Poster

GATA4: a key factor for liver fibrosis regression

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ABSTRACT

Motivation: Several studies have shown that the reversion of active HSCs to a quiescent state is required for liver fibrosis regression. Previous work in our lab have identified GATA4 as key factor for the maintenance of HSCs quiescence. Injection of adenovirus overexpressing GATA4 (Ad-GATA4) via the tail vein in CCl4-treated mice have been used as an approach to revert the fibrosis. Using this method, adenoviruses are sequestered by all hepatic cells. We will quantify the % of Adenoviruses that reach the HSCs and other liver cells to test whether the liver regression is due to the overexpression of GATA4 in active HSCs.

A transcriptional repression of HIF2α has been uncover in the group as a molecular mechanism by which GATA4 inactivates fibrotic HSCs during liver fibrosis regression. Two conserved GATA sites in the Hif2α gene have been identified. We will test whether the repression of Hif2α by GATA4 requires these two GATA sites. Finally, we will develop a vector containing HSCs-specific enhancer from the Gata4 gene (hG2) fused to a luciferase reporter gene. Using this construct (hG2-luc), we will generate a stable cell line to screen FDA-approved drugs that activate Gata4 expression in active (fibrotic) HSCs to induce the quiescence.

Methods: Adenovirus overexpressing GFP were injected in mice via the tail vein. Livers from treated mice were analyzed by immunofluorescence for GFP and Desmin (HSCs marker) or GFP and HNF1 (hepatocyte marker). Quantification of double marked cells will be done using ImageJ software. A region of Hif2α containing wild type or mutated GATA sites was fused to the luciferase reporter. 293T cells were transiently transfected with each reporter plasmids and pCI-GATA4 expression vector. Luciferase activity was measured using a commercial kit. Finally, we have cloned the HSCs-specific enhancer from the Gata4 gene into the pGL4-luciferase vector to generate stable LX2 cell line harboring this construct.

Results: Around 50% of HSCs were positive for GFP and Desmin. Mutation of GATA sites in the Hif2α avoid transcriptional repression by GATA4. Finally, the pGL4-hG2-TK construction has been successfully generated to develop a stable cell line.

Conclusions: Adenoviruses injection via tail vein in mice efficiently reach HSCs. GATA4 transcriptionally repress Hif2α via two identified conserved GATA sites. The hG2-luc construct to generate stable lines will allow us to identified potential drugs to induce liver fibrosis regression.

REFERENCES

Sun, M. and Kisseleva, T. (2015). Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol, 39, S60-S63

Lambrecht, J., van Grunsven, L.A. and Tacke, F. (2020). Current and emerging pharmacotherapeutic interventions for the treatment of liver fibrosis. Expert Opin Pharmacother, 21(13), 1637-1650

Delgado, I., Carrasco, M., Cano, E., Carmona, R., García-Carbonero, R., Marín-Gómez, L.M., Soria, B., Martín, F., Cano, D.A., Muñoz-Chápuli, R. and Rojas, A. (2014). GATA4 loss in the septum transversum mesenchyme promotes liver fibrosis in mice. Hepatology, 59(6), 2358-2370