

Collagenolytic activity in macrophages is dependent on lysosomal protease cathepsin D during liver fibrosis.

Valeria Pistorio^{1,2*}, Paloma Ruiz-Blazquez^{1*}, Maria Fernández-Fernández¹, Susana Núñez³, Carmen García-Ruiz^{1,3,4,5}, José Fernandez-Checa^{1,3,4,5}, Anna Moles^{1,3,4}

¹Institute of Biomedical Research of Barcelona. Spanish Research Council, Barcelona, Spain;

²University of Naples Federico II, Napoli, Italy, ³CiberEHD, Spain; ⁴IDIBAPS, Barcelona, Spain; ⁵USC Research Center for ALPD, Los Angeles, United States.

*both authors contributed equally to this work.

Email: anna.moles.fernandez@gmail.com

Background: Changes in proteolytic activity are essential to liver fibrosis development. During fibrosis there is a dysregulation between extracellular matrix (ECM) synthesis and degradation, resulting in excessive ECM accumulation. Previous reports suggest that macrophages are important effectors for ECM remodelling through phagocytosis and processing of ECM within acidic compartments. However, the role of macrophages in ECM remodelling during liver fibrosis is unknown.

Objectives: Thus, the aim of this study was to investigate the proteolytic and degradative pathways associated to macrophages during liver fibrosis.

Methods: To study the proteolytic and degradative signals contributing to fibrosis in macrophages we focused our attention on the lysosomal protease cathepsin D. Accordingly, we generated and validated a novel macrophage-CtsD knock-out mouse strain by breeding LysMCre (macrophages) with CtsD floxed mice. Peritoneal macrophage polarization from CtsD^{ΔMyel+/+} or CtsD^{ΔMyel-/-} mice was achieved with LPS for M1 or IL4/IL13 for M2 stimulation. Polarization markers and MMP profile were assessed by real-time-PCR. Collagen degradation and endocytosis was studied using DQTM Collagen, type I and 10KDa Dextran probes respectively, and WB for Endo180 and UPAR. Lysosomal colocalization was determined using LAMP2. Finally, fibrosis was established chronically by CCl₄ (0.5μl/g) in CtsD^{ΔMyel+/+} or CtsD^{ΔMyel-/-} mice and determined by SR staining, α-SMA IHP and Col1 WB as well as RT-PCR in total liver.

Results: First, CtsD deletion in macrophages from CtsD^{ΔMyel-/-} mouse was confirmed by WB, CtsD activity in macrophages and dual IHP (F4/80-CtsD) in liver tissue. To note, cathepsin B expression remained unaffected despite deletion of CtsD. M1 and M2 polarization of CtsD^{ΔMyel+/+} and CtsD^{ΔMyel-/-} macrophages using LPS (10 and 50 ng/mL) or IL4/IL13 (20 and 50 ng/mL) resulted in similar and significant induction of iNOS (M1) and CD206 (M2) gene expression respectively. However, while M1 downstream effector CCI2 was similarly induced between CtsD^{ΔMyel+/+} and CtsD^{ΔMyel-/-} macrophages after LPS stimulation, IL10 was defectively induced in M2-primed CtsD^{ΔMyel-/-} macrophages. In addition, both, M1 and M2 CtsD^{ΔMyel-/-} macrophages displayed defective induction of MMP-2, -3 and -7 gene expression. CtsD^{ΔMyel-/-} macrophages showed a significant decrease in DQTM Collagen, type I degradation versus CtsD^{ΔMyel+/+} macrophages at 37C. As expected, no degradation was detected at 4C. Collagen degradative profile colocalized partially with LAMP2, indicating that collagen was degraded within the lysosome. Furthermore, Dextran endocytosis in both CtsD^{ΔMyel+/+} and CtsD^{ΔMyel-/-} macrophages remained unaffected. CtsD^{ΔMyel-/-} demonstrated enhanced liver fibrosis as shown by an increase in Sirius red staining, alpha-SMA and Col1A1 gene and protein expression with no affectation of the Endo180/UPAR collagen internalization receptors (WB).

Conclusion: Lysosomal cathepsin D is essential for a correct collagenolytic activity displayed by macrophages during liver fibrosis.