**Biliary wound healing, ductular reactions, and IL-6/gp130 signaling in the development of liver disease**

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INTRODUCTION

Wound healing in the biliary tract significantly contributes to the development of liver disease. For example, ineffectual wound healing, mural scarring, and stricture development in the large extra-hepatic bile ducts are responsible for diseases such as extra-hepatic biliary atresia and primary sclerosing cholangitis. These are leading indications for liver transplantation (http://www.optn.org). A similar problem occurs in 10%–15% of all liver allografts—the biliary sludge syndrome. Conversely, an exuberant wound healing response, or ductular reaction, contributes to the development of cirrhosis from a variety of causes[1-3].

BEC lining the extra-hepatic and large intra-hepatic bile ducts have a different embryologic origin and are distinct phenotypically from BEC lining small intrahepatic bile ducts. But considerations with respect to wound healing are similar to each other and to wound healing at other sites. Unique aspects of the biliary tract that have the potential to impact significantly on wound healing include: (1) anatomy and physiology; (2) exposure to high concentration of bile; and (3) triggering of reactions in the smallest intra-hepatic bile ducts by insults other than, or in addition to, direct injury and ulceration. In addition, chronic inflammation and persistent wound healing reactions in either the large or small bile ducts often precede the development of cancers.

In the skin and intestines, mechanisms of wound repair depend on the depth of injury. Superficial wounds or simple erosions are healed primarily by a two-step process: restitution and regeneration. Restitution begins immediately after creating a superficial wound in barrier epithelia. Cells near the edge of the defect lose close contacts with neighboring cells, undergo shape changes, spread, migrate, and then contract to close the hole. However, restitution is necessarily limited because remaining cells can cover only so much of the denuded surface area. In large wounds, regeneration or proliferation of the remaining epithelial cells is also needed. Eventually the epithelium is restored to a nearly original state[4-5], although surviving cells may carry a legacy of DNA damage and senescence-related changes[6]. Deeper wounds involve the epithelia and underlying stroma. Processes such as angiogenesis; activation, migration, and proliferation...
of (myo-) fibroblasts and endothelial cells; formation of granulation tissue; and wound contraction are needed to close these larger/deeper defects. These more extensive wounds are also frequently inflamed and, in general, stromal involvement and inflammation greatly increase the risk of subsequent scarring7-10.

Epithelial aspects of wound repair are often studied, in vitro, by producing linear “wound” tracks in confluent epithelial monolayers. The restitution phase is isolated by treating the cultures with chemical mito-inhibitors to prevent cell proliferation from contributing to wound closure. Distances migrated by the epithelial cells from the edge of the wound at predetermined time points measure the effectiveness of restitution. We developed a BEC model using a collagen-matrix substrate to prevent premature BEC senescence, which occurs routinely when BEC are plated on plastic or collagen-coated plates, from interfering with the assay11.

“Front row cells”, or those epithelial cells nearest the defect, experience dissolution of epithelial cell-cell contacts and changes in cell shape15-18. They can also acquire some mesenchymal characteristics and, under some circumstances, can undergo complete epithelial-mesenchymal transition (EMT)19,20. Changes in front row cells and EMT function to dis-aggregate epithelial units and reshape the epithelia for movement. Epithelia in transition lose polarity, adherens junctions, tight and gap junctions, desmosomes, and down-regulate cytoskeleton intermediate filaments in order to rearrange their F-actin stress fibers and express filopodia and lamellopodia21,22. When wound closure is complete and proliferation has replenished the lost cells, re-establishment of inter-epithelial junctions restores the barrier. Coordinating these processes is critically important for barrier adaptation and wound healing23,24.

POTENTIAL INFLUENCES OF BILIARY TREE PHYSIOLOGY AND ANATOMY ON WOUND HEALING

The biliary tree can be thought of as a delicate, relatively complex, self-contained organ that communicates with, and is enveloped by, the liver (Figure 1). It monitors, alters the composition of, and triages bile into the intestine. The tenuous only arterial blood supply can be damaged easily by diseases and by surgical procedures. A close relationship and cross-talk between BEC and periductal (myo-)fibroblasts exists throughout the entire biliary tree: damage to one population usually results in reactive changes in the other. For example, periductal (myo-) fibroblasts often undergo activation and proliferation in response to significant BEC growth, injury, and bile leakage from the small15-21 or large bile ducts22-24. In extra-hepatic and large intra-hepatic bile ducts, this results in mural stricturing and luminal narrowing. In smaller intra-hepatic bile ducts, liver fibrosis and/or obliteration of the bile duct lumen can occur. A rich lymphatic network envelopes bile ducts that drains into regional hilar lymph nodes25,26. Numerous intramural peribiliary glands in the extra-hepatic bile ducts can become walled off after trauma and produce mucoceles. All of these potential sources of problems contribute to the well-deserved moniker of the biliary tree as the “Achilles heel” of liver transplantation.

Bile contains bile salts that can induce27,30 or protect BEC from apoptosis31, cross-activate EGFR via TGFβ ligand binding32, induce COX-2 expression33, or trigger BEC IL-6 and other cytokine production34. Bile also normally contains several growth factors (e.g. HGF), cytokines (IL-6), and other molecules39. Understanding the effect of various bile constituents on wound healing (esp. restitution) is critical because bile composition can be altered therapeutically (e.g. treating patients with ursodeoxycholic acid)35,36.

BEC lining the smallest intra-hepatic bile ducts are derived from hepatoblasts and are thought to contain a population of liver stem cells that can differentiate into either hepatocytes or BEC38-41. Changes in the intra-hepatic environment other than, or in addition to, direct injury and ulceration can trigger wound repair reactions in these smallest ducts. These “ductular reactions” are recognized as BEC and surrounding myofibroblasts at the interface zone of diseased livers42,43. Ductular reactions can be provoked by: (1) local BEC injury and inflammation44,45; (2) increased intra-biliary tract pressure, and (3) the combination of: (a) a strong liver regenerative stimulus, such as partial hepatectomy or chronic necro-inflammatory liver disease and (b) hepatocyte mito-inhibition because of carcinogen exposure or chronic oxidative stress2-3. Insufficient BEC regeneration in the smallest ducts leads to liver diseases such as chronic “ductopenic” rejection and drug-induced ductopenia46.

WOUND HEALING IN THE EXTRA-HEPATIC BILIARY TREE

Importance of arterial blood flow and wound depth

Study of the biliary sludge syndrome in liver allografts

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has been particularly illustrative of pathophysiologic mechanisms involved in biliary wound healing. This relatively common and particularly frustrating complication affects about 10% of all liver allografts. There are many potential causes of ineffectual biliary wound healing including abnormal anatomy created by the operation; suboptimal arterial blood flow because of technical problems, anastomotic narrowing, thrombosis, or antibody mediated rejection; recurrent ascending cholangitis; recurrent primary sclerosing cholangitis; and ischemic-preservation injury\(^{[46]}\). Regardless of the cause, impediments to bile drainage results in progressive intrahepatic fibrosis, which in turn, increases morbidity and decreases organ half-life.

The extra-hepatic biliary tree is sustained only by the hepatic artery, which drains into three terminal classical capillary microvascular networks that supply: (1) the bile ducts (peribiliary plexus), (2) the connective tissue of the portal tracts, and (3) the hilar and perihilar structures\(^{[47]}\). The allograft biliary tree is especially vulnerable to arterial ischemia for the first several months after the operation. Preservation injury damages the microvasculature of the peribiliary plexus. The transplant operation can injure the arterial blood supply and it can also disrupt the normal collateral circulation typical of the arterial cascade arrangement supplying all gastrointestinal organs, including the liver\(^{[49]}\). Interference with arterial flow at any level can result in “ischemic cholangitis” - a succinct phrase used to describe the common association between poor arterial flow and biliary ischemia that manifests as persistent ulcers, inflammation, sludge, and strictures\(^{[48,49]}\).

Cold ischemic-preservation injury depletes energy stores in microvascular endothelial cells and BEC. This results in activation of metalloproteinases, detachment of endothelium and BEC from the underlying matrix, and in the microvasculature, predisposition to thrombosis after reperfusion\(^{[50,51]}\). Reperfusion with blood after transplantation also delivers leukocytes that become activated by tissue damage. Activated leukocytes release effector molecules, which in turn, cause more tissue damage and further promote thrombogenesis\(^{[50,51]}\). Hydrophobic bile salts remaining in the biliary tree also further damage marginally viable BECs\(^{[50,52]}\) already weakened by preservation injury. Damaged BEC are sloughed into the bile\(^{[53]}\). Although patient and allograft survival during the first several weeks after transplantation are dependent primarily on parenchymal function, long term allograft viability is determined primarily by biliary wound healing and adequate bile drainage\(^{[54,55]}\). Allografts that eventually fail show biliary sludge, mucosal ulcers, and inflamed granulation tissue and myofibroblast activation/proliferation in the wall of extra-hepatic and large intrahepatic bile ducts\(^{[55]}\) (Figure 2). Exposure of the underlying stroma to bile appears to serve as a nidus for crystallization of biliary sludge and a stimulus for inflammation and activation of myofibroblasts. This leads to wound contraction and fibrosis, and eventually, to strictures in large caliber ducts and to luminal obliteration of smaller caliber ducts (Figure 2C).

Two observations illustrate the critical and primary importance to wound healing of an adequate arterial blood supply. First, any insult that directly interferes with the arterial flow is usually associated with large bile duct ulcers and strictures. Second, perfusion of the hepatic artery and peribiliary plexus with low viscosity preservation solutions before transplantation dramatically decreases the incidence of biliary complications after transplantation in otherwise susceptible extended criteria donor livers with long cold ischemic times\(^{[56]}\). This maneuver is thought to flush thrombogenic material from the peribiliary plexus and facilitate reperfusion and oxygenation after transplantation. As with many clinical observations, confirmation of this mechanism is lacking. But it is reasonable to conclude that without sufficient arterial flow biliary wound healing is
unlikely to proceed normally. Once adequate arterial flow is ascertained, other factors that also significantly contribute to BEC wound repair can then be studied in greater detail.

Deep wounds of extra-hepatic bile ducts precipitate stromal involvement in wound healing. Granulation tissue and inflammation, local production of interferon-γ[67] and transforming growth factor beta (TGF-β)[58] at this site are of importance in wound contraction and scarring. Our laboratory has focused primarily on BEC aspects of wound repair with an emphasis on interleukin-6/gp130 signaling-dependent cellular processes. This signaling pathway is also critically important for wound healing in the gastrointestinal tract[69] and skin[60,61].

**Cellular and molecular mechanisms of BEC repair involving IL-6/gp130 signaling**

IL-6/gp130 is pleiotropic cytokine signaling system that has diverse effects in many different organ systems and cell types(reviewed in[62,63]), gp130 is one of the most promiscuous cytokine receptors[62], binding to many different ligands including, interleukin (IL)-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and cardiotrophin-like cytokine (CLC)[62,63]. Ligand binding to gp130 generates two major signaling pathways: (1) Jak/STAT3 and (2) SHP2/ERK/MAPK and there is reciprocal negative feedback regulation between them[64,65]. Non-canonical STAT3 signaling pathways also exist, but are not well delineated[66].

In normal livers, IL-6 is produced at low levels by the BEC, perhaps stimulated by the bile salts[59], and secreted into the bile[60]. Active IL-6/gp130/STAT3 signaling can be detected in normal IL-6+/+ but not in normal IL-6−/− mice livers, as evidenced by the detection of phospho-STAT3 by Western blotting and immunohistochemistry. Biliary tree pSTAT3 in normal liver localizes to occasional BEC lining large bile ducts, but more prevalent expression is seen in BEC lining the peribiliary glands[11].

Virtually any bile duct insult, such as obstruction[67-69], infection[69,70] or immunologic damage[66,71,72], triggers sharp increases in IL-6 mRNA and protein production by BEC and peribiliary hematomelymphoid cells[9]. This, in essence, alerts BEC to environmental stimuli and leads to subsequent autocrine, paracrine, and juxtacrine gp130/STAT3 signaling in BEC at the sites of injury[11,67,68,71]. As in the gastrointestinal tract[69] and skin[60,61], an absence of IL-6 in IL-6-deficient (IL-6−/−) mice leads to impaired wound healing[81] and biliary tree integrity[61,73-76]. For the last several years our laboratory has focused on cellular and molecular mechanisms that might contribute to impaired BEC wound healing and biliary barrier defects in the IL-6−/− mice and how the findings might apply to humans. Using a combination of knowledge gained from the gastrointestinal tract and skin, mRNA microarray expression analyses, and pathophysiology studies, we first searched for genes that were: (1) expressed in BECs, (2) regulated by IL-6/gp130/STAT3 signaling, and (3) possibly involved in barrier function and/or repair. Two of the most interesting candidates, studied in greater detail, included intestinal trefoil family factors (TFF)[59,75-78] and small protein rich proteins (SPRR)[11,73]. The reader is referred to recent reviews of IL-6/gp130/ SHP2/ERK/MAPK signaling and other growth factors and cytokines involved in the regenerative phase of BEC wound healing[56,67,69,70-81].

Trefoil family factor (TFF) proteins are comprised of one or more trefoil motifs, which consist of 6 cysteine residues. TFF proteins increase mucous viscosity and thereby contribute to optimal protection of the intestinal mucosa from injury[73,82-84]. By enhancing intestinal epithelial spreading and migration, TFF proteins also stimulate the restitution phase of wound healing[76,85]. Each of the three known TFF proteins is differentially regulated in the gastrointestinal tract[69,73]: TFF1 and TFF2 are expressed primarily in the stomach[80,87]. TFF3 predominates in the small and large intestines[70] and in the biliary tree[11] of mouse and human livers[88-91]. IL-6/gp130/STAT3 signaling is crucial for BEC TFF3 expression. In normal liver, pSTAT3 and TFF3 mRNA and protein expression are significantly higher in IL-6+/+ than in IL-6−/− mice livers. Constitutive expression of TFF3 and pSTAT3 localizes to mucin secreting BEC lining large intrahepatic and extrahepatic bile ducts and peribiliary glands[11]. Medium and small-sized intra-hepatic bile ducts are generally negative for mucin secreting BEC, pSTAT3, and TFF3 expression[11,91,92].

IL-6+/+ BEC consistently show higher levels of TFF3 mRNA and protein expression and significantly better migration and wound healing than IL-6−/− BEC[11], in vitro. Defective migration in the IL-6−/− BEC can be partially, but significantly, reversed by treatment with recombinant TFF peptides[11]. In vivo, biliary TFF3 is dynamically regulated by various factors after bile duct ligation. Included are the reciprocal negative regulation known to exist between the STAT3 and MAPK signaling pathways[9], and other cytokines and growth factors, such as HGF and TGF-β, which can down-regulated BEC TFF3 expression[11]. However, a chronic deficiency of pSTAT3 signaling during bile duct injury, as seen in IL-6−/− mice after bile duct ligation, leads to a chronic deficiency of biliary TFF3 expression and impaired biliary barrier function[11]. In humans, p-STAT3 and TFF3 are newly co-expressed in BEC involved in florid duct lesions in primary biliary cirrhosis and at other sites of BEC injury, but not in similarly-sized normal bile ducts from the same livers[11,89,92]. This likely constitutes a primitive or innate mucosal defense system that guards against injury and stimulates repair.

Our BEC TFF3 studies are consistent with studies focused on the colon and carried out in mice harboring mutations that selectively block all gp130-mediated STAT activity (gp13087-10), but preserve gp130-mediated MAPK signaling. These mice show decreased colonic TFF3 expression, increased sensitivity to sodium dextran sulfate-induced colitis, and impaired mucosal wound healing[59,70]. Thus, it is reasonable to conclude that IL-6/gp130/ STAT3 signaling contributes significantly to normal BEC cytoprotective mechanisms and to migration during wound healing, at least in part, by stimulating BEC TFF3 expression[11].

Small proline-rich proteins (SPRR) are encoded by a tandemly arranged four-member gene family.
contained within a 170-kilobase region of the epidermal differentiation complex (EDC). The EDC is a cluster of more than 50 genes located on chromosome 1q23[93,94] whose products are involved in terminal differentiation of the human epidermis. Included are formation of the cornified envelope that is an effective barrier against the external environment[95,96]. The four SPRR gene families, SPRR1-4, are distinguished on the basis of the number of amino acids in the repeats of the protein's central domain and the consensus of that sequence[98]. There are two Sprr1 genes and one copy each of Sprr3 and Sprr4 genes[98]. SPRR2A genes are the most diversified family; there are seven in humans and eleven in mice[99]. In the skin and other squamous epithelia, SPRR genes are usually regulated coordinately as part of the EDC (i.e. high expression of most EDC genes, as in papillomas, or very low expression of most genes, as in newborn skin). SPRR genes encode for a series of highly homologous proteins that function primarily as critical cross-linkers. They form bridges among other EDC proteins, intermediate filaments, and cornified envelope constituents[94,95,97], such as desmoplakin, loricrin, and trichohyalin, through the catalytic action of transglutaminases[98].

The diverse SPRR2 genes are also non-coordinately expressed, or expressed preferentially, without similar upregulation of other EDC family members[73,97]. This occurs most commonly in non-keratinizing epithelia in curious situations that cannot be explained by squamous differentiation or formation of a cornified envelope. Examples include greater than 100-fold increases of SPRR2A in the intestine after small bowel resection[99] or after introduction of commensal bacteria into germ-free mice[100,101] or after intentional infection with intestinal parasites[102]. In uterine epithelium SPRR2A mRNA and protein expression is, at least in part, regulated by estrogen[103]. Therefore, it is highly and non-coordinately upregulated during certain stages of the oestrous cycle[104,105] and is especially high at the blastocyst implantation site[103]. SPRR2A mRNA and protein are also expressed in bronchial and intestinal epithelium during allergic reactions[106]. Barrier remodeling, as a response to stress[94,105], inflammation, and/or growth, is a common condition of these diverse circumstances. Potential molecular and cellular processes affected by non-coordinate SPRR2A expression are currently under investigation in our laboratory.

In the liver, we have shown that SPRR2A mRNA and protein are not expressed in normal mouse liver, but are non-coordinately upregulated only in BEC after the stress of bile duct ligation[73]. Expression after bile duct ligation is not related to squamous metaplasia and shows strong dependence on IL-6/gp130/STAT3 signaling. In BEC lining the large bile ducts, SPRR2 protein localizes subjacent to the apical plasma membrane. SPRR2 expression is more diffusely distributed throughout the cytoplasm of cholangiocytes participating in ductular reactions and in large duct BEC engaged in the restitution phase of mucosal wound healing. Deficient BEC SPRR2A expression in IL-6−/− mice after bile duct ligation is associated with impaired barrier function[73]. IL-6 replacement therapy restores SPRR2A expression to levels seen in wild type controls and reverses the barrier defect in IL-6−/− mice. In a series of ongoing investigations, preliminary data suggest that BEC SPRR2A expression is associated with BEC restitution.

**WOUND HEALING IN SMALL INTRA-HEPATIC BILE DUCTULES-DUCTULAR REACTIONS**

Wound healing responses can be triggered in BEC lining the smallest intrahepatic bile ducts by environmental changes other than, or in addition to, direct injury and ulceration. This often occurs in chronic necro-inflammatory liver disease regardless of the underlying cause. For several years we were puzzled by the observation that ductular reactions represent a survival advantage for BEC and myofibroblasts over hepatocytes, yet hepatocytes and BEC share the same responses to many cytokines and growth factors that are upregulated in chronic liver disease (e.g. HGF, EGF, IL-6, TGFβ, etc[81,107]). Why then do BEC and myofibroblasts survive preferentially under these circumstances? To answer this question it is helpful to view chronic necro-inflammatory liver disease as a “Darwinian” selection pressure applied to the liver. A survival advantage for BEC can occur because of a relative increase in the rate of proliferation, a relative decrease in the rate of death, transformation of hepatocytes into BEC, or various combinations of the above. Regardless of the mechanism, the end result is a relative decrease in volume percentage of hepatocytes and a relative increase in biliary epithelial cells and myofibroblasts-a pattern typical of evolving cirrhosis[17,14]. When combined with sufficient time[82,83], even a small deficit of hepatocyte survival is enough to evoke a ductular reaction that distorts the hepatic architecture.

Using an established mouse model of decompensated biliary cirrhosis[70] and p21-deficient mice, we tested the hypothesis that hepatocyte mito-inhibition combined with the regenerative stimulus of bile duct ligation would accentuate the ductular reaction and accelerate architectural distortion. Results showed that after long-term (12-wk) ligation mice prone to downregulation show significantly more oxidative stress and hepatocyte nuclear p21 expression, a cyclin dependent kinase inhibitor and important mediator of hepatocyte mito-inhibition[11]. As expected, mice prone to downregulation also showed less hepatocyte proliferation, an exaggerated ductular reaction, and accelerated architectural distortion compared with compensation-prone controls[8]. We next subjected p21 deficient mice to bile duct ligation for 12 wk with the expectation that p21 deficient mice would be better able than wild-type controls to compensate for long-term BDL because of significantly greater hepatocyte proliferation. Indeed, results of these experiments showed that p21-deficient mice showed a larger liver mass because of more hepatocyte proliferation, a less florid ductular reaction, and less architectural distortion than wild type controls[8].

We next wanted to determine whether this concept was applicable to other, non-cholestatic or non-biliary, liver diseases. To accomplish this task, we first showed that hepatocyte nuclear p21 expression in humans awaiting liver replacement directly correlated with pathological disease stage and model of end-stage liver disease scoring[11]. We
also engaged in a collaborative study with Clouston et al who had previously shown that HCV-related liver disease progresses more rapidly when there is co-existent hepatic steatosis[108]. Liver biopsies from 115 patients with HCV scored for steatosis, inflammation, and fibrosis showed a strong correlation between (a) a ductular reaction and portal fibrosis and (b) steatosis and impaired hepatocyte replication[2]. Steatosis correlated with the ductular reaction and greater hepatic progenitor cell proliferation, but was not an obligate feature. The highly significant correlation between the ductular reaction area and fibrosis stage remained even after multivariate analysis[9]. Impaired hepatocyte replication, as determined by p21 expression, was independently associated with hepatic progenitor cell expansion, increased body mass index, and lobular inflammation.

The observation that ductular reactions often appear when hepatocyte mito-inhibition is combined with a liver regenerative stimulus is not new. It was made originally years ago while treating experimental animals with genotoxic carcinogens and then subjecting them to partial hepatectomy (reviewed in[114,115]). These maneuvers stimulate oval cell, or liver epithelial progenitor cell[115] expansion/proliferation at the interface zone-a ductular reaction. However, in carcinogenesis experiments, the ductular reaction eventually resolves without fibrosis.

Our studies show that this concept is applicable to ductular reactions associated with chronic necro-inflammatory liver diseases and the development of fibrosis/cirrhosis[1] (Figure 3). Hepatocytes are more susceptible to injury and mito-inhibition during chronic necro-inflammatory liver disease because they: (1) produce and secrete bile and are the major site of bile stasis; (2) are more complex metabolically and able preferentially to store lipids and metals, such as iron and copper, which are generators and/or catalysts for free oxygen radical formation; and, (3) support HBV and HCV replication, and (4) maybe most importantly, contain many more mitochondria than BEC and mitochondria are the major site of superoxide production[118]. Diverse disorders such as cholestasis[3], HCV replication[119,113], steatosis[2,115], copper deposition[116] and alcohol[117] preferentially stress or injure hepatocytes and this causes nuclear expression of the cyclin-dependent kinase inhibitor, p21[2,12], which in turn, inhibits hepatocyte proliferation.

When small intrahepatic bile ductules are destroyed (ductopenia), due to drugs or chronic allograft rejection, classical cirrhosis usually does not develop[45,109,118,119]. Despite ongoing immunologic liver injury and fibrosis, regenerative nodularity and the associated complications of portal hypertension rarely occur. BEC survival and proliferation in response to injury in these small ductules is related to a combination of immunologic injury, environmental influences, and importantly, arterial blood flow[123], as in the large bile ducts. Perhaps the arterial disease also inhibits regenerative nodule formation (Figure 4).

PROGENITOR CELL ACTIVATION, PERSISTENT WOUND REPAIR AND CANCER

Persistent ductular reactions in human chronic necro-inflammatory diseases can activate progenitor cell populations, as in the experimental animal carcinogenesis models, discussed above (Reviewed in refs 38, 39, 41). Several groups, including ours, have shown that oval cell expansion in mice is dependent significantly on IL-6/gp130/STAT3 signaling[20,122]. This raises the possibility that ductular reactions accelerate the development of cirrhosis and potentially increase the risk of hepatocellular carcinoma. Since oval cells eventually differentiate into hepatocytes[115], exposure to carcinogens or genotoxic damage from oxidative stress imprint genetic mutations in putative liver stem cells. These cells then divide, differentiate, and spread initiated cells more widely throughout the entire hepatocyte population. Liver cancers occur when initiated hepatocytes are subjected to tumor promoters that generally cause hepatocyte proliferation. Chronic liver disease is an excellent cancer-promoting environment.

In the skin, STAT3 signaling enables epidermal stem cells to the escape apoptosis induced by exposure to cutaneous carcinogens[123]. Initiated stem cells survive, divide, differentiate, and subsequently give rise to skin cancers in a promoting environment[23]. Similar processes might occur in hepatocellular and cholangiocarcinomas. Interleukin-6/gp130/STAT3 signaling might provide important survival signals for initiated liver epithelial progenitor cells that later give rise to liver cancers in the context of chronic necro-inflammatory disease. In evolutionary biology[124] this process is referred as “antagonistic pleiotropy” - a short term survival benefit at the expense of long-term increased risk of cancer.

IL-6/gp130/STAT3 signaling is indeed increased in a very wide variety of neoplasms, including hepatocellular carcinomas, melanomas, leukemias and myelomas, and
lung, breast cancer, kidney, prostate, pancreatic, colon, gastric, cervical, ovarian, and head and neck cancers[125,126]. This signaling pathway participates and/or regulates many pathways important in oncogenesis including cell-cycle progression, apoptosis, tumor angiogenesis, tumor-cell invasion and metastasis, and tumor-cell evasion of the immune system (reviewed in[125,126]).

Whether this concept is applicable to cancers arising in extrahepatic and large intrahepatic bile ducts is uncertain because the mechanisms of BEC renewal at these sites have not been studied in any great detail. In the larger bile ducts there are two potential sources of new BEC: (1) proliferation of mature BEC; and (2) proliferation and/or maturation of progenitor cell populations. These potential sources are not mutually exclusive and only one study in the literature even indirectly addresses this topic. Koike et al[127] used pulse and continuous DNA labeling studies to show, in rats, that a proliferative zone, consistent with a BEC progenitor cell population, localized to peribiliary glands (called “crypts” in their study). Long-term follow-up of the animals showed that labeled BEC migrated gradually to the surface and were shed into the lumen with a transit time of about 30-40 d[127]. They concluded the arrangement and pattern of BEC renewal in the extra-hepatic bile ducts was similar to the intestine and colon, but kinetics of BEC turnover was slower[127]. More definitive work is needed in this area.

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

The following are a few examples of the many unanswered questions in the study of biliary wound healing that have stimulated research in our laboratory. For example, are there progenitor cell populations within the peribiliary glands or crypts of extra-hepatic and large intra-hepatic bile ducts? If progenitor cells exist in the extra-hepatic biliary tree, where exactly do they reside, and how are they recognized? Are they activated during wound repair, and do they contribute to the development of bile duct cancers?

EMT contributes significantly to wound healing and to kidney fibrogenesis. Is EMT an important process in BEC wound healing and hepatic fibrogenesis? Can, and do, BEC transform into myo-(fibro)blasts? BEC appear to migrate and can acquire mesenchymal characteristics during the ductular reaction and hepatic fibrogenesis. They transform from polarized cuboidal epithelial cells into a spindle or dendritic-shaped vimentin-positive cells after bile duct ligation and in PBC[128,129]. In embryonic liver, ductal plate BEC “invade” or migrate into the portal tract connective tissue to form mature intrahepatic bile ducts. During migration BEC express vimentin[130] and down-regulate membranous E-cadherin[131] expression. Once mature bile ducts are formed BEC revert totally to an epithelial phenotype. Preliminary studies from our laboratory suggest that IL-6/gp130/STAT3 signaling triggers BEC changes under some circumstances that can induce BEC EMT. The extent to which EMT contributes to BEC wound healing, however, requires further study. Several studies show that IL-6 pre-treatment can prevent liver parenchymal ischemic-preservation injury[132-136]. We have shown similar results in intestinal allografts: IL-6 pretreatment limits epithelial damage and promotes repair[137]. Would pretreatment of donor livers with IL-6, particularly in the aortic flush, have beneficial effects on preservation injury of the peribiliary plexus and promote wound healing in the biliary tree? We would expect IL-6/gp130 signaling to help lessen the incidence of biliary strictures because it upregulates anti-apoptotic molecules in the microvascular endothelium[133,136], preserves epithelial integrity[11,137], and stimulates BEC restitution[11,73] and regeneration[79] after injury.

Several other strategies might be used to prevent biliary strictures[138,139], such as reducing eosinophil and mast cell accumulation with Tranilast[140,141] and Captopril[142,144], reducing activation and transformation of myofibroblasts with anti-oxidants such alpha tocopherol (vitamin E) and peroxisome proliferator-activated receptors-γ (PPAR-γ) ligands, such as the thiazolidinedione family of drugs[145-147].

REFERENCES

1 Lunz JG 3rd, Tsuji H, Nozaki I, Murase N, Demetris AJ. An inhibitor of cyclin-dependent kinase, stress-induced p21Waf-1/Cip-1, mediates hepatocyte mito-inhibition during the evolution of cirrhosis. Hepatology 2005; 41: 1262-1271

www.wjgnet.com
2 Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology* 2005; 41: 809-818

3 Demetris AJ, LaRusso NF, Gores GJ, Starzl TE. Replicative senescence of biliary epithelial cells in human liver in hepatic injury. *Am J Pathol* 2002; 161: 173-179

4 lng D, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* 2003; 4: 721-732

5 Jacinto A, Martin P. Morphogenesis: unravelling the cell biology of cell contact. *Cytobiol Cell Biol* 2003; 31: S532-S537

6 Carlson MA, Longaker MT. The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. *Wound Repair Regen* 2004; 12: 134-147

7 Hinz B, Gabbiani G. Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. *Thromb Haemost* 2003; 90: 993-1002

8 Nozaki I, Lanzu-Grandi A, Maimon DB. Mechanisms of epithelial fusion and repair. *Nat Cell Biol* 2001; 3: E117-E123

9 Poli G, Parola M. Oxidative damage and fibrogenesis. *Free Radic Biol Med* 1997; 22: 287-305

10 Redd MJ, Cooper L, Wood W, Stramer B, Martin P. Wound healing and inflammation: embryos reveal the way to perfect repair. *Philos Trans R Soc Lond B Biol Sci* 2004; 359: 779-784

11 Mammen JM, Matthews JB. Mucosal repair in the gastrointestinal tract. *Crit Care Med* 2003; 31: S532-S537

12 Kalluri R, Madri JA. Portal tract fibrogenesis in the liver: correlation between portal myofibroblasts and development of intrahepatic bile ducts and arterial branches in human liver. *Liver* 2002; 22: 252-258

13 Kinnunen P, Broscoz C, Barbou V, Wendum D, Rey C, Hultcrantz R, Poupon R, Housset C. The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis. *Lab Invest* 2003; 83: 163-173

14 Rickert F, Fuchsbiichler A, Wagner M, Zollner G, Kaser A, Tilg H, Krause R, Lammert F, Langner C, Zatloukal K, Marschall HU, Dekh H, Trauner M. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abx4) knockout mice. *Gastroenterology* 2004; 127: 261-274

15 Ramadori G, Saile B. Portal tract fibrogenesis in the liver. *Lab Invest* 2004; 84: 153-159

16 Jhander MN, Kruglov EA, Lavoie EG, Sévigny J, Dranoff JA. Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPase D2. *J Biol Chem* 2005; 280: 29999-29992

17 Sobesky R, Chollet JM, Prat F, Karkouche B, Pelletier G, Fritsch J, Choury AD, Allonier C, Bedossa P, Buffet C. Inflammatory pseudotumor of the common bile duct. *Endoscopy* 2003; 35: 698-700

18 Dehner LP, Coffin CM. Idiopathic fibrosclerotic disorders and other inflammatory pseudotumors. *Semin Diagn Pathol* 1998; 15: 161-173

19 Inaba K, Suzuki S, Yokoi Y, Ota S, Nakamura T, Konno H, Baba S, Takehara Y, Nakamura S. Hepatic inflammatory pseudotumor mimicking intrahepatic cholangiocarcinoma: report of a case. *Surg Today* 2003; 33: 714-717

20 Yamamoto K, Phillips MJ. Three-dimensional observation of the intrahepatic lymphatics by scanning electron microscopy of corrosion casts. *Lab Invest* 1984; 49: 67-70

21 Szabó G, Magyar Z, Szemirsák A, Jakab F, Mihály K. Bile constituents in blood and lymph during biliary obstruction. II. The absorption and transport of bile acids and bilirubin. *Lymphology* 1975; 8: 36-42

22 Tsuboi K, Tazuma S, Nishioka T, Chayama K. Partial charac
terization of cytoprotective mechanisms of leukin against bile salt-induced bile duct damage. *J Gastroenterol* 2004; 39: 955-960

23 Barone M, Maiorano E, Ladisa R, Pecce A, Berloco P, Strazzabosco M, Caruso ML, Valenti AM, Jerardi E, Di Leo A, Fra
cavilla A. Ursodeoxycholate further increases bile-duct cell proliferative response induced by partial bile-duct ligation in rats. *Vireochs Arch* 2004; 444: 554-560

24 Patel T, LaRusso NF, Gores GJ. Interleukin-6 suppresses chol
gangioctye apoptosis by down-regulation of Bax. *Hepatology* 1997; 26: 226A

25 Que FG, Gores GJ, LaRusso NF. Development and initial ap
plication of an in vitro model of apoptosis in rodent cholan
giocytes. *Am J Physiol* 1997; 272: G106-G115

26 Komichi D, Tazuma S, Nishioka T, Hyogo H, Une M, Chayama K. Unique inhibition of bile salt-induced apoptosis by leckithins and cytoprotective bile salts in immortalized mouse cholangioctyes. *Dig Dis Sci* 2003; 48: 2315-2322

27 Wernerb NW, Yoon JH, Higuchi H, Gores GJ. Bile acids ac
tivate EGFR receptor via a TGF-alpha-dependent mechanism in human cholangioctye cell lines. *Am J Gastroenterol Gastrointest Liver Physiol* 2003; 285: C31-C36

28 Yoon JH, Higuchi H, Wernerb NW, Kaufmann SH, Gores GJ. Bile acids induce cytoxygenase-2 expression via the epi
dermal growth factor receptor in a human cholangiocarcinoma cell line. *Gastroenterology* 2002; 122: 985-993

29 Lamiere T, Zoltowska M, Levy E, Yousef I, Rosenbaum J, Tuchweber B, Desmoulière A. Effects of bile acids on biliary epithelial cells: proliferation, cytotoxicity, and cytokine secretion. *Life Sci* 2003; 72: 1401-1411

30 Kristiansen T2, Bunkenborg J, Gronborg M, Molina H, Thtu
luth PA, Arzini P, Goggin MG, Maitra A, Pandey A. A proteomic analysis of human bile. *Mol Cell Proteomics* 2004; 3: 715-728

31 Paumgartner G, Beuers U. Ursodeoxycholic acid in cholesta
tic liver disease: mechanisms of action and therapeutic use revis
ted. *Hepatology* 2002; 36: 529-531

32 Colombo C, Cossignani A, Assasao M, Battezzati PM, Podda M, Giunta A, Zimmer-Nechemas L, Setchell KD. Ursodeoxy
colic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. *Hepatology* 1992; 16: 924-930

33 Strain AJ, Crosby HA, Nijjar S, Kelly DA, Hubscher SG. Hu
man liver-derived stem cells. *Semin Liver Dis* 2003; 23: 373-384

34 Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in dis
eyed human liver. *Semin Liver Dis* 2003; 23: 385-396

35 Saxena R, Theise N. Canals of Hering: recent insights and cur
rent knowledge. *Semin Liver Dis* 2004; 24: 43-48

36 Thorgeirsso NS, Grisham JW. Overview of recent experimental studies on liver stem cells. *Semin Liver Dis* 2003; 23: 303-312

37 POPPER H, KENT G, STEIN R. Ductular cell reaction in the liver in hepatic injury. *J Mt Sinai Hosp N Y* 1957; 24: 551-556

38 Demetris AJ, Nakamoto T, Liu Z, Yokomuro S, Ezure T, Murase N, et al. The ductular reaction in liver disease emphasis on a type I response. In: Fleig WE, editors. Normal vs. Pathologic repair and inflammation: embryos reveal the way to perfect repair. Philos Trans R Soc Lond B Biol Sci 2004; 359: 779-784

39 Mammen JM, Matthews JB. Mucosal repair in the gastrointestinal tract. *Crit Care Med* 2003; 31: S532-S537

40 Carlson MA, Longaker MT. The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. *Wound Repair Regen* 2004; 12: 134-147

41 Hinz B, Gabbiani G. Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. *Thromb Haemost* 2003; 90: 993-1002

42 Nozaki I, Lanzu-Grandi A, Maimon DB. Mechanisms of epithelial fusion and repair. *Nat Cell Biol* 2001; 3: E117-E123

43 Poli G, Parola M. Oxidative damage and fibrogenesis. *Free Radic Biol Med* 1997; 22: 287-305

44 Redd MJ, Cooper L, Wood W, Stramer B, Martin P. Wound healing and inflammation: embryos reveal the way to perfect repair. *Philos Trans R Soc Lond B Biol Sci* 2004; 359: 779-784

45 Mammen JM, Matthews JB. Mucosal repair in the gastrointestinal tract. *Crit Care Med* 2003; 31: S532-S537
Demetris A, Crawford J, Nalesnik M, Randhawa P, Wu T, Minervini M. Transplantation Pathology of the Liver. In: Odze RD, Goldblum JR, Crawford JM, editors. Surgical Pathology of the GI Tract, Liver, Biliary Tract, and Pancreas. Philadelphia: WB Saunders, 2004: 909-966.

Vanless I. Physioanatomic Considerations. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Diseases of the Liver. Philadelphia: Lippincott Williams & Wilkins, 1999: 3-37.

Demetris AJ. Ischemic cholangitis. Mayo Clin Proc 1992; 67: 601-602.

Ludwig J, Batts KP, MacCarty RL. Ischemic cholangitis in hepatic allografts. Mayo Clin Proc 1992; 67: 519-526.

Kukan M, Haddad PS. Role of hepatocytes and bile duct cells in preservation-reperfusion injury of liver grafts. Liver Transpl 2001; 7: 381-400.

Teoh NC, Farrell GC. Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol 2003; 18: 891-902.

Noack K, Bronk SF, Kato A, Gores GJ. The greater vulnerability of bile duct cells to reoxygenation injury than to anoxia. Implications for the pathogenesis of biliary strictures after liver transplantation. Transplantation 1993; 56: 495-500.

Carrasco L, Sanchez-Bueno F, Sola J, Ruiz JM, Ramirez P, Robles R, Rodriguez JM, Parrilla P. Effects of cold ischemia time on the graft after orthotopic liver transplantation. A bile cytological study. Transplantation 1996; 61: 393-396.

Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. Liver Transpl 2003; 9: 651-663.

Demetris AJ, Fontes P, Lunz JG, Specht S, Murase N, Marcos A. Wound healing in the biliary tree of liver allografts. Cell Transplant 2006; 15 Suppl 1: S57-S65.

Pirenne J, Gunson B, Khaleef H, Hubscher S, Afford S, McLaren P. The role of ischemia-reperfusion injury on rejection after liver transplantation. Transplant Proc 1997; 29: 366-367.

Shivakumar P, Campbell KM, Sabla GE, Mieathe A, Tiao G, McNeal MM, Ward RL, Bezerra JA. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFNgamma in experimental biliary atresia. J Clin Invest 2004; 114: 322-329.

Geng ZM, Yao YM, Liu QG, Niu XJ, Liu XG. Mechanism of benign biliary stricture: a morphological and immunohistochemical study. World J Gastroenterol 2005; 11: 295-295.

Tebbutt NC, Giraud AS, Inglese M, Jenkins B, Waring P, Clay PJ, Malki S, Alderman BM, Grail D, Hollande F, Heath JK, Ernst M. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 deficient mice. Cell Death Differ 2003; 10: 1592-1601.

Demetris AJ, Fontes P, Lunz JG, Specht S, Murase N, Marcos A. Wound healing in the biliary tree of liver allografts. Cell Transplant 2006; 15 Suppl 1: S57-S65.

Pirenne J, Gunson B, Khaleef H, Hubscher S, Afford S, McLaren P. The role of ischemia-reperfusion injury on rejection after liver transplantation. Transplant Proc 1997; 29: 366-367.

Shivakumar P, Campbell KM, Sabla GE, Mieathe A, Tiao G, McNeal MM, Ward RL, Bezerra JA. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFNgamma in experimental biliary atresia. J Clin Invest 2004; 114: 322-329.

Geng ZM, Yao YM, Liu QG, Niu XJ, Liu XG. Mechanism of benign biliary stricture: a morphological and immunohistochemical study. World J Gastroenterol 2005; 11: 295-295.

Tebbutt NC, Giraud AS, Inglese M, Jenkins B, Waring P, Clay PJ, Malki S, Alderman BM, Grail D, Hollande F, Heath JK, Ernst M. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 deficient mice. Cell Death Differ 2003; 10: 1592-1601.

Lin QZ, Kondo T, Ishida Y, Takayasu T, Mukaida N. Essential role of vascular cell adhesion molecule-1 in the ductular reaction in mice: interactions between the periductal inflammatory and stromal cells and the biliary epithelium. Hepatology 1998; 28: 1260-1268.

Li Z, Sakamoto T, Ezure T, Yokomuro S, Murase N, Michalopoulos G, Demetris AJ. Interleukin-6, hepatocyte growth factor, and their receptors in biliary epithelial cells during a type I ductular reaction in mice: interactions between the periductal inflammatory and stromal cells and the biliary epithelium. Hepatology 1998; 28: 1260-1268.

Liu Z, Sakamoto T, Yokomuro S, Ezure T, Subbotin V, Murase N, Contrucci S, Demetris AJ. Acute obstructive cholangiopatya in interleukin-6 deficient mice: compensation by leukemia inhibitory factor (LIF) suggests importance of gp-130 signaling in the ductular reaction. Liver 2000; 20: 114-124.

Rosen HR, Winkle PJ, Kendall BJ, Diehl DL. Biliary interleukin-6 and tumor necrosis factor-alpha in patients undergoing endoscopic retrograde cholangiopancreatography. Dig Dis Sci 1997; 42: 1290-1294.

Scottie M, Daveau M, Hiron M, Delers F, Lemel JD, Teniere P, Lebreton JP. Interleukin-6 (IL-6) and acute-phase proteins in rats with biliary sepsis. Eur Cytohokine Netw 1991; 2: 177-182.

Kimmings AN, van Deventer SJ, Obertop H, Rauws EA, Huibregtse K, Gouma DJ. Endotoxin, cytokines, and endotoxin binding proteins in obstructive jaundice and after preoperative biliary drainage. Gut 2000; 46: 725-731.

Akiyama T, Hasegawa T, Seijima T, Sahara H, Seto K, Saito H, Takashima S. Serum and bile interleukin 6 after percutaneous transhepatic cholangio-drainage. Hepatogastroenterology 1998; 45: 665-671.

Nozaki I, Lunz JG 3rd, Specht S, Stolz DB, Taguchi K, Subbotin VM, Murase N, Demetris AJ. Small proline-rich proteins 2 are noncoordinately upregulated by IL-6/STAT3 signaling after bile duct ligation. Lab Invest 2005; 85: 109-123.

Ezure T, Sakamoto T, Tsuji H, Lunz JG 3rd, Murase N, Fung JJ, Demetris AJ. The development and compensation of biliary cirrhosis in interleukin-6-deficient mice. Am J Pathol 2000; 156: 1627-1639.

Ernst M, Inglese M, Waring P, Campbell IK, Bao S, Clay FJ, Alexander WS, Wicks IP, Tarlinton DM, Novak U, Heath JK, Dunn AR. Defective gp130-mediated signal transducer and activator of transcription (STAT) signaling results in degenerative joint disease, gastrointestinal ulceration, and failure of uterine implantation. J Exp Med 2001; 194: 203-209.

Kanai M, Mullen C, Podolsky DK. Intestinal trefoil factor induces inactivation of extracellular signal-regulated protein kinase in intestinal epithelial cells. Proc Natl Acad Sci U S A 1998; 95: 178-182.

Mashimo H, Wu DC, Podolsky DK, Fishman MC. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science 1996; 274: 262-265.

Podolsky DK, Lynch-Devaney K, Stow JL, Oates P, Murgue B, De Beaumont M, Sands BE, Mahida YR. Identification of human intestinal trefoil factor. Goblet cell-specific expression of a peptide targeted for apical secretion. J Biol Chem 1993; 268: 6694-6702.

Matsumoto K, Fuji H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epithelial growth factor promote DNA synthesis in vitro. Hepatology 1994; 20: 376-382.

Yokomuro S, Lunz JG 3rd, Sakamoto T, Ezure T, Murase N, Demetris AJ. The effect of interleukin-6 (IL-6)/gp130 signaling on biliary epithelial cell growth, in vitro. Cytokine 2000; 12: 727-730.

Yokomuro S, Tsuji H, Lunz JG 3rd, Sakamoto T, Ezure T, Murase N, Demetris AJ. Growth control of human biliary epithelial cells by interleukin 6, hepatocyte growth factor, transforming growth factor beta1, and activin A: comparison of a cholangiocarcinoma cell line with primary cultures of non-neoplastic biliary epithelial cells. Hepatology 2000; 32: 26-35.

Tomasetto C, Mason R, Linares JL, Wendling C, Lefebvre O, Chenard MP, Rio MC. p52/TFII interacts directly with the WWF cysteine-rich domains of munc18. Gastroenterology 2000; 118: 70-80.

Gun JR, Hicks JW, Gillespie AM, Carlson EJ, Kottmives L, Karmik S, Hong JC, Epstein CJ, Kim YS. Goblet cell-specific expression mediated by the MUC2 mucin gene promoter in the
Demetris AJ et al. Biliary wound healing, ductular reactions, and IL-6/gp130 signaling

3521

intestine of transgenic mice. Am J Physiol 1999; 276: G666-G676

84 Kindh N, Pottholakis C, Thim L, Lynch-Danevant K, Podelsky DK. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. Gut 1995; 39: 516-522

85 Dignass A, Lynch-Danevant K, Kindh N, Thim L, Podelsky DK. Trefoil peptides promote epithelial migration through a transforming growth factor beta-independent pathway. J Clin Invest 1994; 94: 376-383

86 Tomasetto C, Rio MC, Gautier C, Wolf C, Hareveni M, Chambon P, Lathe R. IFP, the domain-duplicated homolog of p52 protein, is co-expressed with p52 in stomach but not in breast carcinoma. EMBO J 1990; 9: 367-372

87 Rio MC, Belloq JP, Daniel JY, Tomasetto C, Lathe R, Chenard MP, Batzenschlagler A, Chambon P. Breast cancer-associated p52 protein: synthesis and secretion by normal stomach mucosa. Science 1988; 241: 705-708

88 Hertel SC, Chwieralski CE, Hinz M, Rio MC, Tomasetto C, Hoffmann W. Profiling trefoil factor family (TFF) expression in the mouse: identification of an antisense TFF1-related transcript in the kidney and liver. Peptides 2004; 25: 755-762

89 Sasaki M, Tsurenaya K, Saito T, Katoaka H, Mollenhauer J, Poustak A, Nakanuma Y. Site-characteristic expression and induction of trefoil factor family 1, 2 and 3 and malignant brain tumor-1 in normal and diseased intrahepatic bile ducts relates to biliary pathophysiology. Liver Int 2004; 24: 29-37

90 Sasaki M, Tsurenaya K, Nakanuma Y. Aberrant expression of trefoil factor family 1 in biliary epithelium in hepatolithiasis and cholangiocarcinoma. Lab Invest 2003; 83: 1403-1413

91 Sivastava G, Giraud AS, Ulaganathan M, Yeomans ND, Dow C, Nicoll AJ. Biliary epithelial trefoil peptide expression is increased in biliary diseases. Histopathology 2002; 40: 261-268

92 Kimura Y, Leung PS, Kenny TP, Van De Water J, Nishioka M, Giraud AS, Neuberger J, Benson G, Kaul R, Ansari AA, Coppell RL, Gershwin ME. Differential expression of intestinal trefoil factor in biliary epithelial cells of primary biliary cirrhosis. Hepatology 2002; 36: 1227-1235

93 Marenholz I, Zirra M, Fischer DF, Backendorf C, Ziegler A, Mischke D. Identification of human epidermal differentiation complex (EDC)-encoded genes by subtractive hybridization of entire YACs to a gridded keratinocyte cDNA library. Genome Res 2001; 11: 341-355

94 Cabral A, Voskamp P, Cleton-Jansen AM, South A, Nizetic D, Backendorf C. Structural organization and regulation of the small proline-rich family of cornified envelope precursors sug- gest a role in adaptive barrier function. J Biol Chem 2001; 276: 19231-19237

95 Patel S, Kartasova T, Segre JA. Mouse Sprr locus: a clustered family of genes encoding structural proteins of epidermal cornification and 5100 calcium-binding proteins form a gene complex (‘epidermal differentiation complex’) on human chromosome 1q21. Invest Dermatol 1996; 106: 989-992

96 Elder JT, Zhao X. Evidence for local control of gene expression in the epidermal differentiation complex. Exp Dermatol 2002; 11: 406-412

97 Tarcsa E, Candi E, Kartasova T, Idler WW, Marekov LN, Steiner PM. Structural and transglutaminase substrate properties of the small proline-rich 2 family of cornified cell envelope proteins. J Biol Chem 1998; 273: 23297-23303

98 Steen LE, Erwin CR, Falcone RA, Huang FS, Kemp CJ, Williams JL, Warner BW. cDNA microarray analysis of adapting bowel after intestinal resection. J Pediatr Surg 2001; 36: 190-195

99 Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon MJ. Molecular analysis of commensal host-microbial relationships in the intestine. Science 2001; 291: 881-884

100 Mueller A, O’Rourke J, Grimm J, Guillen K, Dixon MF, Lee A, Falkow S. Distinct gene expression profiles characterize the histopathological stages of disease in Helicobacter-induced mucosa-associated lymphoid tissue lymphoma. Proc Natl Acad Sci U S A 2003; 100: 1292-1297

101 Knight PA, Pemberton AD, Robertson KA, Roy DJ, Wright SH, Miller HR. Expression profiling reveals novel innate and inflammatory responses in the jejunal epithelial compartment during infection with Trichinella spiralis. Infect Immun 2004; 72: 6076-6086

102 Hong SH, Nah HY, Lee JY, Lee JY, Lee JW, Gye MC, Kim CH, Kang BM, Kim MK. Estrogen regulates the expression of the small proline-rich 2 gene family in the mouse uterus. Mol Cells 2004; 17: 477-484

103 Tan YF, Li FX, Piao YS, Sun XY, Wang YL. Global gene profiling analysis of mouse uterus during the oestrus cycle. Reproduction 2003; 126: 171-182

104 Song HJ, Poy G, Darwiche N, Lichti U, Kuroki T, Steinitz PM, Kartasova T. Mouse Sprr2 genes: a clustered family of genes showing differential expression in epithelial tissues. Genomics 1999; 55: 28-42

105 Zimmermann N, Doepker MP, Witte DP, Stringer KF, Fulkerson PC, Pope SM, Brandt EB, Mishra A, King NE, Nikolaidis NM, Wills-Karp M, Finkelman FD, Rothenberg ME. Expression and regulation of small proline-rich protein 2 in allergic inflammation. Am J Respir Cell Mol Biol 2005; 32: 428-435

106 Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997; 276: 60-66

107 Clouston AD, Powell EE. Interaction of non-alcoholic fatty liver disease with other liver diseases. Best Pract Res Clin Gastroenterol 2002; 16: 767-781

108 Demetris AJ, Sakamoto T, Liu Z, Yokomuro S, Ezeure T, Murase N, Blakolmer K. The Ductular Reaction in Liver Disease emphasis on a type I response. In: Fleig WE, Editor. Normal and Malignant Liver Cell Growth. Dordecht: Kluwer Academic Publishers, 1999: 141-155

109 Sirica AE, Mathis GA, Sano N, Elmore LW. Isolation, culture, and transplantation of intrahepatic biliary epithelial cells and oval cells. Pathobiology 1990; 58: 44-64

110 Zhang M, Thorgerisson SS. Modulation of connexins during differentiation of oval cells into hepatocytes. Exp Cell Res 1994; 213: 37-42

111 Martin KR, Barrett JC. Reactive oxygen species as double-edged swords in cellular processes: low-dose cell signaling versus high-dose toxicity. Hum Exp Toxicol 2002; 21: 71-75

112 Marshall A, Rushbrook S, Davies SE, Morris LS, Scott JS, Vowler SL, Coleman N, Alexander G. Relation between hepatocyte G1 arrest, impaired hepatic regeneration, and fibrosis in chronic hepatitis C virus infection. Gastroenterology 2005; 128: 33-42

113 Kobayashi S, Matsuoka K, Saiago K, Urashima T, Asano T, Hayashi H, Ochiai T. P21WAF1/CIP1 messenger RNA expression in hepatitis B, C virus-infected human hepatocellular carcinoma tissues. Cancer 2001; 91: 2096-2103

114 Torbenson M, Yang SQ, Liu HZ, Huang J, Cange W, Diehl AM. Stat-3 overexpression and p21 up-regulation accompany impaired regeneration of fatty livers. Am J Pathol 2002; 161: 155-161

115 Sawada N, Kojima T, Obata H, Isomura H, Atsumi S, Sawaki M, Tsuzuki N, Tobioka H, Kokai Y, Satoh M, Mori M. Expression of p21(waf-1/cip-1) is significantly induced in the livers of LEC rats with chronic liver injury. Jpn J Cancer Res 1996; 87: 1102-1105

116 Koteish A, Yang S, Lin H, Huang J, Diehl AM. Ethanol induces redox-sensitive cell-cycle inhibitors and inhibits liver regeneration after partial hepatectomy. Alcohol Clin Exp Res 2002; 26: 1710-1719

117 Blakolmer K, Jain A, Ruppert K, Gray E, Duquesnoy R, Murase N, Starzl TE, Fung JJ, Demetris AJ. Chronic liver allograft rejection in a population treated primarily with tacrolimus as baseline immunosuppression: long-term follow-up and evaluation of features for histopathological staging. Transplantation 2000; 69: 2330-2336

118 Demetris A, Adams D, Bellamy G, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga W, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neill D, Pappo O, Parizhskaya

www.wignet.com
M. Randhawa P, Rasoul-Rockenschaub S, Reinhold F, Reyes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. *Hepatology* 2000; 31: 792-799

120 Oguma S, Belle S, Starzl TE, Demetris AJ. A histometric analysis of chronically rejected human liver allografts: insights into the mechanisms of bile duct loss: direct immunologic and ischemic factors. *Hepatology* 1989; 9: 204-209

121 Omori N, Evarts RP, Omori M, Hu Z, Marsden ER, Thorgeirsson SS. Expression of leukemia inhibitory factor and its receptor during liver regeneration in the adult rat. *Lab Invest* 1996; 75: 15-24

122 Sakamoto T, Ezure T, Lunz J, Murase N, Tsuji H, Fung JJ, Demetris AJ. Concanavalin A simultaneously primes liver hematopoietic and epithelial progenitor cells for parallel expansion during liver regeneration after partial hepatectomy in mice. *Hepatology* 2000; 32: 256-267

123 Chan KS, Sano S, Kiguchi K, Anderson J, Komazawa N, Takeda J, DiGiovanni J. Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. *J Clin Invest* 2004; 114: 720-728

124 Campisi J. Cancer and ageing: rival demons? *Nat Rev Cancer* 2003; 3: 339-349

125 Haura EB, Turkson J, Jove R. Mechanisms of disease: Insights into the emerging role of signal transducers and activators of transcription in cancer. *Nat Clin Pract Oncol* 2005; 2: 315-324

126 Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. *Eur J Cancer* 2005; 41: 2502-2512

127 Koike N, Saitoh K, Todoroki T, Kawamoto T, Iwasaki Y, Nakamura K. Cell proliferation kinetics of the bile duct epithelium of the rat. *Cell Prolif* 1993; 26: 183-193

128 Nakamura Y, Kono N. Expression of vimentin in proliferating and damaged bile ductules and interlobular bile ducts in nonneoplastic hepatobiliary diseases. *Mod Pathol* 1992; 5: 550-554

129 Milani S, Herbst H, Schuppan D, Niedobitek G, Kim KY, Stein H. Vimentin expression of newly formed rat bile duct epithelial cells in secondary biliary fibrosis. *Virchows Arch A Pathol Anat Histopathol* 1989; 415: 237-242

130 Haruna Y, Saito K, Spaulding S, Nalesnik MA, Gerber MA. Identification of bipotential progenitor cells in human liver development. *Hepatology* 1996; 23: 476-481

131 Terada T, Ashida K, Kitamura Y, Matsunaga Y, Takashima K, Kato M, Ohta T. Expression of epithelial-cadherin, alpha-catenin and beta-catenin during human intrahepatic bile duct development: a possible role in bile duct morphogenesis. *J Hepatol* 1998; 28: 263-269

132 Camargo CA Jr, Madden JF, Gao W, Selvan RS, Clavien PA. Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent. *Hepatology* 1997; 26: 1513-1520

133 Gao B. Therapeutic potential of interleukin-6 in preventing obesity- and alcohol-associated fatty liver transplant failure. *Alcohol* 2004; 34: 59-65

134 Hong F, Radaeva S, Pan HN, Tian Z, Veech R, Gao B. Interleukin-6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. *Hepatology* 2004; 40: 933-941

135 Selzner M, Graf R, Clavien PA. IL-6: a magic potion for liver transplantation? *Gastroenterology* 2003; 125: 256-259

136 Sun Z, Klein AS, Radaeva S, Hong F, El-Assal O, Pan HN, Jaruga B, Batkai S, Hoshino S, Tian Z, Kunos G, Diehl AM, Gao B. In vitro interleukin-6 treatment prevents mortality associated with fatty liver transplants in rats. *Gastroenterology* 2003; 125: 202-215

137 Kimizuka K, Nakao A, Nalesnik MA, Demetris AJ, Uchiyama T, Ruppert K, Fink MP, Stolz DB, Murase N. Exogenous IL-6 inhibits acute inflammatory responses and prevents ischemia/reperfusion injury after intestinal transplantation. *Am J Transplant* 2004; 4: 482-494

138 Safadi R, Friedman SL. Hepatic fibrosis--role of hepatic stellate cell activation. *MedGenMed* 2002; 4: 27

139 Tillmann HL, Manns MP, Rudolph KL. Merging models of hepatitis C virus pathogenesis. *Semin Liver Dis* 2005; 25: 84-92

140 Watanabe S, Matsuda A, Suzuki Y, Kondo K, Ikeda Y, Hashimoto H, Umemura K. Inhibitory mechanism of tranilast in human coronary artery smooth muscle cells proliferation, due to blockade of PDGF-BB-receptors. *Br J Pharmacol* 2000; 130: 307-314

141 Ikeda H, Iino M, Fujiwara K. Inhibitory effect of tranilast on activation and transforming growth factor beta 1 expression in cultured rat stellate cells. *Biochem Biophys Res Commun* 1996; 227: 322-327

142 Naftalin R. Alterations in colonic barrier function caused by a low sodium diet or ionizing radiation. *J Environ Pathol Toxicol Oncol* 2004; 23: 79-97

143 Wengrower D, Zanninelli G, Pappo O, Latella G, Sestieri M, Villanova A, Faitelson Y, Pines M, Goldin E. Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. *Inflamm Bowel Dis* 2004; 10: 536-545

144 Ramos SG, Montenegro AP, Goisiss G, Rossi MA. Captopril reduces collagen and mast cell and eosinophil accumulation in pig serum-induced rat liver fibrosis. *Pathol Int* 1994; 44: 655-661

145 Yuan GJ, Zhang ML, Gong ZJ. Effects of PPAR agonist pioglitazone on rat hepatic fibrosis. *World J Gastroenterol* 2004; 10: 1047-1051

146 Kawaguchi K, Sakaida I, Tsuichiya M, Omori K, Takami T, Okita K. Pioglitazone prevents hepatic steatosis, fibrosis, and enzyme-altered lesions in rats liver cirrhosis induced by a choline-deficient L-amino acid-defined diet. *Biochem Biophys Res Commun* 2004; 315: 187-195

147 Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF, Motomura K, Anania FA, Willson TM, Tsukamoto H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. *J Biol Chem* 2000; 275: 35715-35722

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