01$). When a 10%-15% cut-off in IgG4/IgG ratio in the ampullary tissue was applied, AIP could be differentiated from other lesions with a sensitivity of 86% and a specificity of 96%. No PCs demonstrated an IgG4/IgG ratio > 10%. The IgG4/IgG ratio in the duodenum was less helpful in discriminating AIP from other conditions, with the highest sensitivity of 77%.

Conclusions: An IgG4/IgG ratio in the ampullary tissue may be useful in differentiating AIP from other lesions, especially PC, which is challenging to discriminate from AIP. Further prospective studies on ampullary biopsy material would help validate the results of the current study.

Ampullary tissue

	IgG4+ plasma cells		IgG4/IgG	
	range	mean	range	mean
AIP	1 - 902	275	0.003 - 0.56	0.33
pancreatitis-NOS	0 - 150	21	0 - 0.18	0.03
PC	0 - 4	1.4	0 - 0.04	0.01

1339 The Expression of E-Cadherin in Solid Pseudopapillary Tumors and Endocrine Tumors of the Pancreas

S Serra, M Fageeh, F Niakosari, S Salahshor, JM Radhi, R Chetty. University Health Network, University of Toronto, Toronto, ON, Canada; University Health Network and University of Toronto, Toronto, ON, Canada; McMaster University, Hamilton, ON, Canada.

Background: Solid pseudopapillary tumors (SPT) of the pancreas are rare neoplasms with a low malignant potential that occur most frequently in young women. Histologically, it is a solid and cystic tumor with a prominent vascular network and degenerative pseudopapillae. Cystic spaces are the result of degenerative changes occurring in the solid neoplasm. Often times they may resemble endocrine tumors of the pancreas (PET) very closely. The neoplastic cells in SPT are consistently vimentin, CD10 and CD56-positive and nuclear β-catenin expression is also a well-recognized feature. Some cases express focal positivity for α1-antitrypsin, α1-antichymotrypsin, neuron-specific enolase and synaptophysin. The aim of this study was to examine E-cadherin expression in SPT and compare this expression to that in PET.

Design: Eleven SPT of the pancreas were retrieved and utilized for tissue microarray analysis (TMA). Fifty cases of PET were also examined by TMA. Tumor size, necrosis, invasiveness/demarcation, lymphovascular invasion, lymph node and liver metastasis were recorded. Mitotic count and MIB1 index were assessed. All the TMA blocks were stained with β-catenin and E-cadherin.

Results: One case of SPT was in a male and 10 were females, ranging in age from 11 to 62 years. The SPTs ranged in size from 4.5 cm to 8.8 cm. Half of the tumors arose in the tail/body of the pancreas. Twenty-four patients with PET were male and 26 female, ranging in age from 23 to 80 years. The tumors ranged in size from 0.8 cm to 8.7 cm. In SPT, both β-catenin and E-cadherin showed nuclear immunoreactivity in all 11 cases (100%). This was associated with a loss of membranous localization of both proteins. In PET, β-catenin showed nuclear positivity only in 1 case (2 %), whilst E-cadherin was nuclear in 12/50 cases (24%). Only 1 case showed both β-catenin and E-cadherin nuclear staining.

Conclusions: Nuclear β-catenin expression is now well recognized in SPT. This study shows that E-cadherin is also expressed in a nuclear location in 100% of cases of SPT. In contrast, PET very uncommonly demonstrate nuclear expression of both these proteins. Thus, the presence of nuclear β-catenin and E-cadherin can distinguish SPT from PET. This study is also the first to demonstrate nuclear E-cadherin in SPT.

1340 Glypican-3 Expression Has Low Sensitivity for the Diagnosis of Extremely Well-Differentiated Variants of Hepatocellular Carcinoma and Fibrolamellar Carcinoma

N Shafizadeh, L Ferrell, S Kakar. VA and UCSF Medical Center, San Francisco, CA.

Background: Glypican-3 (GPC-3) is a membrane anchored heparin sulfate proteoglycan normally expressed in fetal liver and placenta, but not in normal adult liver. Preliminary studies have shown that GPC-3 is expressed in ~80% of HCC and but not in hepatic adenoma (HA). The utility of GPC3 immunohistochemistry in extremely well-differentiated HCC closely mimicking HA is not clear. The oncofetal protein AFP is negative in fibrolamellar HCC (FLC), but GPC-3 expression has not been determined.

Design: Immunohistochemistry for GPC-3 was performed on 55 formalin-fixed, paraffin-embedded resection cases of macro-regenerative nodules (MRN, n=8), HA (n=8), extremely well-differentiated HCC (n=11), conventional HCC (n=17) and FLC (n=11). Monoclonal antibody directed against GPC-3 (Clone 1G12, BioMosaics, Burlington, VT) was used. Most of the cases classified as extremely well-differentiated HCC were sent in consultation as diagnostic dilemmas. In 3 cases, the morphology was nearly indistinguishable from HA and the diagnosis was based on recurrence/metastasis. In the remaining 8 cases, the morphology largely resembled HA, but was accompanied by focal atypical changes (small cell change, widened cell plates, loss of reticulin) to enable the diagnosis of HCC.

Results: GPC-3 expression was absent in all 8 MRN and all 8 HA. 13/17 (76%) conventional HCC, and 7/11 (64%) FLC were positive. In the extremely well-differentiated HCC, 6/11 (55%) showed GPC-3 expression. Even though the staining was diffuse in the latter, the intensity was weaker than conventional HCC. Overall, among the 26 positive cases, the pattern was cytoplasmic (n=21, 81%) or cytoplasmic and membranous (n=5, 19%). GPC-3 expression was also noted in 4/22 (18%) cirrhotic nodules; it was patchy and weak in 3 cases and strong in 1 case.

Conclusions: Glypican-3 is expressed in ~75% of conventional hepatocellular carcinoma. However, GPC-3 is less sensitive (~50%) in the extremely well-differentiated cases of HCC. Since GPC-3 is negative in adenomas, its expression strongly favors HCC over adenoma, but negative staining does not exclude HCC. Caution should be exercised in using GPC-3 in biopsy specimens as cirrhotic nodules can show strong expression. The fibrolamellar variant of HCC shows expression of the oncofetal protein GPC-3 in two-thirds of cases.

1341 Mucus Extravasation in Pancreatic Intraepithelial Mucinous Neoplasms (IPMNs and MCNs): A Finding Not To Be Confused with Invasive Adenocarcinoma

EB Stelow, TO Safo, SE Pambuccian, TW Bauer, JB Stokes, AM Bellizzi, RB Adams, CA Moskaluk. University of Virginia, Charlottesville, VA; University of Minnesota, Minneapolis, MN.

Background: Mucinous intraepithelial neoplasms of the pancreas (intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs)) are benign cystic neoplasms of the pancreas. Because invasion cannot be excluded preoperatively, it is currently recommend that most of these lesions should be resected. Many invasive malignancies associated with these neoplasms have a pancreatobiliary phenotype, however, a distinct subset are characterized by infiltrating pools of mucus with occasional malignant mucinous epithelial fragments. Although these diagnoses are often straightforward, it has come to our attention that some apparently non-invasive lesions will have mucus extravasation that is sometimes extensive and may be mistaken for invasive malignancy.

Design: All IPMNs and MCNs seen over a 15 year period were reviewed. Cases with invasion were excluded. All other lesions were evaluated for histologic phenotype (gastric, pancreatobiliary or intestinal), grade, and the presence of mucus extravasation. Concise clinical and follow-up information were pursued.

Results: Sixty-four IPMNs (42) and MCNs (22) were identified; 12 (19%) were excluded because of invasive malignancy. Of the non-invasive cases, 10 of 30 IPMNs and 2 of 22 MCNs showed mucus extravasation not diagnostic of malignancy. Eight of 10 of these IPMNs had an intestinal phenotype. Most cases showed cystic pools of mucus in continuity with the intraepithelial lesions. Progressive flattening of the epithelium with loss consistent with pressure necrosis was often seen with mucus dissecting into the adjacent parenchyma in a radiating pattern. Some sections showed isolated, circumscribed mucus pools devoid of epithelium. The epithelia seen within extravasated mucus were much less common than those seen in colloid carcinoma and, in general, showed atypia cytologically insufficient for the diagnosis of adenocarcinoma. Follow-up of these patients failed to reveal any metastatic lesions or the development of pseudomyxoma.

Conclusions: Mucinous intraepithelial neoplasms of the pancreas not infrequently show mucus extravasation, similar to appendiceal mucinous lesions, without showing truly invasive colloid carcinoma. These lesions should not be confused with invasive adenocarcinomas as they do not develop metastases or pseudomyxoma.

1342 Loss of Cell-Adhesion Molecule Complexes in Solid Pseudopapillary Tumor of Pancreas

AA Stelter, S French, S Shen, S Qiu, HQ Wang, J Xie, R Venegas, WW Tang. University of Texas Medical Branch at Galveston, Galveston, TX; Harbor & UCLA Medical Center, Torrance, CA; Methodist Hospital, Houston, TX.

Background: Solid pseudopapillary tumor of pancreas (SPT) is a rare neoplasm that occurs most often in young females with the two distinct features: the "solid-cystic" gross appearance, and the "solid-pseudopapillary" microscopic pattern. It has been reported that almost all SPT tumors contain a mutation in the beta-catenin gene, however, the histogenetic origin of this tumor remains largely a mystery. E-Cadherin is a cell adhesion molecule that links to catenins to form cell adhesion junction, which is associated with the cytoskeleton formation. In this study, we examined expression of E-cadherin and beta-catenin of SPT in an attempt to determine the molecular basis for the unusual morphology of this tumor.

Design: Nine cases of SPT were retrieved from Surgical Pathologic Archives of three institutions, including 1 male and 8 female. H&E slides of each case were reviewed to confirm the diagnosis. Beta-catenin gene was sequenced in one case. Electronic microscopy was done on one case to study the apoptosis and ischemic changes. E-cadherin and beta-catenin immunostains, as well as TUNEL stains, were performed on all 9 cases.

Results: Sequencing analaysis on one case showed a point mutation of the beta-catenin gene, confirming previous findings that almost all SPT tumors contain mutation in the beta-catenin gene. Immunostains showed that, in both solid and pseudopapillary areas,

all the tumor cells lost expression of E-cadherin, and the beta-catenin nuclear expression were observed in all cases. Furthermore, in both pseudo-papillary and solid areas, all tumor cells exhibited negative stain for TUNEL. Electronic microscopy on one case showed intact nuclei and cellular organelles, indicating no evidence of apoptosis.
Conclusions: Our results suggest that loss of cytoplasmic beta-catenin protein in the cell adhesion complex due to beta-catenin gene mutation, result in instability of the complex, loss of E-cadherin in cell membrane, and eventually dissociation of the tumor cells to form the pseudopapillary pattern.

1343 Specific Definitions of Fatty Change in Transplant Donor Liver Biopsies Increase Interobserver Agreement

VO Tan, LW Browne, LD Ferrell. UCSF, San Francisco, CA.
Background: The terms macrovesicular and microvesicular steatosis are inconsistently defined in the literature. Yet, the assessment of macrovesicular and/or large-droplet fatty change (LDF), small-droplet fatty change (SDF), and/or microvesicular fat in transplant donor liver biopsies by the pathologist to determine suitability of the graft for transplantation is critical. More precise definitions of the types of fatty change in donor biopsies could increase interobserver agreement between pathologists, improve communication between surgeons and pathologists, and allow for more accurate future assessments of type of fatty change as related to graft outcome.
Design: The frozen and permanent sections on 123 donor liver biopsies from 1998-2004 were reviewed by 2 evaluators, a Transplant Pathology Fellow and a Liver Pathology Fellow. Each reviewed the biopsies independently without agreeing, in advance, on definitions of macrovesicular and microvesicular steatosis. The evaluators subsequently underwent further training, consisting of agreement on specific definitions, after which the biopsies were re-examined. LDF was defined as fat droplets occupying >1/2 of a hepatocyte. SDF was defined as droplets occupying <1/2 the cell size. "True microvesicular steatosis" (TMS) was reserved for very small, uniform fat globules packed within hepatocytes (as seen in fatty liver of pregnancy) visible in patches at 10X . The differences between the evaluators' results were analyzed, and original reported diagnoses (>90% signed by nonliver pathologists) were reviewed.
Results: Average interobserver discrepancies in the evaluation of % volume of LDF for both evaluators, both prior to and after training, were not significantly increased (<4%). Training significantly increased agreement in assessing % of cells with SDF, where the highest pretraining interobserver discrepancy was 12% on permanents, which training reduced to 4%. Before training, the evaluators labeled 39/123 and 0/123 cases as TMS. After training, both agreed that no case had significant TMS. Review of original diagnoses on pathology reports revealed a wide array of subjective terminology that did not translate between reports and could not be objectively subjected to statistical analysis.
Conclusions: Specific definitions of type of fat increased interobserver agreement between the evaluators for SDF and TMS. Our results suggest that stricter, yet simple, definitions could decrease potential miscommunication between pathologist and surgeon as to the volume of specific types of fat in the donor liver.

1344 Clinicopathologic Features of Nodular Regenerative Hyperplasia after Liver Transplantation

VO Tan, JP Roberts, LD Ferrell. UCSF, San Francisco, CA.
Background: Nodular regenerative hyperplasia (NRH) is known to occur after liver transplantation, but the clinical setting for this occurrence is poorly understood, and the histologic diagnosis of this lesion can be difficult. This study aims to establish the incidence of NRH, clarify the clinical setting of NRH in post-transplant livers, and examine for possible related histologic lesions.
Design: H&E slides of all liver transplant biopsies performed in 2003 and 2004 at our institution were reviewed for the presence of NRH, identified as atrophic and compressed liver cell plates adjacent to areas of regenerating hepatocytes. The presence of sinusoidal dilation (a possible early finding of NRH) was also noted. Histologic findings were correlated with clinical features including imaging, type of transplant procedure, primary liver disease, post-transplant ascites or portal hypertension, liver enzyme studies and graft function.
Results: 562 liver transplant biopsies were reviewed on 309 transplant recipients. The incidence of obvious NRH was 6% of all transplant patients (20 of 309) studied. Another 19% of patients (59) had more subtle findings of NRH that needed further confirmation with reticulin stain. 48% of patients (147) showed sinusoidal dilatation on at least one biopsy. The appearance of NRH occurred at any time after liver transplantation with similar frequency. Findings of NRH and sinusoidal dilation were more frequently seen in living donor recipients (37 patients) compared to cadaveric liver recipients (43% vs. 25% NRH, 65% vs. 45% sinusoidal dilation for respective groups). 35 (33%) of 106 patients with post-transplant ascites or portal hypertension had at least one biopsy showing features of NRH. 3 patients with NRH had severe portal hypertension requiring retransplantation. No specific primary liver disease was correlated with a higher incidence of NRH.
Conclusions: NRH is a common finding on liver transplant biopsies. Reticulin stain can be helpful to identify NRH, but its routine use is probably not indicated given that the majority of NRH appears to be clinically insignificant. Sinusoidal dilation is much more common than and is not directly associated with NRH. We suggest that the high incidence of this lesion is likely related to vascular flow abnormalities commonly seen in the transplant setting as a result of liver size discrepancies, particularly prevalent in living donor recipients as compared to cadaver donor recipients.

1345 Viral Eradication of Hepatitis C Virus after Successful Therapy May Be Associated with Long-Term Histologic Persistence of Chronic Inflammation on Liver Biopsies in Liver Transplant Patients

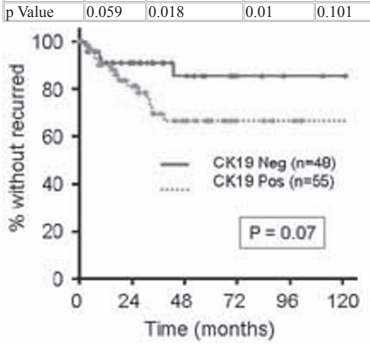
VO Tan, N Terrault, S Feng, LD Ferrell. UCSF, San Francisco, CA.
Background: Current anti-viral therapy for hepatitis C (HCV) can sometimes result in viral eradication, in which the viral load becomes undetectable in the blood. In the setting of ongoing immunosuppression following liver transplantation, we have noted that viral eradication is not always associated with improvement or resolution of inflammatory activity on liver biopsy and some patients have persisting or progessive hepatitis years after viral clearance. Thus, we aimed to determine the short and long-term frequency of persistent hepatitis on liver biopsy in liver transplant patients who have eradicated the virus and the length of time between achievement of viral eradication and complete histologic resolution if it occurs.
Design: 17 HCV liver transplant recipients with biopsy-proven recurrent HCV after transplantation and who had documented undetectable serum levels of HCV RNA for 3 or more months after completion of a course of anti-viral therapy were identified. Liver transplant biopsies were scored for inflammation using the Ludwig-Batts system. Biopsies performed before the start of anti-viral therapy were compared to the last follow-up biopsy after viral eradication.
Results: The median off-treatment histologic follow-up was 500 days (range 154-1999 days). Pre-eradication inflammatory grade ranged from 1 to 3. Compared to pre-eradication biopsies, post-eradication biopsies in 12 patients (71%) showed histologic improvement in grade, with only 4 (24%) patients showing complete resolution of inflammatory activity (grade 0). 2 (12%) patients showed no change in grade, and 3 (18%) patients showed progression of inflammatory activity. The median off-treatment histologic follow-up for the patients with progressive hepatitis was 500 days (range 356-1392 d). In the 4 patients who had complete resolution of hepatitis on biopsy, the median time to histologic resolution was 1203 days after completion of anti-viral therapy (range 333-1999 d).
Conclusions: In patients achieving viral eradication with antiviral therapy, histological benefits are seen in the majority of patients, although persistent low-grade inflammation may still be present. Complete resolution of inflammation on biopsy may occur years after the completion of anti-viral therapy. In a minority of patients, there is worsening of the degree of inflammation even years after serologic clearance of HCV.

1346 Assessment of Prognosis of Pancreatic Endocrine Neoplasm (PEN) by Correlation of Pathological Prognostic Factors with CK19 Immunoreactivity in 112 Cases

LH Tang, P Allen, I Modica, C Ferrone, J Shia, MF Brennan, DS Klimstra. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: While PENs behave in a less aggressive manner than their adenocarcinoma counterpart, synchronous and delayed metachronous disease occur in ~50% cases. Previous investigations have proposed CK19 as a predictor for survival of PENs. We further explored the prognostic potential of CK19 immunoreactivity with correlation of our previously established grading system for PENs, histological parameters, and WHO classification.
Design: Material of 112 patients with PENs who underwent surgery from 1983-2005 were available for this investigation. All patients had complete follow up from 1-166 mon (mean 44 mon). The histopathology of all cases was re-assessed for their accuracy in grading and classification. Immunostain for CK19 was performed on tissue microarray sections prepared from routine histological tissue blocks. Positive stain was defined by a diffuse and specific staining pattern regardless the intensity. Statistical analysis was performed with Pearson Chi-square test, and the significance was assessed by 2-sided Fisher Exact Test. Survival was demonstrated by Kaplan-Meier curve.
Results: The frequency of CK19 immunoreactivity ($CK19^+$) was detected in 55% of 112 PENs. There was no significant correlation between $CK19^+$ and gender, tumor functionality, or tumor location. $CK19^+$ was associated with larger tumors. High mitotic activity, intermediate grade, and WHO classification were significantly associated with $CK19^+$(Table 1). Negative CK19 was associated with a better recurrent free survival (Figure 1).
Conclusions: CK19 immunoreactivity is significantly associated with a number of pathological prognostic factors of PENs. It can be practically used as an additional marker for the assessment outcome of PENs.

Table 1.

	Size (cm)	Size (cut-off 2cm)	Mitoses/ 50HPF	LVI	Grade	WHO Class	LN
CK19 Neg.	3.8±0.4		1.7±0.4				
CK19 Pos.	4.9±0.4	<2cm 23% >2cm 59%	4.4±0.7	45% N 60% Y	43% Low 69% Int	1.1-14%; 1.2-60%; 2.0-60%	52% N 70% Y
p Value	0.059	0.018	0.01	0.101	0.02	0.005	0.65



1347 A Potential Pathogenic Mechanism of Pancreatic Endocrine Neoplasm (PEN) Via p53 Ubiquitin-Protein Ligase MDM2

LH Tang, P Allen, I Modica, J Shia, MF Brennan, DS Klimstra. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Pancreatic endocrine neoplasm (PEN) typically behaves in a less aggressive manner than its adenocarcinoma counterpart. The molecular and genetic alterations that characterize PENs have remained elusive. Gene mutations, which are frequently involved in the pathogenesis of pancreatic ductal carcinoma, *i.e. k-ras, p53, p16,* and *DPC4*, have not been detected in PENs. MDM2 is known to act as a ubiquitin ligase promoting proteasome dependent degradation of p53. MDM2 is also a transcriptional target of p53, such that p53 activity controls the expression and protein level of its own negative regulator, providing for an elegant feedback loop. In this study, we evaluated the expression of MDM2 and p53 protein by immunohistochemistry on tissue microarray sections including 101 PENs with correlation of histopathological and clinical factors.

Design: Material of 101 patients with PENs who underwent surgery from 1983-2005 were available for this investigation. 81 patients had complete follow up (mean 44 months). Immunostain for MDM2 and p53 were performed on tissue microarray sections prepared from formalin-fixed and paraffin-embedded tissue blocks. Positive stain was defined by a diffuse and specific nuclear staining pattern regardless the intensity. Statistical analysis was performed to compare specific immunoreactivity with various histopathological and clinical categories using Pearson Chi-square test, and the significance was assessed by 2-sided Fisher Exact Test. Survival was evaluated by Kaplan-Meier curve.

Results: The frequency of positive MDM2 immunoreactivity was detected in 62% of PENs. In contrast, p53 immunoreactivity was not detected in any PENs in this study. There was no significant correlation between MDM2 immunoreactivity with gender (p=0.823), tumor functionality (p=0.118), tumor size (p=0.461), tumor grade (p=0.702), WHO classification (p=0.440), vascular invasion (p=0.213), lymph node status (p=0.461), or distant metastasis (p=0.366). Overall, MDM2 expression was not associated with recurrent free survival or disease specific survival.

Conclusions: Given its high frequency of expression and negative association with any known histopathological and clinical parameters, MDM2 may represent a novel and ubiquitous pathogenic mechanism of PENs. The finding by immunohistochemistry is being confirmed by quantitative RT-PCR.

1348 Serous Cystadenoma of the Pancreas as a Model of Clear-Cell Associated Angiogenesis and Tumorigenesis

D Thirabanjasak, O Basturk, I Nalbantoglu, JD Cheng, E Levi, R Singh, NV Adsay. Wayne State Univ-Karmanos Cancer Inst, Detroit, MI; New York Univ, New York, NY; St.John Hospital and Medical Center, Detroit, MI.

Background: Similar to the other vHL-related tumors such as renal cell carcinomas and capillary hemangioblastomas, serous cystadenomas of the pancreas (SCAs) are also characterized by clear cells. Over the years, we have also noticed that the epithelium of SCAs shows a prominent capillary network.

Design: 18 cases of SCA were reviewed histologically, and immunohistochemical analysis was performed for CD31 (endothelial marker) and VEGF (angiogenetic factor) as well as the molecules implicated in the clear cell tumorigenesis: GLUT1, and carbonic anhydrase IX (GP250).

Results: There was an extensively rich capillary network in all cases of SCA which was confirmed by CD31 stain that showed 26 capillaries (a capillary is defined as one completely encircled vascular space) per every 100 epithelial cells (range, 12-53). This network was so intimately admixed with the epithelium that it appeared almost intraepithelial. VEGF expression was identified in 10/18 cases (diffuse and strong in 2 and diffuse but weak in 8). Among the "clear-cell" markers, GP250 was detected in all cases (diffuse/strong in 17, and focal/strong in 1), and GLUT-1 in most cases (diffuse/strong in 15, focal in 2, and negative in 1).

Conclusions: As in other vHL-related clear cell tumors, there is a prominent capillary network immediately adjacent to the epithelium of SCAs, confirming that the clear-cell – angiogenesis association is also valid for this tumor type. Molecules implicated in clear-cell tumorigenesis are also consistently expressed in SCAs, which may have biologic and therapeutic implications, especially considering the rapidly evolving drugs against these pathways. These findings may also be helpful diagnostically in small biopsies.

1349 Is Isolated Portal Fibrosis (IPF) a Common Histological Finding in Severely Obese Patients Undergoing Gastric Bypass Surgery?

DG Tiniakos, M Melachrinou, MI Argentou, M Karanikolas, MG Makri, C Kittas, F Kalfarentzos. Medical School, Univ. of Athens, Athens, Greece; Medical School, Univ. of Patras, Patras, Greece.

Background: Isolated portal fibrosis i.e. portal fibrosis associated with hepatic steatosis without zone 3 hepatocellular injury or fibrosis has been proposed as a precursor to nonalcoholic steatohepatitis (NASH) in the spectrum of nonalcoholic fatty liver disease (NAFLD) in severely obese patients. NAFLD is common in these patients and in some cases may progress to cirrhosis. Adipokines may play important role in NAFLD pathogenesis. We aimed to define the prevalence of IPF in severely obese patients undergoing bariatric surgery and correlate it with clinical data, histopathological parameters of NAFLD and biochemical findings, including adipokine serum levels.

Design: Surgical liver biopsies were obtained from 40 severely obese patients (15 male, 25 female, mean age 38.5±9.3, mean BMI 55.7±7.8) undergoing gastric bypass procedure. Patients had no history of hepatitis, exposure to hepatotoxic drugs, prior bariatric surgery or alcohol consumption over 100 grams/week. Demographic data, anthropometric measurements, liver tests and HOMA-IR were recorded. Adipokine serum levels were measured by radioimmunodiffusion assay (leptin and adiponectin) or ELISA (resistin). Hepatic steatosis, lobular inflammation, hepatocellular ballooning and fibrosis were evaluated according to Brunt and scored according to Kleiner et al (2005). IPF, if present, was noted and graded as mild, moderate or severe.

Results: Waist/hip ratio was higher in men (p=0.0001) and leptin serum levels were higher in women (p=0.001). Thirty five of 40 patients (87.5%) had histological NAFLD (activity score: 0-6). Six of 35 patients (17.1%) had simple steatosis, 21 (60%) had steatosis with inflammation and 8 (22.9%) had NASH. IPF was present in 4 (10%) patients and was characterized only by fibrous expansion of portal tracts (mild IPF). All patients with IPF had mild (n=3) to moderate (n=1) steatosis. There was no significant correlation between IPF and demographic, anthropometric, biochemical (liver tests, HOMA-IR, adipokine serum levels) or histological variables.

Conclusions: In severely obese patients undergoing bariatric surgery IPF is not a common histological finding and is not related to clinical and biochemical parameters, adipokine serum levels and histopathological features of NAFLD.

1350 The Potential Role of Claudin-1, Claudin-4, Claudin-5 and Caudin-7, in Hepatocellular Carcinoma

V Tzelepi, AC Tsamandas, A Liava, N Pagoni, T Petsas, C Karatza, CD Scopa. University of Patras School of Medicine, Patras, Greece.

Background: Claudins 1, 4, 5 and 7 are integral tight junction proteins which display structural and regulatory functions, and are dysregulated in a variety of malignancies; however, their role in liver carcinogenesis has not yet been elucidated. This study investigates claudin-1, claudin-4, claudin-5 and claudin-7, expression in hepatocellular carcinoma (HCC).

Design: Formalin-fixed, paraffin-embedded 4μm sections, obtained from 36 HCC hepatectomy specimens with matched non-neoplastic liver, were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal and polyclonal antibodies for claudins 1, 4, 5 and 7 (Zymed, USA). Membranous staining was considered as positive and scored as absent, 1+, 2+, 3+ when 1-30%, 31-50% and >50% of tumor cells were positively immunostaining, respectively. Results were correlated with pathologic data and patients' survival. Mean follow up time was 29.32 months (range 1-86 months).

Results: Claudin-1 was expressed in 21 HCC, claudin-4 in 17, claudin-5 in 19 and claudin-7 in 18 tumors. HCC with coexistent cirrhosis more likely showed 2+ and 3+ expression of claudin-1 (11/13 tumors arising in cirrhosis [84%], compared to 10/23 [43%] tumors in non-cirrhotic liver [p=0.04]), claudin-4 (10/13 [77%] tumors with cirrhosis and 7/23 [30%] without cirrhosis [p=0.019]), claudin-5 (11/13 [84%] tumors with cirrhosis and 8/23 [35%] without cirrhosis [p=0.011]) and claudin-7 (10/13 [77%] tumors with cirrhosis and 8/23 [35%] without cirrhosis [p=0.037]). In addition, cirrhotic non-neoplastic livers more frequently overexpressed claudin-1 (10/13, 77%), and claudin-7 (7/13, 54%), compared to non-cirrhotic liver (7/23, 30% for claudin-1-and 3/23, 13% for claudin-7 [p=0.019 and p=0.018 respectively]). No correlation of claudins-1,-4, -5, -7 expression, with tumor grade and stage, and patients' survival was recorded.

Conclusions: This study demonstrates that aberrant expression of claudins-1, 4, 5 and 7 is frequent in hepatocellular carcinoma. In addition, non-neoplastic regenerative hepatocytes in cirrhosis also overexpress claudins 1 and 7. Overexpression of all claudins studied is also seen in tumors with co-existent cirrhosis. The latter association warrants further research in order to clarify the role of these tight junction molecules in the pathogenesis of liver regeneration and neoplasia.

1351 Increasing Incidence of Hepatic Adenoma in Men

AD Vanderheyden, C Jensen, FA Mitros. University of Iowa, Iowa City, IA.

Background: Classic teaching is that hepatic adenoma (HA) is a tumor of women (female:male reported as > 7:1). The epidemiologic link between exposure to steroid hormones and development of HA is well documented. The use of oral contraceptives has been implicated in the preponderance of these tumors in reproductive age women. Other associations tied to steroid hormone exposure include metabolic disorders and exposure to androgens/anabolic steroids. It has been our observation that the incidence of HA in men is increasing in recent years.

Design: We searched the LIS at the University of Iowa Health Care (UIHC) for HA from 1991 through June of 2006. All patients (N=51) identified were included in the study and a chart review was performed for evaluation of gender, age and known risk factors. Pathologic material on selected cases was analyzed for histologic features.

Results: 51 patients with HA were identified and separated into three time periods to compare incidence: 1991-1995, 9 women and 2 men (4.5 female:male); 1996-2000, 10 women and 2 men (5.0 female:male); and 2001-2005, 14 women and 8 men (1.75 female:male). Graphing the last six years demonstrated that the recent shift in female to male ratio occurred in 2003. The most recent 3 years (2003-2005) show HA in 5 women and 7 men (0.71 female:male) as compared to the prior 3 years (2000-2002), which show 13 women and 1 man (13 female:male); a change that is statistically significant (p-value = 0.0093). This significance remains when comparing the most recent three and a half years (2003 through June of 2006: 9 females, 9 males; 1.0 female:male) to the prior 12 years of the study (1991-2002: 28 females, 5 males; 5.6 female:male) with a p-value of 0.0191. Review of pathologic material showed no significant difference in the histologic features of these tumors.

Conclusions: At UIHC, the number of cases of HA in men appears to be increasing out of proportion to the incidence in women. This change is most pronounced since 2003 where there appears to be a shift from predominantly a tumor of women, to equal proportions between the sexes. Possible etiologies could include 1) the much publicized occult or illicit use of steroid containing substances, 2) increased endogenous estrogens associated with obesity, 3) exposure to environmental steroid hormones or substances which alter steroid synthesis or degradation, or 4) a statistical aberration associated with a short time period. A large scale epidemiologic study is warranted to investigate the possible etiology behind this dramatic alteration of the classical clinical presentation of HA.

1352 HCV Intrahepatic Mutations: A Role for the Innate Immune System?

P Vivekanandan, M Torbenson. The Johns Hopkins University School of Medicine, Baltimore, MD.

Background: The extent of variation in hepatitis C (HCV) nucleic acid sequences within the liver ("quasispecies") has been linked to disease progression, with more extensive quasispecies complexitiy linked to advanced fibrosis. Quasispecies result in part from the error prone HCV reverse transcriptase, but we hypothesized that the host immune system may also play a role. The innate immune system is composed of a number of elements that can protect cells from infection, including the APOBEC family of proteins whose deamination abilities can lead to extensive G to A and C to T changes in viral nucleic acids. Expression of various APOBEC proteins has been shown to lead to massive deamination of the human immunodeficiency virus and hepatitis B virus. However, the role for APOBEC expression in chronic hepatitis C infection (HCV) is unknown.

Design: Using differential DNA denaturing PCR (3D PCR), liver tissues with chronic HCV infection were studied for mutations. 3D PCR is a recently developed method to selective amplify mutated nucleic acids. Intrahepatic HCV levels were quantified and the expression of APOBEC1 and APOBEC2 were studied.

Results: No evidence for massive deamination of type reported for human immunodeficiency virus and hepatitis B viral infections was seen. However, 3D PCR identified a greater number of mutated sequences than regular PCR and separated the cases into two distinct groups, one in which the HCV quasispecies showed few mutations (6/14 liver tissues) and one with numerous mutations (7/14 liver tissues). This latter group also had a higher frequency of APOBEC2 detection and the dinucleotide context for the G to A and C to T changes were non random. No correlation was found between the complexity of the quasispecies and the intrahepatic HCV levels, but the group with higher quasispecies complexity was almost exclusively genotype 1A. APOBEC1 levels were present in a single case and showed no clear relevance to HCV infection.

Conclusions: Massive deamination of the HCV genome was not detected, but 3D PCR was successful in identifying a group of liver tissues with increased quasispecies complexity. This group also demonstrated a higher frequency of APOBEC2 expression. Further studies of the role of the innate immune system in regulating HCV are warranted.

1353 Glypican-3 as a Useful Diagnostic Marker That Distinguishes Hepatocellular Carcinoma from Benign Hepatocellular Mass Lesions

HL Wang, J Zhai, B Adley, F Anatelli, ST Chuang, XJ Yang. Washington University School of Medicine, St. Louis, MO; Weill Medical College of Cornell University, The Methodist Hospital, Houston, TX; Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: Morphologic distinction between hepatocellular carcinoma (HCC) and benign hepatocellular mass lesions, particularly hepatocellular adenoma (HCA), can sometimes be difficult. The currently available ancillary tools are suboptimal in terms of sensitivity and specificity. In this study, we analyzed the diagnostic value of glypican-3 (GPC3), a cell surface proteoglycan that has recently been shown to be overexpressesd in HCC, in the distinction between HCC and benign hepatocellular mass lesions.

Design: Formalin-fixed, paraffin-embedded tissue sections from a total of 193 surgically resected liver specimens were subjected to immunohistochemical staining using a monoclonal antibody specific for GPC3 (clone 1G12; Biomosaics, Burlington, VT). These included 83 HCCs, 48 HCAs, 30 focal nodular hyperplasias (FNH), and 32 macroregenerative nodules (MRN) in the background of cirrhosis. Sections from HCC cases also contained non-neoplastic (cirrhotic or non-cirrhotic) liver tissue. Immunostaining intensity was graded as 1+ (weak), 2+ (moderate) or 3+ (strong).

Results: Cytoplasmic and membranous staining for GPC3 was detected in 58 HCCs (69.9%), among which 32 cases (55%) exhibited a strong (3+) immunoreactivity. In contrast, none of HCAs, FNHs and MRNs (a total of 110 cases) showed detectable GPC3 immunoreactivity. The non-neoplastic liver tissues adjacent to HCCs were also negative for GPC3 expression. In HCCs, GPC3 expression tended to be more frequently seen in moderately and poorly differentiated tumors (78.6%) than in well differentiated ones (58.3%), but this difference did not reach statistical significance (P=0.0535). GPC3 expression did not correlate with the age or gender of the patients, the size or stage of the tumors, the presence or absence of cirrhotic background, or the underlying etiologies.

Conclusions: Positive immunohistochemical staining for GPC3 is highly specific for HCC, which can be reliably used to distinguish from benign hepatocellular mass lesions including HCA. It should be emphasized, however, that GPC3 expression in HCC can be focal and that approximately 30% of the cases can be completely negative. Lack of GPC3 staining thus cannot exclude the possibility of HCC in difficult cases.

1354 Cirrhosis-Like Hepatocellular Carcinoma (HCC): A Rare and Often Clinically-Undetected Variant

A Yabes, B Naini, C Lassman, MM Yeh, S Jakate, M Torbenson, RE Nakhleh, B Portmann, A Tomezzoli, LD Ferrell. UCSF, CA; UCLA, CA; U Washington, WA; Rush, IL; Johns Hopkins, MD; Mayo Clinic, Jacksonville; Kings Col, London, United Kingdom; Verona, Italy.

Background: We have noted a growth pattern of HCC that mimics the gross appearance of cirrhosis and so is often undetected, resulting in lack of timely therapy and poor prognosis. We studied this growth pattern to more clearly define its diagnostic features.

Design: 25 cases of multinodular HCC from cirrhotic explants were subdivided into 2 groups. 8 cases were "cirrhosis-like" (CL): tumor entirely composed of small nodules mimicking cirrhotic nodules. 17 cases were "cirrhosis-like with dominant nodule" (CLDN): a nodule of HCC >3 cm with cirrhosis-like satellite nodules. A dominant nodule accounted for at least 50% of tumor bulk. Evaluation included clinical features (serum AFP, imaging, etiology of cirrhosis), gross/histologic appearance (size, pattern/grade, stromal-vascular invasion, Mallory hyaline, bile) and MIB1.

Results: Serum AFP was not significantly elevated. Cirrhosis etiologies varied. All HCCs were moderately-differentiated and trabecular. Dense, collagenous bands without benign ductular reaction around cirrhosis-like tumor islands were seen. MIB-1 index ranged from 0-15%. *CL*: Pre-transplant imaging was compatible with cirrhosis; 2 cases had enhancing lesions ~1.8 cm. Tumor nodules were typically 0.2 to 0.6 cm, numbering >20 to >100, grossly mimicking cirrhosis. Exceptions included 2 cases with nodules up to 2 cm and 1 case with one 3 cm nodule. Aggregate tumor size ranged from 5-22 cm. Histologically, Mallory hyaline and bile stasis were abundant. Pseudoglands were common. Stromal (100%) and vascular invasion (50%) were identified. *CLDN*: Pre-transplant imaging identified one dominant nodule. Multiple other small nodules of HCC, undetected by prior imaging, were present, significantly increasing the tumor volume from the pre-transplant measurement.

Conclusions: We describe a growth pattern of HCC that evades radiologic detection and has the gross appearance of cirrhosis. Pre-transplant AFP is not reliable for detection. Grossly, the slightly larger tumor nodules with possible bile staining, and microscopically, stromal invasion, abundant Mallory hyaline, bile stasis, pseudoglands, and the dense stromal fibrosis around tumor nodules lacking significant ductular reaction are all features that help differentiate tumor from cirrhotic nodules.

1355 Chromosomal Changes in Fibrolamellar Hepatocellular Carcinoma Determined by Array-Based Comparative Genomic Hybridization

A Yabes, L Burgart, D Jain, S Kakar. UCSF Medical Center, San Francisco, CA; Abbott NW Hosp, Minneapolis, MN; Yale University, New Haven, CT.

Background: Fibrolamellar carcinoma (FLM) is a rare subtype of hepatocellular carcinoma (HCC) that occurs in young patients in the absence of cirrhosis. Although the outcome is better than conventional HCC with cirrhosis, FLM is an aggressive neoplasm with 50% 5-year survival. Conventional HCC reveals a fairly consistent pattern of chromosomal gains and losses. The chromosomal abnormalities in fibrolamellar carcinoma have not been well studied.

Design: Comparative genomic hybridization (CGH) was done on formalin-fixed paraffin-embedded tissue from 11 patients with FLM. Human 1.14 arrays with 2433 bacterial artificial chromosome clones covering the genome at 1.5 Mb resolution were used. 1µg each of tumor and gender-matched reference DNA (lymphocytes) was labeled by random priming using Cy3-dCTP and Cy5-dCTP respectively. Following addition of Cot-1, denaturation, hybridization for 48-72 hrs and mounting in DAPI containing solution, 3 single-color intensity images (DAPI, Cy3 and Cy5) were collected for each array using a charge coupled device camera.

Results: Chromosomal aberrations were seen in 6 cases (54%); the other 5 (46%) yielded normal results. Among the 6 abnormal cases, gains or losses were observed at 3 loci in 2 cases, 7 loci in 1 case, 8 loci in 2 cases and 14 loci in 1 case. Chromosome 7 abnormalities were most common with 7q gain in 5 cases and 7p gain in 4 cases. Abnormalities in chromosome 8 were seen in 3 cases and included 8q gain (2 cases), 8p loss (2 cases) and 8q loss (2 cases). Loss of 18q was present in 3 cases and gain of 19p in 2 cases. Gains in chromosome X and 20 and loss at chromosome 1 were less common (1 case each). There was no correlation of CGH changes with age, gender, tumor size and metastasis. Patients with no CGH abnormalities showed a trend towards better 5-year survival compared to those with chromosomal abnormalities (80% vs. 33%, p=0.06).

Conclusions: Abnormalities in chromosomes 7 and 8 are common in fibrolamellar carcinoma. Aberrations in these loci are well known in conventional HCC. In contrast to HCC, gains at chromosomes 1,20 and X are less prominent while 18q loss and 19p gain may be more frequent. Fibrolamellar carcinoma with chromosomal changes shows a trend towards a more aggressive clinical outcome. There is no correlation of genetic aberrations with age, gender, tumor size, and the presence or absence of metastases.

1356 Evaluation of IgG4 Stain in Autoimmune Pancreatitis Associated Cholangitis and Other Autoimmune Mediated Biliary Diseases

L Zhang, ST Chari, TC Smyrk. Mayo Clinic, Rochester, MN.

Background: Autoimmune pancreatitis-associated sclerosing cholangitis is part of a newly recognized clinicopathological entity of IgG4-related sclerosing disease, incorporating pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis. This entity usually responds to steroid therapy. Increased IgG4-positive plasma cell infiltration in tissue has been approved as a useful diagnostic tool. But the density of IgG4-positive plasma cells infiltration in other autoimmune mediated biliary diseases has not been assessed. We evaluated the IgG4 stain in biopsies from patients with autoimmune pancreatitis-associated cholangitis and other autoimmune mediated biliary diseases.

Design: IgG4 immunostain was performed on core needle liver biopsies from patients with autoimmune pancreatitis-associated cholangitis (5), primary biliary cirrhosis (PBC) (19), autoimmune cholangitis (AMA negative PBC) (19), primary sclerosing cholangitis (PSC) (20), autoimmune hepatitis (AIH) (19), and autoimmune hepatitis-PBC overlap syndrome (OLS) (16). Moderate or marked IgG4-positive plasma cells infiltration was defined as >10 IgG4 positive cells/HPF, which is indicative of autoimmune pancreatitis in pancreatic tissue, according to published criteria. Since majority of the biopsies had sparse IgG4-positive plasma cell infiltration, we also characterized IgG4-positive plasma cells as present or absent; presence of IgG4-positive plasma cells was defined as at least one portal tract with more than 2 positive cells.

Results: All 5 examples of autoimmune pancreatitis-associated cholangitis showed moderate or marked IgG4 positive plasma cell infiltration. None of the biopsies of other biliary diseases had a moderate or marked IgG4-positive plasma cells infiltration. In our absent/present scoring system, IgG4-positive plasma cells were usually absent in PBC (0/19, 0%), AMA negative PBC (2/19, 10.5%), and PSC (1/20, 5%), usually present in AIH (16/19, 84%) and OLS (12/16, 75%).

Conclusions: Increased periductal IgG4-positive plasma cell infiltration is specific for autoimmune pancreatitis-related sclerosing cholangitis. The presence of IgG4-positive plasma cells infiltration in portal tracts in AIH and OLS may correlate with the fact that those conditions respond better to steroid therapy than do PSC and PBC.

1357 p27, Cks1, Skp2 and PTEN Expression in Hepatocellular Carcinoma

V Zolota, V Tzelepi, A Liava, N Pagoni, T Petsas, C Karatza, CD Scopa, AC Tsamandas. University of Patras School of Medicine, Patras, Greece.

Background: $p27^{Kip1}$ is a cell-cycle inhibitory protein and its downregulation is mediated by its specific ubiqitin subunits Cks1 and Skp2. PTEN is a tumor suppressor gene which upregulates p27. This study investigates p27, Cks1, Skp2 and PTEN expression in hepatocellular carcinoma (HCC).

Design: Formalin-fixed, paraffin-embedded 4μm sections, obtained from 67 HCC hepatectomy specimens with matched non-neoplastic liver, were subjected to immunohistochemistry using monoclonal and polyclonal antibodies for p27, Cks1, Skp2 and PTEN. Nuclear staining was considered as positive. Results were correlated with pathologic data and patients' survival. Mean follow up time was 30.12 months (range 1-84 months).

Results: Expression of p27, Cks1, Skp2 and PTEN was recorded in: 51/67(76%) 16/67(24%), 23/67(34%) and 63/67 (94%) cases, respectively. PTEN was also expressed in cirrhotic and non-cirrhotic non-neoplastic livers; however its expression was significantly lower compared to that of tumors (HCC: 70.91±36.46, cirrhotic livers: 31.62±12.04, non-cirrhotic livers: 10.11±9.94-p<0.001). Mean values for Cks1, Skp2 and p27 expression in HCC were: 8.1±15.5, 4.72±9.7, 17±18.4 respectively. Cks1, Skp2 and p27 expression in cirrhotic and non-cirrhotic livers was observed in rare instances. Statistical analysis revealed a loss of PTEN and p27 expression in HCC grade 3: [PTEN: grade 1: 97.3±1.9, grade 2: 71.6±27.4, grade 3: 7.4±2.5-p<0.0001. p27: grade 1+2: 19.2±11.3, grade 3: 5.3±3.1-p=0.029]. Loss of PTEN and p27 expression was also related to presence of vascular invasion (VI): [PTEN: VI(-): 92.1±5.6, VI(+): 12.4±1.2-p=0.0012. p27: VI(-): 23.1±4.5, VI(+): 5.2±2.7-p=0.013]. No association was recorded between Cks1 and Skp2 expression and tumor grade or stage. PTEN and p27 expression were reversibly correlated with disease free survival (r=- 0.61, p=0.0043 and r=- 0.47, p=0.018). Cox regression analysis revealed that vascular invasion (CI:1.231-5.604, p=0.019), tumor stage (CI:0.051-0.690, p=0.012) and PTEN expression (CI: 1.065-41.082, p=0.032), were independent prognostic factors.

Conclusions: This study demonstrates that loss of PTEN and p27 expression is associated with adverse pathological parameters and increased risk for tumor recurrence. These results support the importance of PTEN and p27 loss for the progression of HCC in humans. PTEN increased expression in cirrhotic non-neoplastic livers may reflect an effort for control of hepatocyte regeneration associating liver cirrhosis.

Neuropathology

1358 Diagnostic Utility of Immunohistochemistry in Differentiating Hemangioblastoma from Metastatic Renal Cell Carcinoma

M Acon Laws, JF Silverman, YL Liu, M Tung, RS Saad. Allegheny General Hospital, Pittsburgh, PA.

Background: Differentiating hemangioblastoma (HB) from metastatic clear renal cell carcinoma (CRCC) to the brain is crucial since they have different management and both can occur in patients with Von Hippel Lindau (VHL) disease. Moreover, CRCC can metastasize to hemangioblastoma as a tumor to tumor metastases. In addition, HB can demonstrate vacuolated tumor cells mimicking CRCC. We investigated the diagnostic utility of D2-40, a novel monoclonal antibody, CD10, low-weight cytokeratin (CAM 5.2), epithelial membrane antigen (EMA), RCC, estrogen and progesterone receptors (ER and PR) in HB and CRCC patients.

Design: A computer search of our hospital identified 27 cases of HB between 1997 and 2005, consisting of 10 spinal, 9 cerebellar and 8 cerebral HB. We also included 30 cases of metastatic CRCC, 8 of which were metastases to the central nervous system. Immunostaining was performed on formalin-fixed, paraffin embedded sections using HIER technique. Intensity was graded from 0-3 with a score 0 for no staining and 3 for maximal intensity. The pattern/distribution of reactivity was recorded as focal (5-10%) or diffuse (>10%). Cases which showed weak or <5% staining were considered negative.

Results: Table (I) shows our results. All cases of HB are negative for epithelial markers (Cam 5.2, CD10, EMA, RCC). None of the CRCC was negative for both CD10 and Cam5.2. Cases which were negative or focally positive for CD10, demonstrated strong positivity for Cam5.2 (5 cases) and vice versa (3 cases).

	D2-40	ER	PR	CD10	Cam5.2	EMA	RCC
HB	10/27	0/27	7/27	0/27	0/27	0/27	0/27
CRCC	10/30	0/30	9/30	25/30	24/30	27/30	22/30

HB= hemangioblastoma CRCC= clear cell renal cell carcinoma

Conclusions: In our experience, the monoclonal antibody D2-40 is not a useful marker to distinguish HB from CRCC. Epithelial markers, especially CD10, Cam 5.2 and RCC are superior markers for distinguishing between a HB and a metastatic CRCC. PR immunoreactivity was unable to distinguish between HB and CRCC metastatic to the CNS, and the staining for PR was weak and focal, suggesting that hormonal treatment is not an option for these patients.

1359 Prion Disease in Washington State: A Thirty Month Surveillance Study

CT Allen, J Sonnen, P Gambetti, TJ Montine. University of Washington, Seattle, WA; National Prion Disease Pathology Surveillance Center, Cleveland, OH.

Background: Variant Creutzfeldt-Jakob disease (vCJD), thought to be acquired from eating beef affected by bovine spongiform encephalopathy (BSE), was described following a epidemic in the United Kingdom. In 2003 the first instance of BSE was reported in the USA in a cow slaughtered in Washington. The meat was distributed for human consumption. In 2004, prompted by public concern, we initiated a Center for Disease Control-sponsored collaborative program to enhance autopsy surveillance for clinically suspected prion disease.

Design: The WA Department of Health (DOH) disseminated information about the program to healthcare providers throughout the state. Enrollment was prompted by healthcare providers contacting local or state DOH, the National Prion Disease Pathology Surveillance Center (NPDPSC), or the Univ of WA (UW) to report a case of suspected prion disease. No case was declined and all costs, including transportation of the deceased, were covered. Autopsies were performed by UW Neuropathology and brains were evaluated at this site and the NPDPSC.

Results: During the first 30 months of surveillance, 30 cases of suspected prion disease were referred. Eighteen had prion disease classified as CJD. One case was familial while the remainder had sporadic (s) CJD of the following subtypes: eight M/M isoform 1, two M/M isoforms 1-2, two M/V isoforms 1-2, two M/V isoform 2, one V/V isoforms 1-2, and two V/V isoform 2. There was no case of vCJD. This represents a prevalence of 1.1 sCJD cases per million people per year in WA (population = 6.375 million), a value in close agreement with prevalence estimates in other populations. Eleven of the remaining twelve patients had a variety of structural brain changes that meet criteria for diseases that cause degeneration in cognitive and motor function. One case had no demonstrable pathologic lesions in the tissue examined.

Conclusions: This is the first epidemiologic investigation within a US state based entirely on autopsy-confirmed cases. Our results do not support the hypothesis that vCJD is an emerging illness in WA or that sCJD is more common in this state than in other regions of the world. The findings and lack of evidence for epidemic BSE in the USA is encouraging but continued surveillance for prion diseases is needed because our knowledge about emergence and transmission is inadequate. Our program may serve as an example to other states that wish to enhance surveillance of prion diseases.

1360 Evaluation of Chromosome 7 Alterations Including Epidermal Growth Factor Amplification Status in Pediatric Meningiomas

M Begnami, M Santi, EJ Rushing, M Quezado. National Institutes of Health, Bethesda, MD; Children's Hospital, Washington, DC; AFIP, Washington, DC.

Background: Childhood meningiomas are rare tumors corresponding to less than 3% of all primary CNS tumors. Distinct features include male predominance, infratentorial and intraventricular occurrence, and frequent clear cell and papillary subtypes. Monosomy of chromosome 22 is their best known molecular alteration ; loss of 7p has also been reported. Overexpression of EGFR correlates with enhanced malignant potential of many tumor types, including glioblastomas and astrocytomas. In adult meningiomas, high EGFR expression has been demonstrated by immunohistochemistry (IHC), but is not associated with prognosis. We evaluated 13 pediatric meningiomas utilizing chromogenic in situ hybridization (CISH), fluorescent in situ hybridization (FISH), and IHC to assess chomosome 7 alterations including amplification/expression status of the epidermal growth factor receptor (EGFR).

Design: Thirteen pediatric meningiomas were classified according to the 2000 WHO criteria (6 sporadic, 2 NF2-associated, and 6 radiation-induced). IHC, CISH and FISH were performed for EFGR status and CISH with the chromosome 7 centromere probe was used to assess ploidy status. High EGFR expression by IHC was characterized by complete membranous staining in more than 10% of the tumor cells. EGFR amplification by CISH was detected when 10 or more copies or clusters were observed in more than 50% of the cells; by FISH, when the average ratio of EGFR gene/CEP 7 signal per cell was greater than 2. Tumors with low amplification showed 6-10 copies in the nuclei. Chromosome 7 diploid meningiomas showed 2 copies in more than 50% of the tumor cells (CISH).

Results: High EGFR expression by IHC was observed in 9 (61%) meningiomas. The remaining 4 (39%) cases (1 sporadic, 2 radiation-induced, 1 NF2 associated) were negative. Of the positive cases, two showed low amplification for EGFR by CISH but not by FISH (performed in 3 cases). All cases were diploid for chromosome 7 (two cases showed triploidy in 35% of the cells).

Conclusions: We conclude that (1) amplification of EGFR is not a common feature in pediatric meningiomas, (2) high EGFR expression by IHC does not correlate with EGFR amplification by FISH or CISH, (3) CISH is a better method than IHC for evaluation of true EGFR status amplification, and (4) most pediatric meningiomas show normal chromosome 7 ploidy.

1361 Chromosome 7 Polysomy Detected by Chromogenic In Situ Hybridization (CISH) Is a Common Finding in Sporadic Chordomas

MD Begnami, FA Soares, M Quezado. National Institutes of Health, Bethesda, MD; AC Camargo, Sao Paulo, Brazil.

Background: Chordomas are malignant bone tumors most often located in the axial skeleton. The cytogenetic and molecular features of chordomas are largely unknown but reportedly complex. Copy number gains of chromosome 7 have been detected in some sporadic and familial chordomas by various methods including comparative genomic hybridization, G banding, and FISH. In this study, we evaluated chromosome 7 ploidy status in a group of 11 sporadic chordomas using chromogenic in situ hybridization (CISH).

Design: Eleven sporadic chordomas were analyzed by H&E and immunohistochemistry profile with vimentin, EMA, AE1/AE3, and S100 for confirmation of diagnosis. MIB1 and p53 stains were also obtained. For detection of abnormalities on chromosome 7 we used CISH. Polysomy of chromosome 7 was detected when 3 or more signals were found in the nuclei of more than 50% of the tumor cells.

Results: Chromosome 7 polysomy was detected in 9/11 (82%) sporadic chordomas. All cases showed typical physaliferous cells by H&E and stained positive for all markers. p53 and MIB1 staining was rarely detected and associated with recurrent tumors and necrosis.

Conclusions: Chromosome 7 polysomy is a common event in sporadic chordomas. This finding suggests that this region may harbor an oncogene potentially relevant in the tumorigenesis of sporadic chordomas.