Projekt HRZZ: NOFIBRO

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The Role of Notch Signalling Pathway in Pathogenesis of Hepatic Fibrosis

ACRONIM: NOFIBRO

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Otvori sve

ABSTRACT

Chronic liver diseases are a major health burden associated with severe morbidity and mortality. Hepatic fibrosis is a common feature of various liver diseases such as viral hepatitis, biliary hepatitis, alcohol liver disease, fatty liver disease, which are all characterized by the accumulation of extracellular matrix. Progressive fibrotic processes may lead to liver cirrhosis. Recent fate mapping studies have suggested that activated hepatic stellate cells (HSC) are a major source of myofibroblasts, αSMA positive fibrogenic cells that produce extracellular matrix during fibrogenesis. However, signals that activate quiescent HSC are still not elucidated, recent evidences suggested the possible role of Notch signalling pathway.

The main goal of project proposal is to define the role of Notch signaling in pathogenesis of hepatic fibrosis. To achieve this we will use animal transgenic mice model in which Notch signalling can be activated or inhibited by tamoxifen injection selectively in alphaSMA expressing cells. To these mice hepatic fibrosis will be induced by CCL4 application and DDC feeding. In some experiments we will inhibit Notch pathway pharmaceutically by application of SAHM1 which is Notch specific in comparison with inhibitors used in previous studies. We will also collect human samples from cirrhotic and noncirrhotic liver tissue and assess expression of Notch related genes and proteins. From blood samples DNA will be extracted to assess association between the selected Notch pathway single nucleotide polymorphisms and hepatic fibrosis by comparing their prevalence in general population with one in patients with end-stage liver disease due to alcoholic cirrhosis which require liver transplantation.

INFORMACIJE O PROJEKTU

Chronic liver diseases

Chronic liver diseases are a major health burden associated with severe morbidity and mortality. Hepatic fibrosis is a common feature of various liver diseases such as viral hepatitis, biliary hepatitis, alcohol liver disease, fatty liver disease, which are all characterized by the accumulation of extracellular matrix. Initially, hepatic fibrosis is a part of normal healing response to the liver injury. If the injury is transient, the process of fibrosis is reversible and normal liver architecture may be restored after removal of the injury's cause. However, if the injury is sustained, progressive fibrotic processes may lead to liver cirrhosis, a common endpoint of various liver diseases characterized with substitution of normal liver tissue by scar tissue (Hernandez-Gea). Because there are no effective and clinically approved anti-fibrotic therapies available, liver transplantation is currently the only satisfactory patient therapy that restores organ functionality. For development of therapies targeted at reversing excessive accumulation of fibrous tissue, better understanding of cells and signals involved in the initiation and progression of fibrosis is necessary.

Recent fate mapping studies have suggested that activated hepatic stellate cells (HSC) are a major source of myofibroblasts, αSMA positive fibrogenic cells that produce extracellular matrix during fibrogenesis (Mederacke). Normally, HSC reside in space of Disse in the form of quiescent HSC (qHSC), specialized pericytes of the liver sinusoids that serve as storage for vitamin A. In response to various signals associated with liver damage and inflammation (TGF-β, PDGF etc), qHSC become activated. Activated HSC (aHSC) express alpha-smooth muscle actin (αSMA-positive) and differentiate into extracellular matrix producing myofibroblasts. Studies that specifically targeted αSMA-positive cells during the development of fibrogenesis have confirmed that modulation of their signaling pathways may ameliorate or reverse this pathogenic process (Michelotti). Some other cells such as portal fibroblasts, stromal bone marrow progenitor cells and epithelial cells might also differentiate into αSMA-positive myofibroblasts and contribute to fibrogenesis (Kisseleva, Hernandez-Gea). However, the level of their importance remains controversial. While some studies suggested that nonHSC derived myofibroblasts constitute significant proportion of αSMA-positive myofibroblasts, a study by Mederacke et al by tracking HSC (using Cre expression driven by lecithin-retinol acyltransferase (Lrat) to selectively mark HSCs) suggested HSCs as dominant contributors to myofibroblast population independent of fibrosis etiology, although the possibility that LratCre activity was turned on in non-HSC populations during fibrogenesis remained unsolved. The same study excluded possibility that aHSCs may differentiate into the hepatocytes or epithelial cells during the fibrogenesis as was reported by studies on hGFAP-Cre and αSMA-CreERT2 mice (Michelotti, Yang), thus concluding that nonHSCs positive for hGFAP or αSMA were a source of hepatocytes and epithelial cells in these studies.

Activation and differentiation of qHSC to aHSC and their subsequent differentiation into myofibroblast is a complex, multistep process that involves a number of mediators, different cell-to-cell signals and is still not fully understood. The most important mediators involved in qHSC activation appear to be PDGF and TGF-β, but various other cytokines (Leptin, VEGF, FGF etc) were shown to modulate fate of qHSC activation (Friedman). Recent studies suggested potential involvement of Notch signaling pathway in qHSC activation, suggesting that inhibition of Notch signaling might suppress transformation of qHSC to extracellular matrix producing fibroblasts (Chen, Bansal). However evidences are limited to in vitro experiments or to studies in which Notch signaling was inhibited in vivo pharmaceutically by γ-secretase inhibitors which are not Notch-specific, as they indiscriminately block signaling pathways downstream of γ-secretase.

Furthermore, since Notch modulation can affect many other cell types involved in liver fibrogenesis, most notably macrophages in which Notch signaling determines the M1 versus M2 polarization, such approaches might possibly have off target effects. Further studies are required to clarify the role of Notch signaling selectively in αSMA-positive myofibroblasts population in vivo.

In humans Notch signalling is necessary for normal liver development. Mutations in JAG1 or NOTCH2 result in Alagille syndrome, characterized by intrahepatic bile duct paucity and in some cases biliary atresia. However, most patients do not develop overt fibrosis. Trehanpati et al reported enhanced activation of Notch signaling during progression of HBV infection (Trehanpati). The involvement of Notch in hepatic fibrogenesis development which occurs in alcoholic liver disease is still not sufficiently investigated. Furthermore, Single nucleotide polymorphisms (SNP) of Notch signalling associated genes have been reported to be associated with risk for various diseases such as rheumatoid arthritis, breast carcinoma, schizophrenia etc (Zhang, Orent). However, their association with risk for development of hepatic fibrosis has not been studied yet.

Notch signaling mediates communication between neighbouring cells to control cell fate decisions both during embryogenesis and in postnatal life. The mammalian genome encodes four Notch receptors (Notch1-4) and at least five ligands (Jagged1 and 2 and Delta-like 1, 3 and 4). In the canonical Notch pathway, binding of ligands to Notch receptors expressed on the neighbouring cell triggers two successive intramembrane proteolytic cleavages of the receptors, mediated by the γ-secretase complex, resulting in the release of the Notch intracellular domain (NICD) from the membrane. NICD, then, translocates to the nucleus where it interacts with a transcription factor of the CSL family (RBPjk/CBF-1 in mammals) to activate transcription of target genes. Among the best known targets of Notch/RBPjk signaling are the Hes/Hey family of basic helix-loop-helix transcription repressors (Kopan). In the proposed investigation, we will use a transgenic mouse model enabling us to overexpress or inhibit Notch specifically in αSMA-positive cells, and the effect on overexpression/inhibition on fibrogenesis will be monitored. We also intend to analyse expression of Notch pathway in samples of human cirrhotic and noncirrhotic livers and to analyse asssociatiuon between the SNP of genes included in Notch signalling and liver fibrosis occurrence.

Scientific objectives

The objective of the proposed study is to characterize the effect of modulating Notch signaling in SMA-labeled cells on the process of hepatic fibrosis. We will utilize a transgenic mouse model using αSMA-promoter driving Cre recombinase (αSMACreERT2) in which expression of specific genes can be modulated in αSMA positive cells and cells can be identified and followed. Breeding procedures will be employed to generate transgenic mice with Notch overexpression or inhibition selectively in αSMA positive cells. The expression of Notch signalling components will be determined in human cirrhotic livers.

The overall objective will be studied by the following specific aims:

Specific Aim 1: Using transgenic αSMACreERT2 mice we will label and follow SMA positive cells during fibrogenesis. Different regimens of tamoxifen treatment will enable us to label and follow cells that basally express αSMA or cells that express αSMA following induction of fibrogenesis. Expression of Notch related target genes in αSMA positive cells (basally and at various points of fibrogenesis) will be determined, as well as their potential to differentiate into myofibroblasts and other liver cells (hepatocytes cholangiocytes).

Specific Aim 2: By in vivo and in vitro experiments asses effect of Notch overexpression in αSMA positive cells on development of hepatic fibrosis using two animal models of liver fibrosis: a) toxic model, induced by carbon tetrachloride (CCl4) injections and b) model of feeding with 3,5-Diethoxy-carbonyl-1,4-dihydrocollidine (DDC). Overexpression of Notch signaling will be induced by tamoxifen injection specifically in αSMA positive cells. Potential of differentiation of TdT positive cells and their progeny into the myofibroblasts, hepatocytes or cholangiocytes will be followed.

Specific Aim 3: By in vivo and in vitro experiments asses effect of Notch inhibition in αSMA positive cells on development of hepatic fibrosis. Experimental approach will be similar to aim 1, but this time transgenic mice (ΔRbpjκΔ) in which Notch inhibition can be induced will be used. We will also evaluate effects of treatment with Notch transcription factor complex inhibitor SAHM1 (stapled α-helical peptides derived from MAML1) on development of liver fibrosis

Specific Aim 4: To analyse the expression of Notch signalling components in collected samples of cirrhotic (end stage of alcoholic liver disease) and healthy human livers. The samples of cirrhotic liver will be collected during liver transplantation and samples of noncirrhotic liver from healthy liver tissue obtained during the tumor removal. To asses Notch signalling in earlier stages of alcoholic liver disease we will analyse samples of liver tissue obtained by biopsy of patients suffering from alcoholic hepatitis.

Specific Aim 5: to assess association between the selected Notch pathway single nucleotide polymorphisms and hepatic fibrosis by comparing their prevalence in general population with one in patients with end-stage liver disease due to alcoholic cirrhosis which require liver transplantation.

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