ABSTRACT# 16

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Title: Cyr61 coordinates liver fibrosis through monocyte and macrophage recruitment and polarization
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Background: Obesity is increasing worldwide and can lead to a multitude of GI complications. Major complications of obesity include non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatosis (NASH). Aside from dietary intervention, there are few treatments available. The Hippo signaling pathway and its effector transcriptional coactivator YAP are responsible for fibrotic and inflammatory responses in injured hepatocytes. Our previous work demonstrates that YAP-target Cyr61 is the main mediator of these effects. The fibrotic activity of YAP/Cyr61 is only seen in the presence of circulating and liver-resident monocytes and macrophages. We hypothesize that Cyr61 modulates liver fibrosis primarily through activation of macrophages and could be targeted as a therapeutic for chronic liver injury.

Methods: Mice lacking hepatocytic Cyr61 (Cyr61<sup>ΔHep</sup>) were treated with a NASH-inducing diet for 12 weeks, and Cyr61 expression was induced in hepatocytes using an AAV-Cyr61 vector (AAV-Cyr61<sup>Hep</sup>). Macrophage polarization was examined in vivo using mass cytometry and flow cytometry and in vitro using bone marrow-derived macrophages.

Results: Cyr61<sup>ΔHep</sup> NASH livers showed less fibrosis than control, while AAV-Cyr61<sup>Hep</sup> livers showed more fibrosis than control. RNA sequencing shows decreased inflammatory (TNFa, CCL2, Ly6C, TLR1/2) and fibrotic (Col1a1, Acta2, TIMP1, CTGF) gene expression in Cyr61<sup>ΔHep</sup> NASH livers compared to control, and increased expression of the pro-resolution macrophage marker CD163. Cyr61<sup>ΔHep</sup> NASH livers have fewer infiltrating monocytes (CD11b<sup>+</sup>) that express high levels of inflammatory and fibrotic markers (TNFa, TGFb) and more pro-resolution macrophages (CD11b<sup>lo</sup>, F4/80<sup>+</sup>) that express low levels of inflammatory and fibrotic markers. AAV- Cyr61<sup>Hep</sup> livers have more pro-inflammatory macrophages and fewer pro-resolution macrophages. Furthermore, treatment with Cyr61 protein increases expression of pro-inflammatory genes (iNOS, IL-6) in bone marrow-derived macrophages.

Conclusions: Cyr61 from hepatocytes attracts and polarizes macrophages towards an inflammatory state upon liver injury, leading to fibrosis. Lack of Cyr61 during injury reduces fibrosis and inflammation, indicating that targeting Cyr61 during liver injury could be an effective therapeutic avenue.

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