gamma and PKC delta dependent and proceeds via a mitochondrial pathway.

Disclosures:

The following people have nothing to disclose: Andrea N. Johnston, Kathleen Ponzetti, Simon Hohenester, Mohammed S. Anwer, Cynthia R. Webster

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CARBON TETRACHLORIDE-INDUCED LIVER INJURY AND FIBROSIS CORRELATES WITH OSTEOPONTIN EXPRESSION IN MICE

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Background: Osteopontin (OPN) is a multifunctional matricellular cytokine that plays a significant role in innate immunity, cell survival, tumor invasion, and metastasis. **Aim**: We have previously shown that OPN promotes activation of quiescent hepatic stellate cells and increases collagen I expression and secretion. Here, we elucidated the role of OPN in the pathogenesis of hepatic fibrosis *in vivo* using both OPN transgenic mice (*Opn* Tg) and OPN knockout mice ($Opn^{-/-}$). **Methods**: Liver fibrosis was induced in C57BL/6 WT, *Opn* Tg and $Opn^{-/-}$ mice by i.p. injections of carbon tetrachloride twice a week for 1 month (5 μl CCl_4/10 g b. wt.), which induces significant oxidative stress via generation of $CCl_3^{\cdot}$ radical. Commercially available kits were used for biochemical assays. H&E staining and immunohistochemistry were carried out to determine the extent of liver injury. Samples were scored by an experienced hepatopathologist. **Results**: To decipher the role of OPN in progressive liver injury, we tested whether liver injury and fibrosis under chronic CCl_4 administration could correlate with OPN expression. WT mice under CCl_4 treatment showed marked elevation of serum AST, ALT and γGT, along with striking hepatic inflammation, necrosis, ballooning, activity score, activation of hepatic stellate cells, and scarring. All these pathophysiological markers were significantly elevated by CCl_4 in *Opn* Tg mice but were attenuated in $Opn^{-/-}$ mice compared to WT mice. There was up-regulation of collagen I and OPN proteins in CCl_4-treated *Opn* Tg mice, while the opposite occurred in $Opn^{-/-}$ mice, compared to CCl_4-treated WT mice. *Opn* Tg mice injected with CCl_4 showed elevated collagenous proteins, portal fibrosis, bridging fibrosis, greater collagen I band thickness, and fibrosis score than CCl_4-injected WT or $Opn^{-/-}$ mice. Immunohistochemical analysis revealed massive induction of OPN in biliary epithelial cells, oval cells, and hepatic stellate cells under CCl_4 treatment in WT and *Opn* Tg mice. OPN+ cells were organized in small nests or arborizing duct-like structures, while isolated cells were found at some distance from portal tracts. **Conclusion**: These results suggest that OPN plays a significant role in the pathogenesis of hepatic fibrosis *in vivo*; thus, opening up the possibility of blocking OPN for preventing the development of liver fibrosis.

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DELETION OF C-FLIP IN HEPATOCYTES AUGMENTS CCL4-INDUCED LIVER INJURY AND FIBROSIS

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The mechanisms that contribute to chronic liver injury and promote liver fibrosis are only incompletely understood. The caspase-8 homologue cellular FLICE inhibitory protein (cFLIP) acts to protect cells from apoptosis involving impaired activation of pro-caspase 8 at the level of the death inducing signaling complex (DISC). We have previously shown that deletion of c-FLIP enhances hepatocellular apoptosis from activation of receptors of the TNF-receptor superfamily. Based on these observations, we hypothesized that deletion of c-FLIP would augment acute and chronic liver injury and fibrosis from the hepatotoxin CCl4. To test this hypothesis mice exhibiting a hepatocyte-specific deletion of c-FLIP were generated using the cre-loxP system under control of an albumin promoter (ΔhepFLIP). To induce liver injury wild type (wt) und ΔhepFLIP mice were treated with CCl4 for a total of 8 weeks and liver injury was assessed at an early (acute injury) and late (chronic injury) stage through measurement of serum ALT. We observed increased ALT in ΔhepFLIP at both time points. At 24h ΔhepFLIP mice exhibited 2,5-fold higher serum transaminases compared to wt mice (ALT: 2865±425 vs. 7540±2804 wt vs. ΔhepFLIP, p=0,05). Following 8 weeks of treatment, the absolute levels of ALT were slightly lower, however liver injury from CCl4 was still significantly higher in ΔhepFLIP mice compared to wt mice (ALT: 3317±690 vs. 4914±709, wt vs. ΔhepFLIP). Histological analysis of H&E and Goldner-stained liver sections revealed that ΔhepFLIP mice exhibited significantly more necrotic hepatocytes and hepatic fibrosis. Additionally, transcription of collagen I, measured by real time-PCR was increased by 2.6 compared to wt mice. Since interleukin 6 (IL6) was previously shown to augment liver injury and fibrosis from CCl4 we examined mRNA levels of this pro-inflammatory cytokine and found that IL6 was increased 6-fold in ΔhepFLIP mice compared to wt mice. In summary, deletion of c-FLIP in hepatocytes augments CCl4-induced acute and chronic liver injury and hepatic fibrosis potentially involving increased levels of IL6. These findings imply that c-FLIP could potentially contribute to liver regeneration and fibrosis in addition to the regulation of cell death pathways.

Disclosures:

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