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BILE ACID INDUCED SIGNAL TRANSDUCTIVE PATHWAYS INVOLVED IN REGULATION OF CHOLESTEROL 7A-HYDROXYLASE (CYP7A1) TRANSCRIPTION.

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Background: The hepatic biosynthesis of bile acids constitutes a major excretory pathway of cholesterol from the body. The gene encoding CYP7a1, the rate-limiting enzyme in the neutral pathway of bile acid synthesis, is feedback inhibited at the transcriptional level by hydrophobic bile acids. Evidence from our laboratory suggests that relatively hydrophobic bile acids may repress CYP7a1 transcription by activating isoforms of PKC. The objective of the present studies was to identify the mitogen activated protein kinase (MAPK) cascade(s) downstream of PKC which may mediate the repression of CYP7a1 transcription by bile acids. Methods: *In vitro* kinase assays were performed to identify the bile acid mediated post-PKC signaling events in primary rat hepatocyte cultures. CYP7a1 mRNA levels were determined by RNase protection assay. Results: Bile acids (50 μM) activated Raf-1 kinase and p42/p44^{MAPK} (2-3 fold) independently of bile acid hydrophobicity. Inhibition of p42/p44^{MAPK} by PD98059 (50 μM for 30 min) failed to block the down-regulation of CYP7a1 mRNA by TDCA (50 μM). TCA (50 μM) strongly activated (3-4 fold) c-Jun N-terminal kinase (JNK) in a time- (5-60 min) and concentration (13.5-100 μM) dependent manner. The activator of JNK by bile acids of varying hydrophobicities mirrored their ability to repress CYP7a1 mRNA levels in culture. Pretreatment of hepatocytes with chelerythrine (7 μM for 30 min), a PKC inhibitor, blocked the activation of JNK by both TCA and TDCA. Overexpression of dominant negative JNK completely blocked the ability of TCA to activate JNK and to down-regulate CYP7a1 mRNA. Infection of hepatocytes with a dominant negative c-Jun recombinant adenovirus (TAM67) blocked the repression of CYP7a1 mRNA by TCA. Conclusions: In primary rat hepatocytes, hydrophobic bile acids may repress CYP7a1 gene by sequentially activating PKC and JNK, leading to phosphorylation of c-Jun. Phosphorylated c-Jun may mediate this effect by acting as a transcriptional repressor of the CYP7a1 gene.

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RADIOLOGICLAL IMAGES, HISTOLOGICAL FINDINGS, AND BIOLOGICAL FACTORS IN THE GALLBLADDER MUCOSA WITH AN ANOMALOUS JUNCTION OF THE PANCREATICOBILIARY DUCT.

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Objectives. : We have reported that an anomalous junction of the pancreaticobiliary duct (AJPBD) is an important risk factor for gallbladder carcinoma. In this study, we evaluated the radiological images, histological findings, and biological factors of gallbladder with AJPBD. *Methods.* We examined 36 surgically operated gallbladders with AJPBD (20 of hyperplasia, 10 of stage I carcinoma, and 6 of stage II to IV carcinoma). In all cases, the methods of endoscopic retrograde cholangiopancreatography (ERCP) were performed preoperatively. Histological findings, the labeling index of proliferative cell nuclear antigen (PCNALI), and K-*ras* mutations were compared with the preoperative radiological images obtained by ERCP. *Results.* In 20 cases of hyperplasia, there were 8 cases with whom the major pancreatic duct joined the common bile duct (P-C type), and 12 cases with whom the common bile duct joined the major pancreatic duct (C-P type). The dilatation of common bile duct was detected in 16 cases. The PCNALI in cases with P-C type was greater than that with C-P type ($p<0.05$). K-*ras* mutation was detected in 3 cases with P-C type. In 10 cases of stage I carcinoma, there were 9 cases with P-C type, and 1 case with C-P type. K-*ras* mutation was detected in 6 cases with P-C type. The spectrum of K-*ras* mutation in hyperplasia and stage I carcinoma was GGT to GAT in codon 12. In 6 cases of stage II to IV carcinoma, there were 5 cases with P-C type, and 1 case with C-P type. K-*ras* mutation was detected in all cases. *Conclusions.* These results suggest that gallbladder mucosae with AJPBD those with P-C type may have increased cellular kinetics, high prevalences of K-*ras* mutation, and that the surgical resection for the patients with AJPBD especially those with P-C type should be considered actively.

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TGF BETA SIGNALING BY SMAD2/3 AND ELF SPECTRINS RESULTS IN BILE DUCT FORMATION AND IS DISRUPTED IN PRIMARY BILIARY CIRRHOSIS.

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Background: Transforming growth factor-β (TGF-β) is a major cytokine involved in multiple cellular processes including differentiation, proliferation, migration, extracellular matrix composition, and apoptosis. SMAD proteins serve as intracellular signaling molecules of TGF-β and activins. We have recently found that mice doubly heterozygous for disruptions of the smad2 and smad3 genes display a novel phenotype of bile duct and liver hypoplasia. We have also shown that inhibition of ELF3, a Beta spectrin also results in a Primary Biliary Cirrhosis (PBC) phenotype in embryonic explant cultures, (Oncogene 1999, 18, 353-364). Similar loss of ELF3 is seen in PBC tissue, suggesting that ELF is important in the pathogenesis of PBC. Aim: To determine the role of TGF Beta and ELF in bile duct formation and PBC, we 1: Cultured embryonic liver explants from smad2/3 mutants in the presence of TGFBeta. 2: Analyzed SMAD 2/3 and ELF expression by confocal microscopy in the normal, PBC, and tissue from other diseases. 3:Analyzed ELF expression in the smad2/3mutants, and functional cooperativity of ELF with SMAD 2 and SMAD3 in normal and PBC liver tissue. 4: Analyzed ELF expression and phosphorylation in different cell lines. Methods and Results: 1. The addition of TGF Beta resulted in the formation of a prominent limiting plate, budding bile ducts with increased and localized expression of SMAD 2and3. 2.Immunofluorescence confocal microscopy utilizing antibodies to SMAD2 and SMAD3 was performed in liver tissues from PBC, primary sclerosing cholangitis, and hepatitis C patients. A marked suppression of SMAD 3 in PBC, absent nuclear localization of SMAD 2 were seen only in PBC liver tissue. 3. Immunofluorescent labeling with antibody to ELF was performed in the smad2/3 mutant liver tissue. An identical pattern of ELF expression is seen in smad2/3 mutants and PBC tissue, not seen tissues from patients with Hepatitis C, sclerosing cholangitis, alcoholic liver disease and hemochromatosis. 4:Coimmunoprecipitation studies were performed examining ELF binding to SMAD 2 and SMAD 3 using normal, PBC and Hepatitis C liver lysates. SMAD2, and to a lesser extent, SMAD 3 both bind to ELF in PBC tissue. 5: Immunoblotting analysis with ELF 3 and phospho-Ser, Thr-specific antibodies. Conclusions: 1: TGF Beta induces bile duct formation through SMAD2 and 3 activation. 2. The smad2/3 mutants have a severe but similar phenotype to that of ELF, and this pathway may play a crucial role in the pathogenesis of PBC.

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IS ADENOMYOMATOSIS OF THE GALLBLADDER A RISK FACTOR FOR GALLBLADDER CANCER?

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Purpose: Adenomyomatosis of the gallbladder (ADM) is considered to be a type of benign proliferative gallbladder disease or tumorous lesion. However, in recent years, a large number of patients with ADM and gallbladder cancer have been reported. It is suggested that cancer frequently develops on the fundal mucosa in patients with segmental type ADM. However, the association between these factors remains to be clarified in many respects. In this study, we examined the possibility of carcinogenesis in patients with ADM. Methods: This study included 28 patients in whom various diagnostic imaging procedures suggested ADM at our department. In 19 patients who underwent cholecystectomy were histopathologically diagnosed as having ADM (M : F = 14 : 5, mean age = 51.5 years). Immunohistochemical staining with cancer-suppressor / associated gene proteins, p53 and bcl-2, was performed using paraffin sections. Expression of each protein was compared. For evaluation, cells with DAB-stained were regarded as positive. The extent of positive cells was classified into 3 grades (+: focal, ++: partially aggregated, +++: diffuse) . Grades ++ and +++ were regarded as showing overexpression. In 9 patients (M : F = 9 : 0, mean age = 59.4 years, mean follow-up period; 39.4 months), symptoms and morphological changes were followed. Results: On immunohistochemical examination, p53 and bcl-2 were detected in 26.3% and 36.8% of patients, respectively. Overexpression of p53 was observed in 5.3% of patients. None of the patients showed overexpression of bcl-2. During the follow-up period, there were no additional symptoms in any patient. None of the patients underwent cholecystectomy during the follow-up period. Diagnostic imaging did not reveal any marked changes in gallbladder wall thickening or morphology during the follow-up period. Cholecystolithiasis was detected in 63 . 2% of patients undergoing resection and in 44.4% of followed patients. Conclusion: Cancer-suppressing gene proteins are also detected in patients with benign lesions such as cholecystitis. Neither immunohistochemical nor clinical examination suggested that ADM is a precancerous lesion of gallbladder cancer. The association between cholecystolithiasis and gallbladder cancer has been indicated. The incidence of concurrent cholecystolithiasis was high in

patients with ADM, suggesting that ADM is an indirect risk factor for gallbladder cancer.

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LIPOPOLYSACCHARIDE STIMULATION OF TNFA SECRETION BY HUMAN BILIARY EPITHELIUM.

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Epithelial cells of the biliary tract may play a role in control of infection through cytokine production. To understand the contribution of *human* biliary epithelium to the cytokine profile of the bile ducts, we examined the ability of cultured *human* biliary duct epithelial cells to produce tumor necrosis factor-α (TNFα) constitutively and in response to lipopolysaccharide (LPS) treatment. Methods: Epithelial cells isolated from human common bile ducts were cultured to confluency on Transwell inserts. At the start of experiments, the epithelial cells were transferred to serum free medium. For LPS treatment, E. coli LPS at 100 or 10 μg/ml was added to the apical medium. At 2, 4, 6, and 24 hours after treatment, the medium from both apical and basolateral compartments was removed, and the TNFα concentration measured by immunoassay. Results: Untreated *human* biliary epithelial cells secreted detectable amounts of TNFα into the apical and basolateral medium by 4 hours. By 6 hours, the cells secreted up to 14 pg TNFα into the apical compartment, with a comparable amount of TNFα in the basolateral medium. At 24 hours, up to 21 pg and 43 pg TNFα was detected in the apical and basolateral medium, respectively, in untreated cells. A distinct alteration in TNFα secretion was detected after treating the cells with E. coli LPS. LPS treatment doubled the apical secretion of TNFα compared to controls by 6 hours, with similar results seen at 24 hours. In contrast, LPS did not significantly alter the amount of TNFα secreted into the basolateral medium compared to controls for up to 24 hours. Discussion: Cultured mouse biliary cells secrete both TNFα and endothelin-1 in a polarized manner when treated with LPS. We have extended some of these observations to cultured *human* bile duct epithelial cells. These epithelial cells are a uniform population, devoid of other cell types such as lymphocytes normally present in the sub-mucosa. The *human* cells slowly secreted TNFα from both their apical and basolateral surfaces. In all experiments, treating the apical surface of the cells with E. coli LPS caused increased secretion of TNFα from the apical surface but had no affect on basolateral secretion. These experiments demonstrate that *human* biliary epithelial cells can produce and secrete cytokines. The distinctive apically-directed secretion of TNFα in response to LPS suggests that the epithelial cells of the *human* biliary tract can contribute to the response to bacterial infection and may mediate hepatocystic secretory function during sepsis.

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FUNCTIONAL EXPRESSION OF NA^+/H^+ EXCHANGE IN PRIMARY CULTURES OF PRAIRIE DOG GALLBLADDER EPITHELIUM.

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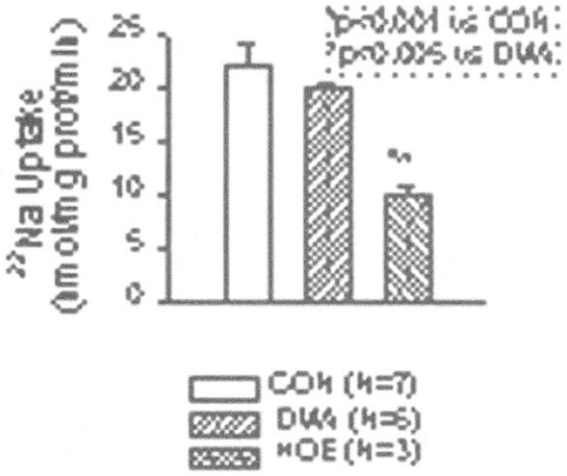
The prairie dog has emerged as an important animal model for the study of human cholesterol gallstones(GS). Several studies have shown that gallbladder(GB)Na^+ absorbtion is increased prior to GS formation and may promote cholesterol nucleation in animals-fed lithogenic diet. Most of the previous studies have utilized either *in vivo* or isolated GB tissues to examine the mechanisms of Na^+ transport and its potential role in GS formation. Recently Chapman et al. have described the establisment of primary GB epithelial cell(GBEC)cultures from the prairie dog (JSR 80: 35-43, 1998). However, no studies on Na^+ transport have been done in these GBECs. Na^+/H^+ exchange (NHE)is one of the major pathways for Na^+ transport in GB epithelia. The aim of the present study was to measure Na^+/H^+ exchange activity in primary GB epithelial cells. Methods: GBs were obtained from prairie dogs fed nonlithogenic diet. Cell harvest and isolation were performed following Chapman et al. Cells were seeded on 24-well plates and examined for Na^+/H^+ exchange activity after 8-10 days when they reached confluency. $^{22}Na^+$ uptake was measured for 3 min within linear range at an acid gradient(pH in 6.4, pH out 7.4)in the absence(CON)or in the presence in the transport buffer of either 100 μM dimethylamiloride(DMA)or 10 μM HOE-694. Na^+/H^+ exchange activity was determined as the difference in Na^+ uptake in the control minus the uptake in the presence of 100 μM DMA. The contribution of NHE1, NHE2 and NHE3 to NHE activity was determined by use of HOE-694.(HOE-694 *Ki* for NHE1, 0.16 μM; for NHE2, 5 μM; for NHE3, 650 μM in PS120 transfected cells; *Mol Pharmacol* 44:1041,1993). Results: Initial rates of $^{22}Na^+$ uptake in the control were 22.2±1.8 nmol/mg prot/min (Mean±SEM; Fig). Approximately, 90% of Na^+ uptake was mediated through 100 μM DMA-inhibitable Na^+/H^+ exchange. HOE data showed that Na^+/H^+ exchange contained roughly 50/50 of 10 μM HOE-sensitive and HOE-insensitive NHE isoforms. Conclusions: These data demonstrate that Na^+/H^+ exchange mediates most apical Na^+ uptake by the primary GBECs from the prairie dog. These data also suggest that HOE-sensitive and HOE-insensitive isoforms contribute to GBEC Na^+/H^+ exchange under basal conditions. These primary GBECs may serve as a novel tool for studying the mechanisms of GB ion transport under physiologic conditions and in GS formation.



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MAGNETIC RESONANCE CHOLANGIOPANCREATOGRAPHY IN PRE-OPERATIVE BILIARY IMAGING: THE NEW GOLD STANDARD?

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AIMS: Magnetic Resonance Cholangiopancreatography (MRCP) is a safe non-invasive method of imaging the pancreatico-biliary tract which avoids the risks associated with conventional biliary imaging modalities (ionizing radiation and iodine exposure). This study prospectively evaluates the efficacy of MRCP in imaging the common bile duct (CBD) in patients at risk of choledocholithiasis prior to, or after previous, cholecystectomy. METHODS: A consecutive prospective series of patients with a history of epigastric pain and abnormal liver function tests (LFT s), jaundice or pancreatitis were referred for MRCP. Patients with CBD calculi diagnosed at MRCP were referred for endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy (ES) if indicated. Patients with negative MRCP findings underwent cholecystectomy with or without intraoperative cholangiography (IOC). Patients with a negative MRCP undergoing laparoscopic cholecystectomy (LC) were followed up to exclude CBD calculi. RESULTS: A consecutive series of 106 patients (74 females, 58[50-71]*) were referred for MRCP over a 2 year period. The commonest indications were epigastric pain and abnormal LFT s (61%), jaundice (13%) and gallstone pancreatitis (12%). Twenty-seven (25%) patients had a previous cholecystectomy 28 (22-96)* months earlier. Adequate images were obtained in 102 (96%) patients. Eighty-two (80%) scans revealed no evidence of CBD calculi (true negatives). There were 14 (14%) scans with unequivocal evidence of CBD calculi (true positives) confirmed in 13 cases by ERCP and ES (6), OC and exploration of CBD (OECBD) (5) and percutaneous transhepatic cholangiography (2). In 6 (6%) cases the MRCP report suggested CBD calculi (confidently in 3, possible in 3). Subsequent ERCP (3), LC and IOC (2) and OECBD (1) failed to confirm the presence of calculi (false positives). Thirty seven (67%) in the true negative group went on to have an uncomplicated cholecystectomy (LC 30; OC 7). In 13 patients clinically unsuspected pathology was identified on MRCP (hepatobiliary malignancy [3], choledochal cysts [2]). No patients with a negative MRCP have re-presented with choledocholithiasis during a follow-up period of 18 (10-23)* months. CONCLUSIONS: MRCP is safe and accurate in the pre-operative diagnosis of CBD calculi, reducing the requirement for more invasive risk-associated imaging techniques. Clinically unsuspected pathology may also be identified. * median (Interquartile range).