GATA4: a key factor for liver fibrosis regression

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Motivation

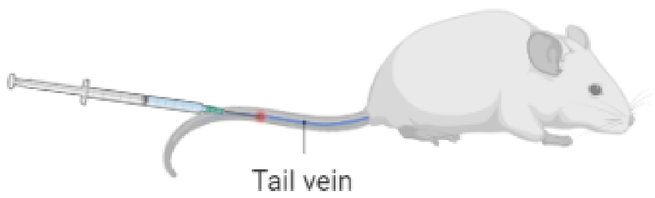
Several studies have shown that the reversion of active HSCs to a quiescent state is required for liver fibrosis regression. Previous work in our lab have identified GATA4 as key factor for the maintenance of HSCs quiescence. Injection of adenovirus overexpressing GATA4 (Ad-GATA4) via the tail vein in CCl_4-treated mice have been used as an approach to revert the fibrosis. Using this method, adenoviruses are sequestered by all hepatic cells. As the first objective of this project 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.

Transcriptional repression of HIF2α by GATA4 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 *Hif2a* gene have been identified. As a second objective we will test whether the repression of *Hif2a* by GATA4 requires these two GATA sites. Finally, as a third objective, 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.

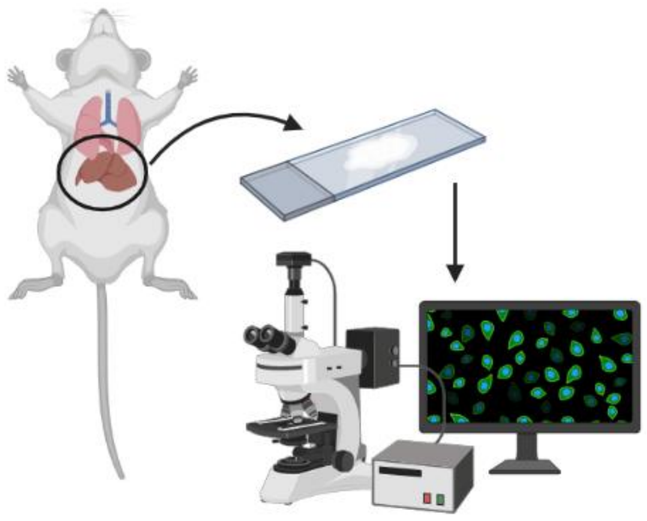
Methods

Objective 1

Intravenous administration of Ad-GFP



1 week later mice were sacrified



Immunofluorescence in liver sections of mice treated with Ad-GFP for

GFP and Desmin (HSCs marker)

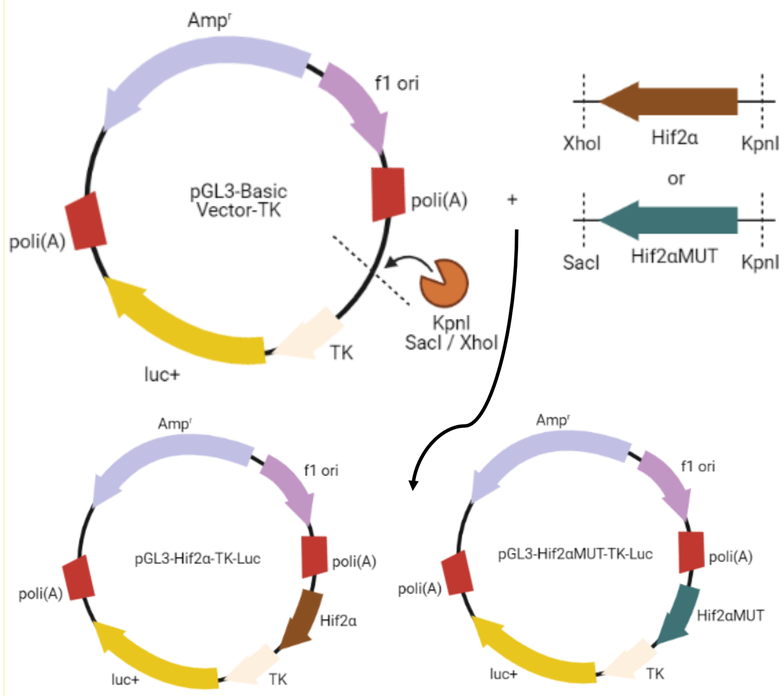
or

GFP and HNF1 (hepatocyte marker)

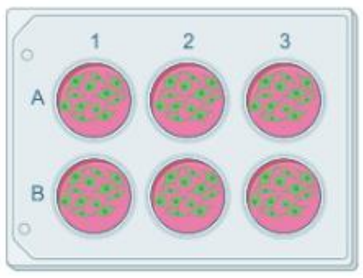
Quantification of double marked cells will be performed using ImageJ software

Objective 2

Cloning process of pGL3-Hif2α(MUT)-TK-Luc and transfection experiments



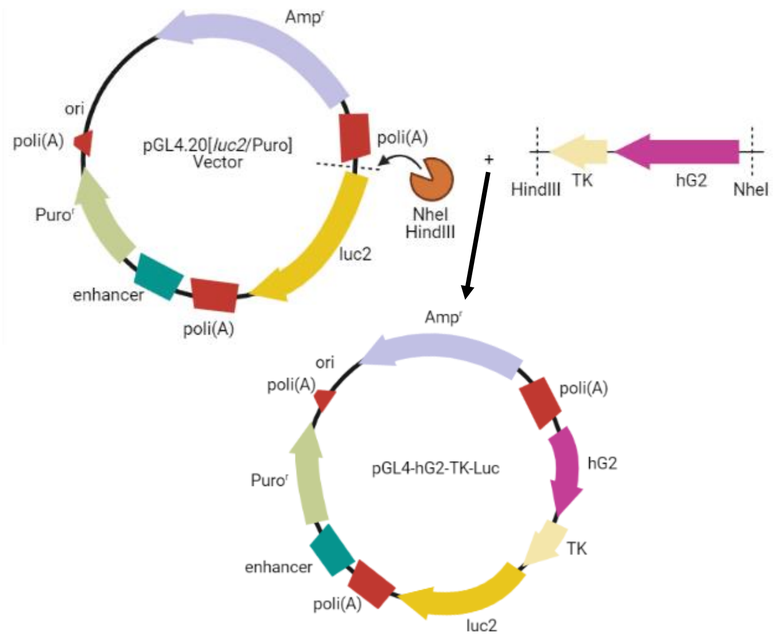
293T cells were transiently transfected with
- each reporter plasmid,
- pCI-GATA4 or pCI-Ø expression vector, and
- control vector for Renilla luciferase expression



Luciferase activity was measured using a commercial kit

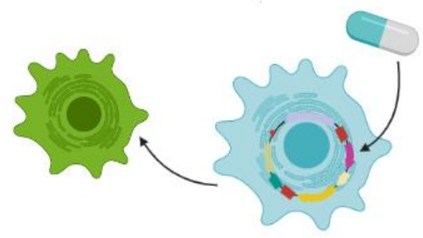
Objective 3

Cloning process of pGL4-hG2-TK-Luc and generation of a stable cell line



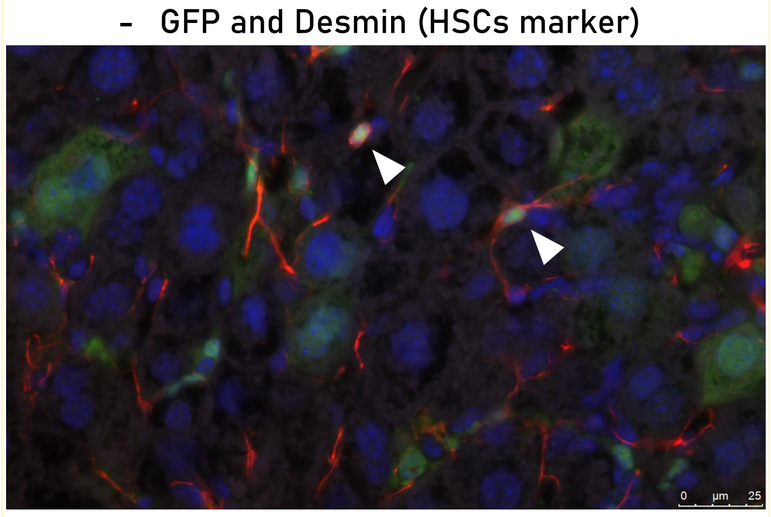
LX2 cells were transiently transfected with the reporter plasmid containing the HSCs-specific enhancer from the *Gata4* gene in order to generate stable LX2 cell line harboring this construct. This construct will allow the FDA-approved drugs screening to identify activators of *Gata4* expression in HSCs

Luciferase activity will be measured using a commercial kit

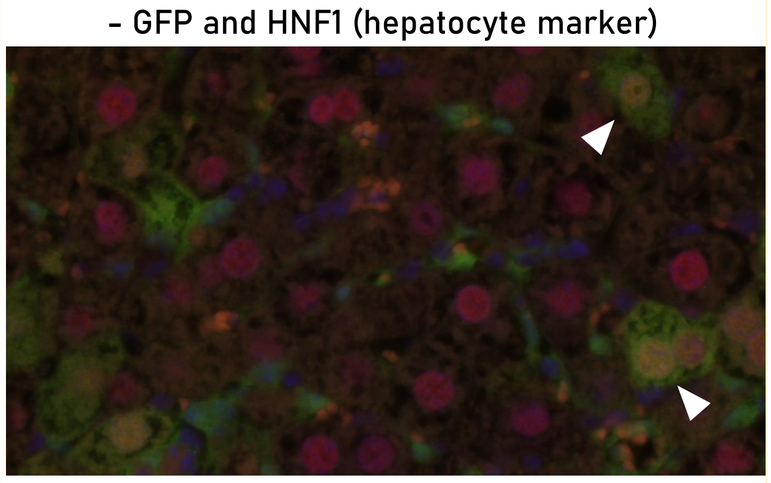


Results and conclusions

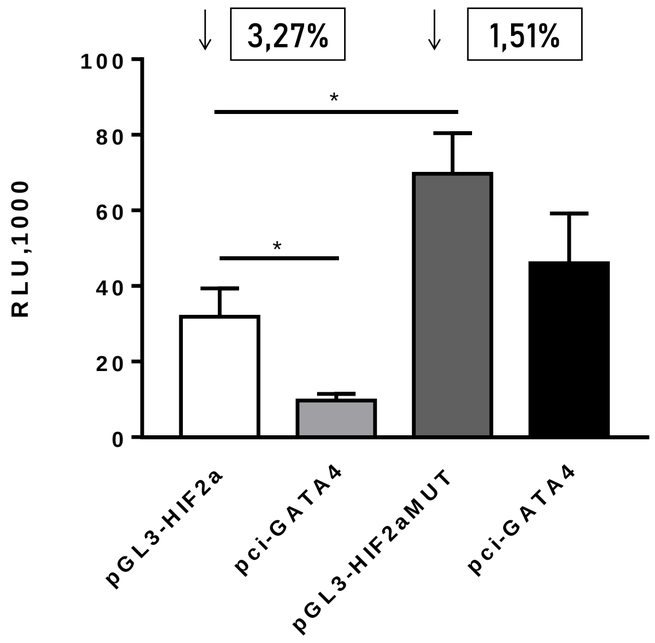
- GFP and Desmin (HSCs marker)



- GFP and HNF1 (hepatocyte marker)



Data from preliminary quantification showed that around 50% of HSCs were positive for GFP and Desmin. So that, adenoviruses injection via tail vein in mice efficiently reach HSCs.

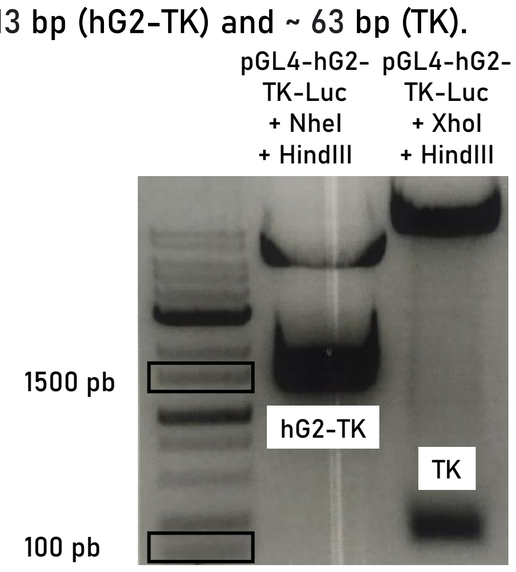


As its seen in the graphic, mutation of GATA sites in the *Hif2a* avoid transcriptional repression by GATA4, letting to know that GATA4 transcriptionally repress *Hif2a* via two identified conserved GATA sites.

The difference between basal groups can be explained by the activation of endogenous GATA4 that exists in the 293 cell line.

The 1,51% of repression observed in HIF2αMUT must be due to a third GATA site non mutagenized in the *Hif2a*, as well as GATA4 is forced to bind to the endogenous GATA sites of 293 cells.

pGL4-hG2-TK-Luc construction has been successfully generated to develop a stable cell line. As shown, digestion of the vector obtained with restriction enzymes NheI/HindIII and XhoI/HindIII released, respectively, a fragment of ~ 1513 bp (hG2-TK) and ~ 63 bp (TK).



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