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Research Article

Adenoviral overexpression of Lhx2 attenuates cell viability but does not preserve the stem cell like phenotype of hepatic stellate cells

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ABSTRACT

Hepatic stellate cells (HSC) are well known initiators of hepatic fibrosis. After liver cell damage, HSC transdifferentiate into proliferative myofibroblasts, representing the major source of extracellular matrix in the fibrotic organ. Recent studies also demonstrate a role of HSC as progenitor or stem cell like cells in liver regeneration. Lhx2 is described as stem cell maintaining factor in different organs and as an inhibitory transcription factor in HSC activation. Here we examined whether a continuous expression of Lhx2 in HSC could attenuate their activation and whether Lhx2 could serve as a potential target for antifibrotic gene therapy. Therefore, we evaluated an adenoviral mediated overexpression of Lhx2 in primary HSC and investigated mRNA expression patterns by qRT-PCR as well as the activation status by different *in vitro* assays. HSC revealed a marked increase in activation markers like smooth muscle actin alpha (αSMA) and collagen 1α independent from adenoviral transduction. Lhx2 overexpression resulted in attenuated cell viability as shown by a slightly hampered migratory and contractile phenotype of HSC. Expression of stem cell factors or signaling components was also unaffected by Lhx2. Summarizing these results, we found no antifibrotic or stem cell maintaining effect of Lhx2 overexpression in primary HSC.

Abbreviations: αSMA, alpha smooth muscle actin; ECM, extracellular matrix; eGFP, enhanced green fluorescence protein; HSC, hepatic stellate cells; Lhx2, LIM homeobox protein 2; PDGF, platelet derived growth factor; p.i., post infection; Sox9, sex determining region Y-box 9; TGF-β, transforming growth factor beta

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Introduction

Hepatic stellate cells (HSC) are – beside their role as vitamin A-storing cells – major players in regenerative and fibrotic processes within the liver [1,2]. As a result of liver cell damage and injury by toxic reagents (alcohol, acetaminophen, carbon tetrachloride, etc.), viral infection (hepatitis B and C), primary and secondary cholestasis (bile duct ligation, Mdr2$^{-/-}$, etc.) or fatty degeneration (methionine-choline-deficient diet) HSC transform to myofibroblast-like cells [3]. This is characterized by a change in gene and protein expression patterns including a switch from inhibitory to activating transcription factors [4] as well as from epithelial to mesenchymal marker genes [5,6]. The activation process is divided into two phases. The initiation phase is triggered by secretion of transforming growth factor (TGF)-β and platelet derived growth factor (PDGF)-BB and is followed by the perpetuation phase, which results in an increased expression of alpha smooth muscle actin (αSMA) and extracellular matrix proteins such as collagen 1α and 3α [7].

Beside the fact that HSC undergo transdifferentiation during activation, the expression of stem and progenitor cell markers like CD133 (prominin 1), Octamer binding factor 4 (Oct4, the product of Pou5f1) and Nanog supports the assumption that HSC can function as progenitor or stem cell like cells in liver regeneration [8]. Additional features of HSC underline this hypothesis, as e.g. their localization in the liver stem cell niche or the presence of stem cell related signaling pathways (wnt, hedgehog, notch) that are essential for maintenance of HSC quiescence [9,10]. Furthermore, similar to hematopoietic stem cells, quiescent HSC proliferate in response to CD95 ligand instead of becoming apoptotic [11]. Moreover, several groups already showed that HSC are capable to differentiate into hepatocyte like cells *in vitro* or *in vivo* after liver damage and thus represent an important source for epithelial progenitors in the regenerating liver [8,12,13].

The transcription factor Lhx2 is part of the family of LIM homeobox proteins that are defined by the presence of a DNA-binding (homeodomain) and a protein binding (LIM) domain [14]. Lhx2 has a prominent role in developmental processes of the central nervous system, the optic cup and in establishment of the hepatic microenvironment [15]. As a consequence, Lhx2 knockout mice are not viable [16]. Examination of fetal Lhx2$^{-/-}$ livers showed a fibrotic phenotype with accumulation of extracellular matrix and an increased number of αSMA-positive and thus activated HSC [17]. Therefore, Lhx2 is presumed to function as an inhibitory transcription factor in HSC activation, maintaining HSC quiescence [4].

Furthermore, Lhx2 is described as occupying a "central switch-position" between proliferation and differentiation of stem cells [18]. In the hair follicle bulge [19,20] as well as in the neuroepithelium during corticogenesis [21] Lhx2 regulates the maintenance and identity of stem and progenitor cells by coordinating expression of stem cell determining factors like sex determining region Y-box 9 (Sox9) or Notch signaling components.

Here, we hypothesized that overexpression of Lhx2 in primary HSC could inhibit the activation process during cultivation on plastic surface and could preserve their stem cell like phenotype *in vitro* by regulating expression of stem cell factors. Therefore, we used an adenoviral vector to generate a continuous expression of Lhx2 in primary HSC over a time period of seven days in culture.

Table 1 – List of genes used for Fluidigm qRT-PCR analysis and network construction.

NCBI symbol	Gene name [Mus musculus]	NCBI reference sequence
BMP-9	Bone morphogenetic protein 9	NM_019506.4
Brcp1	ATP-binding cassette (Abcg2)	NM_011920.3
CD105	endoglin (Eng), transcript variant 3	NM_001146348.1
CD133	Prominin-1 (Prom1)	NM_001163583.1
CD146	melanoma cell adhesion molecule (Mcam)	NM_023061.2
CD29	integrin beta 1 (Itgb1)	NM_010578.2
CD34	CD34 antigen (Cd34), transcript variant 1	NM_001111059.1
CD73	5′ nucleotidase, ecto (Nt5e)	NM_011851.4
c-kit	kit oncogene (Kit)	NM_001122733.1
Col1a1	collagen, type I, alpha 1	NM_007742.3
Col3a1	collagen, type III, alpha 1	NM_009930.2
CXCR4	chemokine (C-X-C motif) receptor 4	NM_009911.3
Dkk3	dickkopf homolog 3	NM_015814.2
Edn1	endothelin-1	NM_010104.3
Foxf1	forkhead box F1	NM_010426.2
Fzd2	frizzled homolog 2	NM_020510.2
GFAP	glial fibrillary acidic protein (Gfap)	NM_001131020.1
Ldb2	LIM domain binding 2 (Ldb2), transcript variant 1	NM_010698.3
Ldb3	LIM domain binding 3 (Ldb3), transcript variant 1	NM_011918.4
Lgr5	leucine rich repeat containing G protein coupled receptor 5	NM_010195.2
Lhx2	LIM homeobox protein 2	NM_010710.3
Mki67	antigen identified by monoclonal antibody Ki 67	NM_001081117.2
MMP10	matrix metallopeptidase 10	NM_019471.2
Musashi1	musashi RNA-binding protein 1	NM_008629.1
Nanog	Nanog homeobox	NM_028016.2
Nes	Nestin	NM_016701.3
Notch1	Notch gene homolog 1	NM_008714.3
Notch3	Notch gene homolog 3	NM_008716.2
Oct4	POU domain, class 5, transcription factor 1 (Pou5f1)	NM_013633.3
p75NTR	nerve growth factor receptor (Ngfr)	NM_033217.3
Pdgfb	platelet derived growth factor, B polypeptide	NM_011057.3
PITX2c	paired-like homeodomain transcription factor 2	NM_001042502.1
PPARgamma	peroxisome proliferator activated receptor gamma	NM_001127330.1
Survivin	baculoviral IAP repeat-containing 5 (Birc5)	NM_001012273.1
Rac1	RAS-related C3 botulinum substrate 1	NM_009007.2
SCGF	C-type lectin domain family 11, member a (Clec11a)	NM_009131.3
Shh	sonic hedgehog	NM_009170.3
Slain1	SLAIN motif family, member 1	NM_198014.2
Smad6	SMAD family member 6	NM_008542.3
Smad7	SMAD family member 7	NM_001042660.1
Sox9	SRY-box containing gene 9	NM_011448.4
Flt3	FMS-like tyrosine kinase 3 (Flt3)	NM_010229.2
Tcf4	transcription factor 4	NM_013685.2
Tgfb2	transforming growth factor, beta 2	NM_009367.3