1109
PROTECTION AGAINST HEPATOCYTE MITOCHONDRIAL DYSFUNCTION DELAYS FIBROSIS PROGRESSION IN MICE

Claudia Mitchell[1], Marie-Anne Robin[2], Meriem Mahrouf-Yorgov[1], Alicia Mayeuf[1], Abdellah Mansouri[3], Bernard Fromenty[2], Helene Gilgenkrantz[1]; [1]Cochin, INSERM U567, Paris 5 University, Paris, France; [2]INSERM U.620, University Rennes 1, Rennes, France; [3]INSERM U.773, Centre de Recherche Biomédicale Bichat Beaujon, Paris, France

Aim of the study: Accumulating evidence indicates that oxidative stress is involved in the physiopathology of liver fibrogenesis. However, amid the global context of hepatic oxidative stress, the specific role of hepatocyte mitochondrial dysfunction in the fibrogenic process is still unknown. The aim of this study was to determine whether a targeted protection of hepatocytes against mitochondrial dysfunction could modulate fibrosis progression. Methods: We induced liver fibrogenesis by biweekly intraperitoneal injections of CCl4 as 5 ul of a 10% solution in mineral oil during 3 or 6 weeks in two groups of male mice: wild type C57Bl/6 mice (WT) and transgenic mice expressing Bcl-2 (Bcl-2 Tg) specifically in their hepatocytes. Two control groups were injected with mineral oil. We analyzed mitochondrial dysfunction by quantifying complexes I and IV of the mitochondrial electron respiratory chain activity and we measured the level of liver lipid peroxidation and glutathione content. We evaluated the extension of fibrosis by picrosirius red staining, α-SMA immunohistochemistry and real-time PCR for TGF-β and collagen α-I. Results: Complexes I and IV of the mitochondrial respiratory chain were found significantly decreased in WT mice. These results indicate that Bcl-2 transgenic animals are protected against mitochondrial dysfunction and oxidative stress resulting from CCl4 injury. This protection is correlated with a reduced extension of fibrosis and activation of hepatic stellate cells at early stages of fibrogenesis. After 3 weeks of CCl4 treatment, there was a significant induction of lipid peroxidation in WT mice, while MDA levels in the liver of Bcl-2 Tg mice were still low. At this step, we found no modification of glutathione content and liver aconitase activity in both experimental groups. However, after 6 weeks of treatment, there was no more significant difference between Bcl-2 Tg mice and WT mice for fibrosis markers. This loss of protection against fibrosis progression was correlated with a reduction in glutathione content and aconitase activity both WT and Bl-2 Tg mice suggesting that at later stages, increased non-mitochondrial/non-hepatocytic oxidative stress eventually overcame the capacity of Bcl-2 overexpression to prevent the fibrotic process. Conclusion: We demonstrate for the first time that a specific protection against hepatocyte mitochondrial dysfunction plays a preventive role in early stages of fibrogenesis, delaying its onset. However, with the persistence of the aggression, this protection is no longer sufficient to impede fibrosis progression.

Disclosures:

The following people have nothing to disclose: Claudia Mitchell, Marie-Anne Robin, Meriem Mahrouf-Yorgov, Alicia Mayeuf, Abdellah Mansouri, Bernard Fromenty, Helene Gilgenkrantz

1110
EGR-1 DEFICIENCY ENHANCES FIBROSIS AND PROMOTES ACTIVATION OF THE OVAL CELL RESPONSE AFTER CARBON TETRACHLORIDE EXPOSURE IN MICE

Michele T. Pritchard[1], Laura E. Nagy[1,2]; [1]Pathobiology, Cleveland Clinic, Cleveland, OH; [2]Gastroenterology, Cleveland Clinic, Cleveland, OH

Egr-1 is a critical mediator of hepatoprotection after acute carbon tetrachloride (CCl_4)-induced liver injury in mice; mice deficient in Egr-1 exhibit profound progression of liver injury. The purpose of this study was to determine if progressive liver injury in *egr-1-/-* mice was associated with an altered wound-healing response. Acute liver injury was induced in wild type and *egr-1-/-* mice by a single CCl_4 injection (1μL/g body weight after 1:3 dilution in olive oil), while fibrosis was achieved by repeated injections of CCl_4, at the same dose, twice a week for 5 weeks. Seventy-two hours after acute exposure to CCl_4, hepatic mRNA accumulation of α smooth muscle actin (SMA) and desmin, markers of hepatic stellate cell (HSC) activation and number, respectively, were greater in *egr-1-/-* mice compared to wild type mice. αSMA protein expression was also increased in *egr-1-/-* mice. In addition, hepatic mRNA accumulation of COL1A1 and COL1A2, type-I collagen molecules produced by activated HSC, was 5-fold more in *egr-1-/-* mice. Both 3 and 7d after cessation of chronic CCl_4 exposure, hepatic Sirius red staining was greater in *egr-1-/-* mice compared to wild type mice. In contrast to wild type mice, the pattern of extracellular matrix (ECM) deposition was diffuse in livers from *egr-1-/-* mice; a marked hypercellularity contributed to the diffuse ECM deposition. Some cells in this hypercellular region exhibited morphological features consistent with the presence of oval cells. Hepatic mRNA accumulation for alpha fetoprotein, thymocyte differentiation antigen-1, hepatocyte growth factor and connective tissue growth factor, genes expressed during oval cell-mediated liver regeneration, were strongly induced in livers of *egr-1-/-* mice compared to wild type mice 3 and 7d after chronic CCl_4 exposure. Expression of laminins α, β and γ, ECM molecules associated with oval cell activation, was also enhanced in livers of *egr-1-/-* mice compared to wild type mice. Finally, the number of cells expressing A6, a mouse oval cell marker, was more than 3-fold greater in livers from *egr-1-/-* mice compared to wild type controls, both 3 and 7d after CCl_4. In summary, progressive CCl_4-induced liver injury in *egr-1-/-* mice early in the time course of chronic CCl_4 exposure is associated with enhanced fibrosis and activation of the oval cell compartment. Inhibited or insufficient hepatocyte replication is required for oval cell-mediated hepatic reconstitution; therefore, these data suggest that Egr-1 likely plays a critical role in normal hepatocyte proliferative responses after CCl_4-induced liver injury. This work was funded by grants from the NIAAA (AA015833 & AA0138868).

Disclosures:

The following people have nothing to disclose: Michele T. Pritchard, Laura E. Nagy

1111
MMP-13 DELETION PROTECTS MONOCROTALINE INDUCED LIVER INJURY IN MICE

Joseph George[1], Mikihiro Tsutsumi[2], Jeanine D'Armiento[1]; [1]Medicine, Columbia University, New York, NY; [2]Gastroenterology, Kanazawa Medical University, Ishikawa, Japan

Monocrotaline (MCT) is both hepatotoxic and pneumotoxic in several species including human. Since matrix metalloproteinases-1 (MMP-1) is absent in mice, MMP-13 is responsible for cleavage and maturation of connective tissue growth factor

(CTGF). This study was aimed to elucidate the molecular events associated with hepatic fibrogenesis in MCT induced toxic liver injury. Hepatic injury was produced through subcutaneous administrations of MCT in doses of 60 mg/100 g body weight once a week for 4 weeks. Serial administrations of MCT resulted in marked elevation of AST, ALT, and hyaluronic acid (HA) in the serum and extensive activation of hepatic stellate cells, massive hepatic necrosis, bridging fibrosis and deposition of collagen fibers in the liver. However, these changes were significantly attenuated in MCT administered MMP-13 knockout mice. Staining for CTGF showed marked upregulation in MCT-treated wild-type mice but not in the knockout. Semiquantitative and real-time RT-PCR for CTGF, TGF-beta1, and type-I collagen mRNA showed remarkable upregulation in MCT administered wild-type mice, but not in similarly treated MMP-13 knockout mice. Protein levels of CTGF, TGF-beta1, MMP-2, MMP-9 and type I collagen were also increased in MCT treated wild-type mice. All these molecules were significantly reduced and CTGF was absent in MCT treated MMP-13 knockout mice compared to the similarly treated wild-type. Taken together, these results show that MMP-13 plays a crucial role in the pathogenesis of MCT induced liver injury through activation of CTGF. Furthermore, our study indicates that blocking of CTGF could pave the way for the therapeutic intervention of hepatic fibrogenesis.

Disclosures:

The following people have nothing to disclose: Joseph George, Mikihiro Tsutsumi, Jeanine D'Armiento

1112
THIOACETAMIDE-INDUCED HEPATIC FIBROENESIS IS AMELIORATED IN MICE GENETICALLY DISRUPTIVE OF CD1D

Sachiko Ishikawa[1], Kenichi Ikejima[1], Hisafumi Yamagata[1], Tomonori Aoyama[1], Kazuyoshi Kon[1], Kazuyoshi Takeda[2], Sumio Watanabe[1]; [1]Department of Gastroenterology, Juntendo University School of Medicine, Tokyo, Japan; [2]Department of Immunology, Juntendo Universty School of Medicine, Tokyo, Japan

Bacground: The liver contains a variety of innate immune cells, predominating natural killer (NK) and invariant NKT cells. Lines of evidence support the hypothesis that innate immune response is profoundly involved in the mechanisms of hepatic fibrogenesis, however, the precise role of NKT cells in hepatic fibrogenesis remains unclear. In this study, therefore, we investigated the differences in progression of hepatic fibrosis caused by thioacetamide (TAA) in mice lacking mature NKT cells due to genetic disruption of the CD1d molecule, which is essential for maturation and differentiation of NKT cells. Methods: Male CD1d-knockout (KO) and wild-type (WT) C57Bl/6 mice 8 weeks after birth were given repeated intraperitoneal injections of TAA (3 times/week; 0.1 mg/g BW for the initial week, 0.2 mg/g BW for latter weeks) for up to 9 weeks. Mice were sacrificed at 5 and 9 weeks, and serum and liver samples were obtained. Serum ALT was measured, and liver histology was assessed by H-E and picro-Sirius red staining. The expression of activated hepatic stellate cells (HSCs) was determined by smooth muscle α-actin (SMA) immunohistochemistry. Further, mRNA levels of TGFβ1, αI(1)procollagen (COL1A1), and tissue inhibitor of matrix metalloproteinase (TIMP)-1 were measured by real-time RT-PCR. Results: Half number of WT mice died during the initial week of TAA administration, whereas all of CD1d-KO mice given the same treatment survived. CD1d-KO mice treated with TAA for 5 weeks demonstrated milder necro-inflammatory changes in the liver, as well as significantly lower serum ALT levels, in comparison with WT mice given TAA. At the same time point, TAA-induced increases in α-SMA-positive cells were markedly prevented in CD1d-KO mice. Further, TAA-induction of TGF-β mRNA in the liver was also blunted significantly in CD1d KO mice, the levels being nearly 38% lower than those in WT mice. WT mice developed overt bridging fibrosis in 9 weeks of TAA administration; however, CD1d-KO mice showed minimal fibrotic changes with the same treatment. In fact, TAA-induced increases in mRNA for COL1A1, as well as TIMP-1, were largely blunted in CD1d-KO mice, the levels only reaching 30% and 14% of those in WT mice, respectively. Conclusions: These findings clearly demonstrated that TAA-induced fibrogenesis was remarkably ameliorated in CD1d-KO mice, in which mature NKT cells are depleted. Since CD1d plays a key role in lipid antigen presentation, it is postulated that this molecule facilitates oxidative injury-triggered innate immune responses involving NKT cells, thereby participating in the development of TAA-hepatotoxicity and subsequent profibrogenic responses.

Disclosures:

The following people have nothing to disclose: Sachiko Ishikawa, Kenichi Ikejima, Hisafumi Yamagata, Tomonori Aoyama, Kazuyoshi Kon, Kazuyoshi Takeda, Sumio Watanabe

1113
HEPATIC STELLATE CELLS CONTRIBUTE TO THE MAINTENANCE OF CANCER STEM CELL-LIKE POPULATIONS VIA WNT SIGNALING IN HUH7 HEPATOCELLULAR CARCINOMA CELL LINE

Masayuki Adachi, Hajime Higuchi, Ayano Kabashima, Gen Sakai, Motoko Izumiya, Hiromasa Takaishi, Hidetsugu Saito, Toshifumi Hibi; Medicine, Keio University School of Medicine, Tokyo, Japan

BACKGROUND: Cancer stem cells play crucial roles in cancer development and progression. The function and survival of stem cells appears to be regulated by local microenvironment or niche. In many tumors, tumor cells actively recruit a variety type of cells including myofibroblasts and create a tumor niche that participates in the process of cancer progression, chemo-resistance, and metastasis. During liver injury, hepatic stellate cells (HSCs) transdifferentiate into myofibroblasts and the resulting fibrosis is associated with the development of hepatocellular carcinoma (HCC). However, the possible contribution of HSCs to tumor-stroma interactions is totally unknown. Therefore, our **AIM** is to investigate precise role of HSCs on interactions with tumor cells, especially with cancer stem cells. **METHODS:** Hepatoma cell line Huh7 was used in this study. FACS analysis was used to evaluate stem cell markers such as side population (SP) and CD133. Each subpopulation was isolated and stem cell-like properties were evaluated by *in vivo* tumorigenesity and *in vitro* sphere-forming activity. To evaluate the role of HSCs on tumor-stroma interactions, Huh7 cells (at lower dish) and immortalized HSC line hTERT HSCs (at upper dish) were co-cultured in Transwell culture dishes. In some experiments, Huh7 and hTERT HSCs were mix-cultured in the upper dish and Huh7 cells in the lower dish was analyzed by FACS. **RESULTS:** We identified a small percentage (approximately 0.6%) of Huh7 cells as a side population (SP) relative to the main population (MP) cells by Hoechst 33342-based FACS profiles. In SP subpopulation, CD133-positive cells were enriched as compared with MP subpopulation (60.3% in total cells, 81.3% in SP, and 58.0% in MP). Only CD133-positive SP cells, but not CD133-negative SP cells nor CD133-positive MP cells, displayed tumor forming capacity and sphere forming activity, suggesting that distinct CD133-positive SP subpopulation have stem cell-like properties in Huh7 cells. The SP subpopulation of Huh7 is increased after the co-culture with hTERT HSCs for 4 days (7.6 hold), suggesting that HSCs maintains self-renewal of cancer stem cells in Huh7. CD133-positive cells were not increased after the co-culture. An increased number of SP subpopulation