Biliary senescence affects the transcriptomic landscape of murine hepatocytes

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Background and aims: Biliary diseases are often characterised by biliary epithelial cell (BEC) senescence which, together with its senescence-associated secretory phenotype (SASP), is proposed to regulate liver injury. However, the effects of BEC senescence and its SASP on the modulation of hepatocyte biology remain unexplored. Here, we aimed to generate an in vitro model of BEC senescence and study its impact on hepatocyte transcription dynamics.

Method: A murine primary BEC line (Ep-CAM+CD24+CD133+) was treated with etoposide (a DNA topoisomerase inhibitor) to induce senescence, which was assessed via microscopy and RT-qPCR. Conditioned medium from senescent BECs was subsequently added to naïve BECs to confirm transmitted BEC senescence. A non-contact insert-based system was then used to co-culture senescent BECs and naïve primary hepatocytes. Bulk RNA-seq and transcriptomic analysis were performed to assess the effects of the BEC SASP in hepatocytes.

Results: Senescence and SASP in etoposide-treated BECs were confirmed by a significant increase of senescence-associated beta-galactosidase activity and gene expression of *Cdkn1a*, *Trp53bp1*, *Il6*, *Ccl2*, *Cxcl2*, *Cx3cl1*, *Serpine1*, *Vegfa* and *Pdgfa*. These were accompanied by a decrease in proliferation (EdU fluorescence and *Mki67* gene expression). The secretome of senescent BECs promoted secondary senescence and SASP-related gene expression in naïve BECs, including significantly increased *Cdkn1a*, *Cdkn1b*, *Cx3cl1* and *Vegfa*. Differential expression and gene set enrichment analyses of RNA-seq data from treated BEC donors confirmed the presence of senescence and SASP-related factors. In hepatocytes exposed to senescent BECs, differential expression of RNA-seq data revealed a significant increase of the SASP-related factor *Vcam1*. Functional enrichment analysis using the STRING database retrieved the highest enriched terms (false discovery rate [FDR] <0.0001): (1) decreased “fatty acid omega-oxidation” (enrichment score [ES] 7.65); (2) increased “neutrophil extravasation, and myeloperoxidase” (ES 6.16); and (3) increased “cathelicidin, and neutrophil aggregation” (ES 5.56). Additional terms (FDR <0.01) included decreased “drug metabolism-cytochrome P450” (ES 4.01); increased “leukocyte migration involved in inflammatory response” (ES 2.58); and increased “regulation of chemokine production” (ES 1.57).

Conclusion: Murine BEC senescence was modelled in vitro. In our experimental setting, senescent BECs present SASP features typical of human biliary diseases. Our model revealed several BEC-dependent SASP-induced changes in hepatocytes that reflect structural, metabolic and functional alterations. Together, this data suggests that, in the context of biliary disease, hepatocytes exposed to senescent BECs display decreased metabolism and enhanced immunomodulatory abilities.