203

Mesenchymal Stem Cell Transplantation Improves Chronic Colitis-Associated Cholangitis Through Inhibiting the Activity of LPS/TLR4

Xiaolan Zhang, Guochao Niu, Lei Liu, Hui Li, Jinbo Guo, Jia Song, Yufeng Liu, Shuang Chen

Background: The hepatobiliary manifestation, cholangitis, is frequently encountered in inflammatory bowel disease (IBD). Toll receptor 4 (TLR4) signaling pathway plays a pivotal role in the pathogenesis of various chronic liver diseases. Mesenchymal stem cells (MSCs) are important means for the treatment of IBD and liver diseases. This study investigated the protective role and mechanism of MSCs in the chronic colitis-associated cholangitis. Methods: Mouse chronic colitis model was established by administration of dextran sodium sulfate (DSS) drinking water and treated with MSCs. Mice were grouped as follows: DSS+Vehicle group (n=10), DSS+MSCs group (n=10) and control group (n=10). Severity of colitis was evaluated by disease activity index (DAI), body weight (BW), colon length, histopathology. Histology and function of mouse liver were checked correspondingly. Serum LPS levels and bacterial translocation of mesenteric lymph nodes were detected. Pro-inflammatory cytokines including TNF-α, IFN-γ, IL-1β, IL-17A, TLR4, TRAF6, and NF-κB were detected by immunohistochemical staining, western blot analysis and real-time PCR, respectively. Results: DSS-induced chronic colitis model was characterized by reduced BW, higher DAI, worsened histologic inflammation, and enhanced levels of LPS and bacterial translocation. Chronic colitis-associated hepatobiliary complications revealed histomorphological signs of cholangitis and the impaired liver function. The more severer the degree of colitis showed, the more severer cholangitis were showed. The protein and mRNA levels of TNF- α, IFN-γ, IL-1β, IL-17A, TLR4, TRAF6 and NF-κB significantly increased after DSS administration. MSCs transplantation markedly ameliorated the pathology of colon and liver by reduction of LPS level, and proteins and mRNA expressions of TNF- α, IFN-γ, IL-1β, IL-17A, TLR4, TRAF6 and NF-κB as well. Conclusions: Our results reveal that MSCs may be a novel therapeutic drug for the treatment of chronic colitis-associated cholangitis, which correlated to downregulating the LPS/TLR4 signaling pathway.

207

MicroRNA-29b Prevents Liver Fibrosis by Attenuating Hepatic Stellate Cell Activation and Inducing Apoptosis *In Vitro* and in Mice

Jia Wang, Eagle SH Chu, Hui-Yao Lan, Joseph J. Y. Sung, Jun Yu

Background & Aims: microRNA-29b (miR-29b) is known to be associated with transforming growth factor-β (TGF-β)-mediated liver fibrosis, but the mechanistic action of miR-29b in liver fibrosis remains unexplored. We aimed to examine the role and molecular regulators of miR-29b in liver fibrosis. Methods: Male C57BL6 mice were injected with carbon tetrachloride (CCl4) to induce liver fibrosis. The anti-fibrotic effect of miR-29b was evaluated by delivering miR-29b through tail vein injection into the liver. Primary rat hepatic stellate cell (HSC) culture was established to evaluate the change of miR-29b expression in quiescent and activated HSCs. Human HSC cell line (LX-1) and rat HSC cell line (HSC-T6) were transfected with miR-29b to study its effect on HSCs activation *in vitro*. The upstream regulator of miR-29b was identified by CHIP-PCR. The downstream targets of miR-29b were screened using in silico searches and validated by luciferase reporter assay. The role of miR-29b-modulated targets in liver fibrosis was examined by loss-of-function assays. Results: miR-29b was significantly downregulated i) in human fibrotic liver tissues compared with normal liver tissues ($P<0.01$); ii) in activated HSCs compared with quiescent HSCs ($P<0.05$); and iii) in CCl4-induced liver fibrotic tissues compared to the normal liver tissues in mice ($P<0.05$). Gene transfer of miR-29b into the liver prevented fibrogenesis induced by CCl4 ($P<0.01$). Such delivery of miR-29b in liver decreased expression of α-SMA, collagen I and TIMP-1, indicating that the anti-fibrotic effect by miR-29b was associated with the suppression of HSC activation. This was further confirmed by *in vitro* experiments. Ectopic expression of miR-29b in activated HSCs (LX-1, HSC-T6) inhibited cell viability ($P<0.05$) and colony formation ($P<0.05$), and caused cell cycle arrest in G1 phase ($P<0.01$). miR-29b also induced apoptosis in HSCs ($P<0.05$). The underlying mechanisms of miR-29b in liver fibrosis were therefore investigated. CHIP-PCR assay revealed that Smad3 bound to the promoter of miR-29b and down-regulated its expression. Whilst, miR-29b could in turn suppress Smad3 expression. TargentScan prediction and luciferase reporter assay showed that miR-29b targeted the 3'UTR of PIK3R1 and AKT3, and inhibited the expression of these two genes at translation level. A significant up-regulation of PIK3R1 and AKT3 was detected in human fibrotic liver tissues ($P<0.05$). Knockdown of PIK3R1 or AKT3 which blocked the phosphorylation of AKT induced apoptosis in HSCs ($P<0.05$). Conclusions: miR-29b prevents liver fibrogenesis by inhibiting HSC activation and inducing HSC apoptosis though down-regulation of PIK3R1 and AKT3 to block AKT phosphorylation. These results provide novel mechanistic insights for the anti-fibrotic effect of miR-29b in liver.

208

Deletion of PPARγ in Hepatic Stellate Cells Attenuates Regression of Liver Fibrosis

Tae Jun Park, Xiao Liu, Mingjun Zhang, Tatiana Kisseleva, David A. Brenner

BACKGROUND: Liver fibrosis is characterized by extensive accumulation of extracellular matrix (ECM). Activated HSCs are the majour source of collagen, they downregulate proliferator-activated receptor gamma (PPARγ), and induce expression of ECM proteins. Upon removal of the etiological source of the chronic injury, regression of liver fibrosis is associated with the disappearance of activated HSCs/myofibroblasts either by apoptosis or by HSC inactivation into a quiescent-like phenotype. Inactivation of HSCs is accompanied by abrogation of fibrogenic gene expression (Collagen Type I and α-SMA) and re-expression of PPARγ. AIM: We hypothesized that PPARγ is not only a marker of HSC inactivation but actually plays an important role in mediating HSC inactivation. Thus, we predict that selective deletion of PPARγ in HSCs inhibits regression of liver fibrosis. METHODS: PPAR γ floxed mice (PPARγfloxP-Stop-floxP mice) were crossed with GFAPCre mice (expressing Cre under control of human glial fibrillar acidic protein promoter) to generate HSC-specific PPAR γ knockout GFAPPPARγ-/- mice. We assessed the specific function of PPARγ in HSC in the development of HSCs and HSC inactivation during regression of liver fibrosis. RESULTS: Mice developed normally, and the liver architecture observed in GFAPPPAR γ-/- knockout mice was indistinguishable from that in wild type GFAPPPARγ+/+ littermates. PPARγ-deficient HSCs did not spontaneously exhibit an activated phenotype, and livers from GFAPPPAR γ-/- mice stained negative for the myofibroblast-marker α-SMA. Deletion of PPARγ was confirmed by RT-PCR, demonstrating >90% reduction of PPARγ expression in isolated PPARγ-deficient qHSCs (vs wild type qHSCs). Compared to wild type qHSCs, PPAR γ-deficient qHSCs contained less lipid droplets and slightly induced Col1a1 mRNA expression. Expression of PPARγ target genes (Insig1, CEBPd) was > 50% reduced in PPARγ deficient HSCs, indicating that PPARγ may regulate HSC phenotype. Next, GFAPPPAR γ-/- and GFAPPPARγ+/+ mice were subjected to CCl4, and then allowed to recover for 1 month. As expected, GFAPPPAR γ-/- mice developed more fibrosis than GFAPPPAR γ-/- mice. More importantly, GFAPPPAR γ-/- mice were markedly defective in the resolution of fibrosis compared to GFAPPPAR γ+/+ littermates. Regression of liver fibrosis was assessed by Sirius Red staining, and quantified as fold downregulation of Sirius Red positive area compared to that in CCl4-injred mice. Reduced regression of liver fibrosis in GFAPPPAR γ-/- mice was accompanied by persistence of αSMA+ and Pro-Collagen Type I+ myofibroblasts, suggesting that PPAR γ is required for HSC inactivation. CONCLUSION: HSC-specific deletion of PPAR γ in mice does not cause liver fibrosis due to spontaneous HSC activation, but results in impaired resolution of liver fibrosis due to a defect in HSC inactivation.

209

Uric Acid-Activated NLRP3 Inflammasome Is a Therapeutic Target in Alcoholic Liver Disease

Jan Petrasek, Shashi Bala, Karen Kodys, Victoria Menashy, Matthew Barrieau, Evelyn A. Kurt-Jones, Gyongyi Szabo

Background: Steatosis and increase in inflammatory cytokines, including interleukin (IL)-1β, are key components of alcoholic liver disease (ALD). NOD-like receptors, such as NLRP3, are intracellular sensors of danger signals that activate the inflammasome complex. The NLRP3 inflammasome is activated by host-derived molecules, including uric acid, and triggers activation of Caspase-1 (Casp1) that subsequently cleaves pro-IL-1 β into mature IL-1β. A critical role of inflammasome activation in ALD has recently been shown, raising a possibility that inflammasome represents a therapeutic target in ALD. The mechanisms of inflammasome activation in ALD are unknown. **Aim:** To determine the mechanisms of inflammasome activation in ALD. **Methods:** Wild-type (WT), NLRP3-deficient mice, and two strains of uricase transgenic (Tg) mice were fed with Lieber-DeCarli ethanol or control diet for 4 weeks. Some mice received allopurinol or probenecid. **Results:** Alcohol feeding to WT mice resulted in liver injury (ALT), steatosis and upregulation of inflammatory cytokines and multiple inflammasome components, including NLRP3, the adaptor molecule ASC and the active p10 fragment of the effector Casp1. We found that NLRP3-deficient mice were protected from alcohol-induced inflammasome activation and IL-1 β production, and showed significantly lower levels of serum ALT, inflammatory cytokines and liver triglycerides compared to WT mice. As this data suggested the role for NLRP3 inflammasome in ALD, we evaluated the potential activators of NLRP3. Alcohol-fed mice demonstrated a significant increase in the levels of uric acid in both serum and liver compared to pair-fed controls. To evaluate the role of endogenous uric acid in ALD, we used mice depleted of uric acid owed to overexpression of the uric acid-degrading enzyme uricase in the serum (ssUOX-Tg) or in the cells (intUOX-Tg). We observed that alcohol-fed, ssUOX-Tg or intUOX-Tg mice failed to activate the inflammasome, and that uric acid-depleted mice had significantly attenuated liver injury, inflammation and steatosis compared to WT mice. To translate this finding into a clinically relevant scenario, we treated alcohol-fed mice with allopurinol, a xantine oxidase inhibitor, or with probenecid, a uricosuric agent, both of which are used as gout medication. We found that pharmacological depletion of uric acid prevented activation of the inflammasome and significantly attenuated alcoholic liver injury, inflammation and steatosis. **Conclusions:** Our novel data identify endogenous uric acid as a pro-inflammatory trigger that activates the NLRP3 inflammasome leading to inflammation in ALD. Our results reveal a crosstalk between metabolic and inflammatory signals in the liver, and demonstrate that pharmacological depletion of uric acid attenuates inflammation, steatosis and liver damage in ALD. (Supported by NIAAA)

210

Genetic Inhibition of Keratin Digestion by Caspases Promotes Hepatocyte Injury via Apoptosis and Necrosis Pathways

Sujith Weerasinghe, Nam-On Ku, Peter J. Altshuler, David Moons, Bishr M. Omary

Background: Type-I keratins, including keratins 18 and 19 (K18/K19), among other cytoskeletal intermediate filament proteins (IFs) such as desmin and lamins, undergo caspase-mediated cleavage during apoptosis in association with keratin filament collapse. Mutations in IFs cause or predispose to >70 human diseases, and in some cases the mutations reside within the caspase cleavage site and render the keratin noncleavable by caspases. However, the significance of caspase-mediated keratin cleavage during apoptosis is unknown. Methods: The two K18 caspase cleavage sites (D238E/D397E) were mutated in the human K18 gene followed by overexpression in transgenic mice. The response to liver injury in the transgenic mice that overexpress wild-type (WT) or K18 D238E/397E (K18-DE), or in isolated hepatocytes from these rmice was characterized. Cell or tissue injury was induced by exposing mice or isolated primary hepatocytes to Fas ligand (Fas-L) and/or hyposmotic stress. Liver injury and hepatocyte damage was monitored by immunostaining, biochemical, histochemical, serologic, and apoptosis or necrosis marker testing. Results: K18-DE mice develop more dramatic liver damage as compared to WT mice, based on measuring caspase activation, serum ALT, TUNEL staining and liver histology. Immunofluorescence staining of WT livers, or hepatocytes challenged with Fas-L showed disrupted keratin filaments and degraded K18, whereas livers or isolated hepatocytes from the K18-DE mice had relatively intact keratin filaments. In contrast, primary hepatocytes from K18 WT and DE mice had similar caspase activation and mitochondrial damage in responses to Fas-L. In stark contrast, K18-DE

hepatocytes were more susceptible than WT hepatocytes to necrosis when exposed to Fas-L or hyposmotic conditions, as measured by LDH and HMGB1 release. Upon hyposmotic stress of hepatocytes, cell death occurred in the absence of caspase activation. Similar to primary hepatocytes, K18-DE mice treated with Fas-L had elevated serum necrosis markers (LDH and HMGB1) as compared to their WT counterparts. K18-DE mutation renders the K18 obligate heteropolymer, K8, less susceptible to phosphorylation at its p38 stress kinase site K8 S79, without altering the binding of K8/K18 to p38 kinase. Notably, K8 S79 phosphorylation plays an important role in mediating keratin filament reorganization upon stress. Conclusions: Caspase digestion of K18 plays an essential role in promoting apoptosis-associated keratin filament collapse in the liver. Keratin phosphorylation-mediated filament reorganization appears to be critical in protecting hepatocytes from necroapoptosis, and provides a mechanism for susceptibility to tissue injury in human IF mutations that involve caspase cleavage sites.

211

Concanavalin A-Induced Hepatitis in Mice Is Prevented by Depletion of Gut Bacteria Using Antibiotics

Beth Mallard, Leo R. Quinlan, Ayako Mabuchi, Antony Wheatley

Background: Concanavalin (Con) A administration induces an immune-mediated hepatitis in mice, the mechanism of which involves the activation of T cells and Kupffer cells, which produce the cytokines interferon (IFN)-γ and tumour necrosis factor (TNF)-α. The lipopolysaccharide (LPS)/Toll-like receptor (TLR) 4 signalling pathway is involved in several forms of liver injury. Furthermore, several forms of experimental liver injury involve increased gut permeability. Con A-induced hepatitis is attenuated in mice deficient in TLR4. In the current study, the effect of depleting gut bacteria on Con A-induced hepatitis was investigated. The effect of Con A on gut permeability was also examined. Methods: Groups of mice (C57BL/6) were treated for 5 days with Polymyxin B (150 mg/kg/day) and Neomycin (450/mg/kg/day) via drinking water to deplete Gram-negative bacteria in the gut. Control mice were given normal drinking water. Following antibiotic treatment, Con A (20 mg/kg i.v.) was administered. Blood and tissue were harvested 8 or 24 hours later. Liver injury was assessed by measuring plasma ALT and histologically. In wild-type (C57BL/6), FITC-dextran was administered by oral gavage and its subsequent appearance in plasma was measured to assess gastrointestinal tract permeability. Permeability was further examined in wild-type mice using an Ussing chamber to measure transepithelial resistance. Results: Control mice developed severe injury following Con A administration, indicated by elevated plasma ALT and confirmed by histology. However, mice treated with antibiotics prior to Con A administration showed no significant elevation in plasma ALT at either 8 or 24 hours. Wild-type mice treated with Con A displayed increased gut permeability, indicated by an increase in plasma FITC-dextran levels compared control (1578 ±370 ng/mL in Con A treated vs. 160 ± 26 ng/mL in control mice). Furthermore, Con A treated mice displayed a 20% reduction in transepithelial resistance compared to control mice, which is consistent with increased permeability. Discussion: Our results show that Con A causes liver injury in mice with a normal bacterial population resident in the gastrointestinal tract. However, following the depletion of Gram-negative bacteria, Con A does not elicit liver injury. Con A also increased gut permeability. Together, these results suggest that the mechanism for Con A-induced liver injury involves increased translocation of LPS from the gastrointestinal tract by an as yet unknown mechanism and that the liver injury is dependent on activation of the LPS/TLR4 pathway.

212

Exome Sequencing in Families Identifies Rare Variants in Cell Junction Associated Proteins Representing a Potential Link Between Genetic Variation and Liver-Targeted Autoimmunity in Primary Biliary Cirrhosis

Brian D. Juran, Eric W. Klee, Ying Li, Erik Schlicht, Konstantinos Lazaridis

Purpose: Genome-wide association studies (GWAS) of primary biliary cirrhosis (PBC) suggest the role of common genetic variants in PBC risk to largely center on a generalized propensity to autoimmunity, and the factors contributing to liver targeting in PBC remain obscure. Here we employ exome sequencing in PBC families to explore the impact of rare genetic variants in PBC, laying groundwork towards an individualized understanding of the genetic architecture underlying this complex disease. Methods: 3 PBC families (Fam1, Fam2, Fam3) were selected from our registry and the exome of 2-affected and 1-unaffected female member of each was sequenced on an Illumina HiSeq 2000. Variants were called using an established pipeline based on Best Practice Variant Detection v4. Lists of genes harboring candidate variants for each family were generated using a probabilistic method implemented in the Variant Annotation, Analysis and Search Tool (VAAST) followed by inheritance and QC based filtering. Familial gene lists were then compared for overlaps, prioritized on predicted impact, and tested for gene set enrichment (GSE) using ConsensusPath. Results: Gene lists comprised of 74, 136, and 83 variants for Fam1, Fam2 and Fam3 respectively. All variants were non-synonymous (i.e. protein changing), present only in the 2 affected family members, and no overlap between the lists was found. GSE analysis identified significant enrichment of gene-ontology (GO) based gene sets implicating cell junctions in 2 of the 3 families. Specifically, the GO term "GO:0005913 cell-cell adherens junction" (P=4.6e-5, Pcor=0.001) was enriched in Fam1, due to variants in the genes SHROOM2, ITGA6, and TJP1. In Fam2, the GO term "GO:0002934 desmosome organization" (P=3.6e-4, Pcor=0.068) was enriched, due to variants in the genes DSP and JUP, which comprise the desmoplakin/gamma catenin complex, which was also significant in the ConsensusPath analysis (P=3.1e-5, Pcor=9.4e-5). In Fam3, the GSE analysis identified enriched sets indicative of immune function, due to rare variants in HLA-C, HLA-DRB1, and KIRDL3. Conclusions: Family-based exome sequencing found many rare genetic variants potentially contributing to PBC, but no familial overlaps. Of interest, 2 of the 3 families carried multiple non-synonymous variants involved with aspects of the cell junction. This finding is in line with hypotheses of pathogenic "leaky bile" due to diminished biliary epithelia integrity in PBC, which were not supported by GWAS. While additional studies are needed, our finding provides novel evidence linking genetic variation to liver-targeted autoimmunity in PBC. Moreover, this finding, in combination with the rare genetic variants in HLA and KIR genes found in the third family, serves to illustrate the complexity inherent in moving from a population-based to an individualized understanding of PBC genetics.

292

Collagen Endocytosis Regulates Matrix Synthesis and Degradation by Hepatic Stellate Cells

Yan Bi, Dhriti Mukhopadhyay, Vijay Shah

Background and Aims: Hepatic stellate cells (HSC) are a major matrix producing cell during liver fibrosis. Recent studies indicate that matrix also in turn regulates HSC function. We hypothesized that HSC may endocytose matrix proteins to sense and respond to changes in their matrix microenvironment. Method: Primary human HSC (P2-6) were incubated with fluorescent labeled collagen I or gelatin. FACS analysis and confocal microscopy were used for measuring cellular uptake of fluorescent labeled matrix proteins. Target mRNA levels were measured by PCR array and confirmed by real-time PCR. Results: HSC endocytose both collagen I and gelatin in a concentration- and time- dependent manner. Collagen matrix endocytosis was associated with a decrease in collagen I mRNA levels by 31.3±6.8% (n=3, $p<0.01$) and increase in MMP9 mRNA levels by 3.65±1.13 fold (n=3, $p<0.05$). We next explored endocytic mechanisms by which HSC internalize collagen. In confocal microscopy studies, endocytosed matrix co-localized with endocytosed Dextran, a marker of macropinocytosis. Furthermore, amiloride, an inhibitor of macropinocytosis reduced matrix uptake by 46.1±3.6% (n=3, $p<0.001$). In contrast, matrix uptake was not reduced by pharmacological inhibition of either clathrin- or caveolea- dependent endocytosis. The inhibitor of actin polymerization cytochalasin D, and the myosin light chain kinase inhibitor ML7, blocked collagen uptake by 46.5±8.9% (n=3, $p<0.05$) and 45±12%(n=3, $p<0.05$), respectively, indicating that actin dependent macropinocytosis is critical for collagen endocytosis. The PI3K inhibitor wortmannin and AKT inhibitor blocked collagen uptake by 69.7±11.1% (n=5, $p<0.001$) and 89.2±14.5% (n=4, $p<0.001$), respectively indicating that macropinocytosis of matrix proteins requires PI3K/AKT signaling. Finally, overexpression of the dominant negative dynamin II K44A construct blocked matrix uptake by about 77±10.9% (n=3, $p<0.01$) indicating a role for dynamin in matrix macropinocytosis. Conclusion: HSC endocytoses matrix proteins through macropinocytosis that requires PI3K, AKT, and dynamin. Matrix endocytosis reduces collagen synthesis while activating matrix degradation. These observations indicate that HSC sample their matrix microenvironment to guide matrix production and degradation kinetics.

293

Adiponectin Disrupts Focal Adhesion Assembly by Adaptor Protein Containing Pleckstrin Homology Domain, Phosphotyrosine Binding Domain, Leucine Zipper Motif 1 (Appl1): Activation of a Novel SHP2 Dependent Mechanism

Pradeep Kumar, Ping P. Fu, Jamie E. Mells, Frank A. Anania

BACKGROUND: Adiponectin is known to possess anti-fibrotic properties in the liver; however the molecular mechanism to explain these beneficial events are lacking. **PURPOSE**: To demonstrate molecular details for how adiponectin reverse fibrotic mechanisms by disrupting focal adhesion assembly, a key component of initiation and perpetuation of hepatic fibrosis. **METHODS**: *In vivo* approaches—adiponectin global knock-out (KO) mice and their wild-type littermates were exposed to either saline or carbon tetrachloride (CCl_4) by gavage for eight weeks. Some of the mice were administered adenovirus containing an adiponectin expression vector concomitantly following 8 weeks of CCl_4 gavage. After sacrifice qRT-PCR, immunofluorescence and immunoblot were performed to examine focal adhesions related proteins. *In vitro* approaches—rat hepatic stellate cells (HSCs) were isolated, plated, and cultured in the presence or absence of recombinant high molecular weight adiponectin. Lentivirus transfection using siRNAs for either adiponectin receptor isoforms (AdipoR1 or AdipoR2) were created for stable knockdown. Src homology 2 domain containing non-transmembrane protein tyrosine phosphatase (Shp2) siRNA was transiently transfected to knockdown the expression of Shp2. Compound C was used to inhibit adenosine monophosphate kinase (AMPK) phosphorylation in the presence or absence of adiponectin. Immunoprecipitation and immunoblot studies were conducted from HSCs lysates *in vitro* to elucidate signal transduction mechanisms to account for *in vivo* observations. **RESULTS**: Adiponectin delivery in mice gavaged with markedly reduced hepatic mRNA for key integrins— αv, β3, β1 and α-smooth muscle actin (αSMA), as well as focal adhesion protein vinculin. Adiponectin also diminished phosphorylation of paxillin, another key focal adhesion protein component. Adiponectin treatment resulted in dephosphorylation of FAK at Tyrosines (Tyr)-577/576, and Tyr-925 in HSCs. Adiponectin increased the phosphorylation of the tyrosine phosphatase, Shp2 and Shp2 siRNA attenuated adiponectin-induced FAK dephosphorylation. Physical association of p-Shp2 and p-FAK was enhanced in the presence of adiponectin. Adiponectin also induced preferential binding of adipoR1 and APPL1 and shp2 associated with APPL1. Adiponectin enhanced phosphorylation of Shp2 associated with APPL1. shRNA adipoR1 inhibited phosphorylation of Shp2 and subsequent dephosphorylation of FAK. FAK desphosphorylation, despite chemical inhibition of AMPK, persisted in the presence of adiponectin. **CONCLUSIONS**: We describe a novel Shp2 dependent mechanism that disrupts focal adhesion assembly, underscoring the hypothesis that tyrosine phosphatases play a key role in the resolution of liver fibrosis. We are currently exploring the role of small molecule adiponectin-like agonists as a potential anti-fibrotic therapy.

294

Palmitoleic Acid Prevents Palmitate-Induced Cholangiocyte Lipoapoptosis

Sathish Kumar Natarajan, Mary A. Smith, Sohini Roy, Anuttoma Ray, Justin L. Mott

Background: Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver disease starting from simple steatosis to non-alcoholic steatohepatitis (NASH). NASH can lead to liver fibrosis and liver cirrhosis. These patients have elevated plasma saturated free fatty acid levels. Recently, a cholestatic variant of NAFLD with portal inflammation and ductular