Macrophage cell-specific deletion of cathepsin D amplifies liver inflammation and fibrosis

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Background and Aims: Changes in proteolytic activity are essential to liver fibrosis development. Proteases control not only matrix turnover but also, the activation and repression of growth factors and chemokines influencing disease progression. Beyond metalloproteases, our knowledge of the proteolytic enzymes contributing to liver fibrosis is limited. The importance of lysosomal protease cathepsin D (CtsD) in kidney fibrosis has been recently demonstrated but its role in liver fibrosis remains elusive. Thus, the aim of this study was to analyse CtsD cells-specific role during liver fibrosis.

Method: To study the cell-specific role of CtsD we generated two novel knock-out mouse strains by breeding LysM-Cre (macrophages) or Albumin-Cre (hepatocytes) mice with CtsD floxed mice. CtsD cell-specific deletion was confirmed in primary isolated hepatocytes and peritoneal macrophages by PCR and WB. Fibrosis was established chronically by CCl₄ (0.5µl/g) in CtsD^{AlbCre+/+} and CtsD^{AlbCre-/-} or CtsD^{LysMCre+/+} and CtsD^{LysMCre-/-}. Liver damage was determined by serum ALT and H&E staining. Fibrotic and inflammatory genes were determined by real-time-PCR in total liver. CtsB, CtsD and α-SMA were determined by immunohistochemistry or WB.

Results: First we determined CtsD expression in WT after chronic CCl₄ challenge, demonstrating CtsD presence in hepatocytes and macrophages. CtsD cell-specific deletion was validated by protein expression (WB) and floxed allele recombination (PCR), using primary mouse hepatocytes and peritoneal macrophages from CtsD^{AlbCre+/+} and CtsD^{LysMCre+/+} respectively and in comparison, with their floxed counterparts. To note, cathepsin B expression remained unaffected in hepatocytes or macrophages from CtsD^{AlbCre+/+} or CtsD^{LysMCre+/+} respectively. Neither CtsD deletion in macrophages nor in hepatocytes affected liver damage after chronic CCl₄ administration. CtsD cell-specific deletion in macrophages, but not in hepatocytes, increased liver fibrosis as shown by an increase in Sirius red staining, alpha-SMA protein expression and increased hepatic alpha-SMA, TGF-beta and Col1A1 mRNA. Furthermore, inflammation was affected only in macrophage-CtsD deficient mice showing significant increase in CCL2, CCL3 and CCL4. Finally, lack of CtsD in macrophages during liver fibrosis resulted in an upregulation of MMPs, such as MMP7 and MMP13.

Conclusion: Specific deletion of CtsD in macrophages, but no in hepatocytes, triggers upregulation of inflammation and matrix remodelling, leading to an amplification of the fibrotic response.