SPOCK1 EXPRESSION IN LIVER REGENERATION, CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

PhD thesis

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1. Introduction

Proteoglycans (PG) are core proteins covalently attached to glycosaminoglycan (GAG) chains. These molecules are synthesized in the endoplasmic reticulum and the Golgi apparatus. GAGs are repetitive sulphated disaccharide units composed of a galactose or a uronic acid (glucuronic acid or iduronic acid) and an amino-monosaccharide (N-acetylglucosamine or N-acetylgalactosamine).

PGs play an important role in the restoration of normal liver structure during liver regeneration. Undulating changes in the ribonucleic acid (RNA) expression of syndecan-1, perlecan, syndecan-2, and decorin have been observed after partial hepatectomy in rats. For example, decorin a dermatan sulfate PG, peaked at 30 minutes, 24 hours, and 96 hours, while perlecan peaked at 4 hours and 24 hours after partial hepatectomy. Syndecan-1 showed oscillatory dynamics during the first 24 hours, whereas syndecan-2 displayed decreasing levels throughout the tested period. Endocan is a circulating dermatan sulphate PG, and its hepatic levels decrease after partial hepatectomy, accompanied by an increase in serum levels.

Sparc/ osteonectin, cwcv, and kazal-like domains proteoglycan 1 (SPOCK1) is a chondroitin sulphate-heparan sulphate proteoglycan (CSHS-PG) that was first isolated and characterized from seminal plasma in 1992.

The human SPOCK1 gene is located on chromosome 5q31.2 and consists of 11 exons. SPOCK1 encodes a protein precursor of 439 amino acids organized in modular structures. The six identified domains are as follows: N-terminal hydrophobic signal sequence, N-terminal region, follistatin-like domain, extracellular (EC) calcium binding domain, Thyropin domain homology, Domain V.

Under normal conditions it is highly expressed in certain regions of the brain, it has been also detected in the heart muscle, skeletal muscle, prostate and testis. It is also involved in the development of the blood-brain barrier and in the adipocyte differentiation and maturation. Its known transcription factors are CHD1L and POU2F1 and interacts with the integrin α5β1 receptor. It is involved in the epithelial to mesenchymal transition (EMT), remodeling of the extracellular matrix (ECM) trough activation of matrix metalloproteinase 2 and 9 (MMP2 and MMP9) and activates the AKT and Wnt/β-catenin signaling pathways.

It has recently attracted considerable interest because of its involvement in several types of cancer, but little is known about its role in the normal liver, in the controlled proliferation of the hepatocytes during liver regeneration or in liver cirrhosis and hepatocellular carcinoma (HCC).

Acute ingestion of toxins (e.g. acetaminophen), trauma and fulminant hepatitis can cause extensive parenchymal necrosis which initiates liver regeneration. In human liver two distinct morphological patterns of regeneration have been distinguished after fulminant hepatic failure, depending on the cause and the extent of the parenchymal necrosis. (a) If significant viable parenchyma remains the surviving hepatocytes dedifferentiate, organize into acinar structures, and start to proliferate. (b) If the surviving parenchyma is negligible, the regeneration is accomplished by the progenitor cells. Prominent periportal ductular reaction is characteristic for this subtype of regeneration.

Cirrhosis is the diffuse nodular transformation of the liver after a long period of inflammation resulting in replacement of the normal liver parenchyma with fibrotic tissue and cirrhotic nodules. The strongest risk factor for HCC is chronic liver disease leading to cirrhosis.

Cirrhosis of any etiology can promote hepatocarcinogenesis, and in 41.0% of the HCC cases occur in the setting of hepatitis B-virus (HBV) infection, 28.5% in hepatitis C-virus (HCV) infection, 18.4% in alcoholic liver disease, 6.8% in metabolic dysfunction-associated steatotic liver disease (MASLD) and in 5.3% in other less-common risk factors

Primary liver cancer is the sixth most common neoplasm and the third leading cause of death worldwide. HCC is the most common primary liver cancer representing 80% of the cases.

Increased SPOCK1 expression was observed in human cirrhotic livers and in HCC, compared to normal controls. SPOCK1 was overexpressed in the tumor area compared to adjacent tissue and increased expression was associated with advanced clinical stage and metastasis. CHD1L is a potent anti-apoptotic and pro-proliferative factor coded in the 1q21 region, that is frequently amplified in HCC. It activates transcription by binding to the promoter region of SPOCK1 (nt-1662 to +34). In human HCC samples, a positive correlation between SPOCK1 and CHD1L has been established.

Several studies have found that SPOCK1 is involved in EMT. The addition of TGF-β to the culture medium induced the expression of SPOCK1 in A549 non-small cell lung cancer (NSCLC) cell line. Similar findings have been observed in lung adenocarcinoma, breast cancer and pancreatic ductal adenocarcinoma cells. Silencing of SPOCK1 in pancreatic cancer cell lines increased the expression of E-cadherin and decreased the expression of vimentin and the EMT transcription factors (Snail, Slug, ZEB1 and ZEB2). SPOCK1 overexpression induced Snail and Slug expression in the Caki-1 clear cell renal carcinoma cell line.

In SPOCK1 transgenic mice staining for type IV collagen revealed altered integrity of the gingival basement membrane. Higher levels of active-MMP2 and active-MMP9 were observed, with no change in the levels of the inactive form of these proteins. Similar effects were observed in the QGY-7703 HCC cell line, where SPOCK1 overexpression promoted the expression and activation of MMP9. Transfection of an MMP2 expressing plasmid into the Caki-1 clear cell renal carcinoma cell line reversed the effects of SPOCK1 silencing on invasion. String analysis revealed that MMP14 and MMP16 were two of the top ten interactors of SPOCK1, both of which contribute to the activation of MMP2 and MMP9.

Knockdown of SPOCK1 in U87 MG glioma cell line significantly suppressed the expression of the Wnt and its downstream targets c-Myc and cyclin D1. This resulted in an increased cell fraction in G1 phase. Similar findings were observed in NSCLC cell lines, where SPOCK1 silencing decreased the levels of β-catenin, c-Myc and cyclin D1 together with decreased proliferation, migration and invasion rates.

In-vitro SPOCK1 overexpression promoted proliferation and foci formation in QGY-7703 and PLC-8024 HCC cell lines trough Akt. Knockdown of SPOCK1 in the U87MG glioma cell line significantly downregulated the p-PI3K and p-Akt levels. Similar findings were observed in HCT116 colorectal cancer cells. Recombinant SPOCK1 protein activated the Akt signaling pathway in clear cell renal carcinoma cells. SPOCK1 overexpression or recombinant SPOCK1 promoted Akt phosphorylation and consequently the expression of Cyclin B1, cyclin D1, MMP2, and MMP9.

2. Objectives

Based on previous studies showing that overexpression of SPOCK1 is associated with poor prognosis in various cancer types, our hypothesis was that SPOCK1 is actively involved in the development and progression of the HCC. To address this hypothesis, first we aimed to evaluate the localization and physiological function of SPOCK1 in the liver. Second, we studied the role of SPOCK1 in carcinogenesis and progression of HCC, deciphering the underlying signaling pathways, and evaluated its potential as a diagnostic marker.

The specific objectives of our study were:

- To investigate the cellular localization and expression of SPOCK1 in normal human, mouse and rat livers.
- To study the SPOCK1 expression in human liver regeneration following massive liver necrosis.
- To evaluate the SPOCK1 expression in cirrhotic human livers of various etiology, in HCC and in mouse DEN hepatocarcinogenesis model.
- To analyze the serum concentration of SPOCK1 in HCC patients.
- To develop SPOCK1 knock-in and knock-down in vitro models to perform functional assays and analyze the changes in signaling pathways.

3. Methods

3.1. Cell Culture and Experimental Models

In out in-vitro experiments HepG2, HLE, and Huh7 HCC cell lines were utilized. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics.

Silencing SPOCK1 expression in HLE and Huh7 cells was performed using ON-TARGETplus siRNA and Lipofectamine™3000. SPOCK1 expression was measured using quantitative reverse transcription PCR and Automated Western Blotting System, proliferation was assessed using BrdU incorporation assay, and the and relative site-specific phosphorylation of 43 kinases was determined using Human Phospho-Kinase Array. For SPOCK1 overexpression studies, the full-length SPOCK1 open reading frame was amplified from HLE cDNA using a two-step PCR protocol and cloned into pcDNA™4/TO expression plasmid. HepG2 cells were transfected with Neon electroporation system, followed by Zeocin selection to establish stable SPOCK1-overexpressing clones. SPOCK1 expression was measured using quantitative reverse transcription PCR and Automated Western Blotting System, proliferation was assessed using BrdU incorporation assay.

3.2. Diethyl-nitrosamine Induced Hepatocarcinogenesis (DEN)

DEN hepatocarcinogenesis model was carried out by a single intraperitoneal dose of DEN in 15 days old C57/Black mice at 15 μg/g body weight. 26 DEN-treated wild-type C57/Black mice and 16 untreated C57/Black controls were studied and sacrificed at 9-months post-exposure. The liver samples were formalin-fixed paraffin-embedded (FFPE) and used for SPOCK1 and CHD1L immunohistochemistry.

3.3. Human Liver and Serum Samples

Human HCC, cirrhotic and normal liver tissue samples were collected and handled following the guidelines of the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (TUKEB, permit number: 155/2012) and the Medical Research Council Committee of Science and Research Ethics (permit number: 61303-2/2018/EKU). SPOCK1 immunohistochemistry was carried out, the stained slides were scanned and quantitative analysis was conducted with CaseViewer DensitoQuant 2.2 (3DHISTECH Ltd., Budapest, Hungary).

Human serum samples were collected from patients with HCC and from those without HCC diagnosis and were analyzed via ELISA to quantify SPOCK1 levels.

4. Results

4.1. SPOCK1 expression in normal human liver

We evaluated the baseline SPOCK1 expression in normal human livers. For this purpose, we used normal area from peritumoral tissues where the normal lobular structure of the liver was preserved (Figure 1A, 1C). Hepatocytes adjacent to both central and portal vein exhibited intense granular SPOCK1 expression (Figure 1B, 1D). The smooth-muscle cells in the arterial wall showed intense positive reaction as well, thus we used this as internal positive control (Figure 1E, 1F)

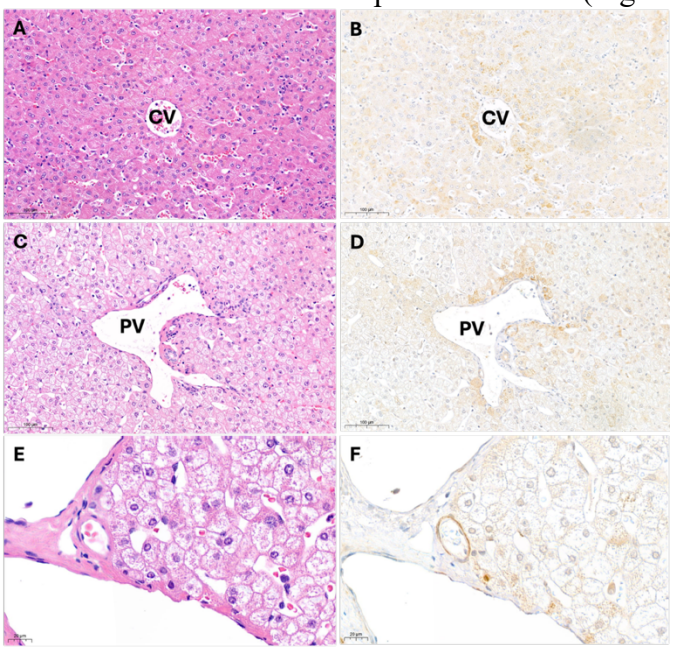

Figure 1. Expression of SPOCK1 in normal liver. The normal, lobular structure of the liver was preserved (A, C). Intense, granular staining pattern was seen in hepatocytes around the central vein (CV) and the portal vein (PV) on SPOCK1 IHC (B, D). Arterial wall at higher magnification (E), which showed intense positivity on SPOCK1 IHC (F), and we used as an internal positive control. Scale bar A-D 100 µm; E-F 20 µm.

4.2. Subcellular localization of SPOCK1

The granular staining pattern suggests that the intracellular localization of the SPOCK1 is related to a cellular organelle. To verify this, we labeled the mitochondria using MitoTracker® Red CMXRos in Huh7 human cells line (Figure 2B) and carried out SPOCK1 fluorescent immunostaining (Figure 2A) on these samples. We observed the co-localization of the two markers (Figure 2C), suggesting that the SPOCK1 is localized to the mitochondria.

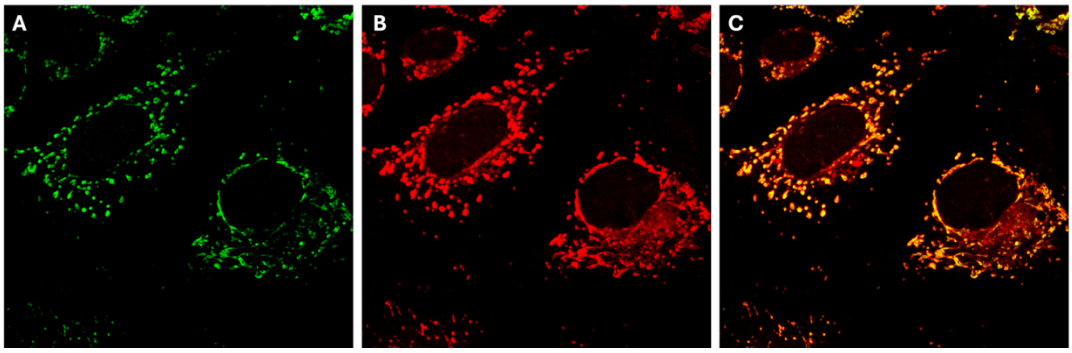

Figure 2. Co-localisation of SPOCK1 (green) and MitoTracker® (red) mitochondrial marker is shown. Representative images display SPOCK1 fluorescent immunostaining (A) and MitoTracker® Red CMXRos mitochondrial marker (B) in Huh7 cells. The merged image (C) shows co-localisation (appearing yellow), suggesting that SPOCK1 localizes to mitochondria. Images were taken at 100x magnification.

4.3. SPOCK1 expression during human liver regeneration

We assessed the SPOCK1 expression during the controlled hepatocyte proliferation in regenerating human livers. We collected FFPE human liver samples from our archive, explanted due to acute liver failure caused by massive hepatic necrosis, and grouped them based on the histologic pattern: 1) regeneration from dedifferentiated hepatocytes (n=5); 2) regeneration trough progenitor cells, forming regenerative foci (n=3). In the first group of regeneration, surviving hepatocytes dedifferentiate and they are organized into acinar structures containing bile in their lumens (Figure 3A, 3C). These hepatocytes showed intense, diffuse staining on SPOCK1 IHC without preferential periportal or pericentral localization (Figure 3B, 3D)

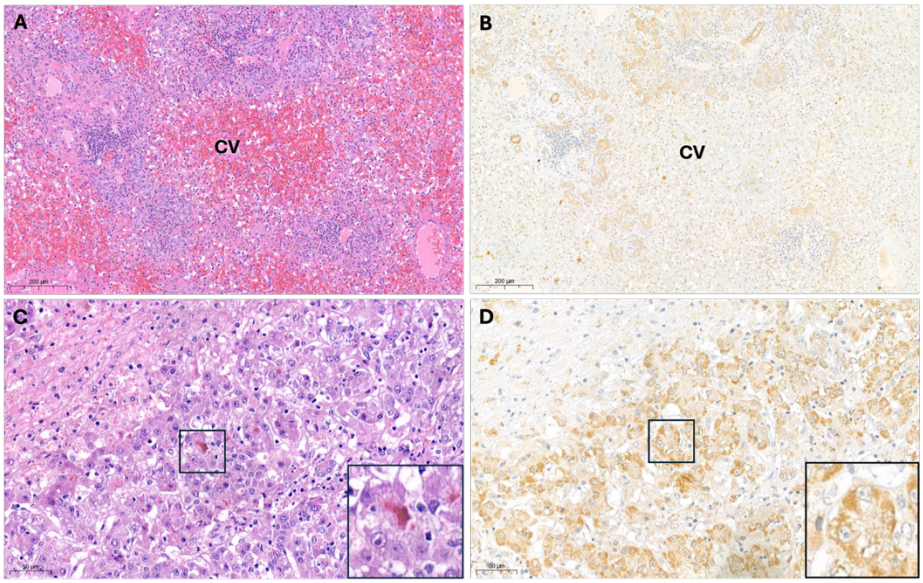

Figure 3. Hematoxylin and eosin (HE) and SPOCK1 IHC in the regeneration group from dedifferentiated hepatocytes. (A) HE staining of massive hepatic necrosis. (B) Low magnification image of SPOCK1 IHC. (C) Higher magnification image showed surviving hepatocytes arranged in acinar structures (inset) which expressed high level of SPOCK1 (D). Scale bar (A)(B) 200μm, (C)(D) 50μm.

In the regeneration group from progenitor cells intense ductular reaction was observed in the periportal area. In the ductules, larger cells were seen with pale eosinophilic cytoplasm resembling hepatocyte morphology (Figure 4A, 4C). These cells showed increased SPOCK1 expression compared to the surrounding ductular cells (Figure 4D). Besides the intense positive reaction of the arterial wall used as an internal control, faint staining was observed in the regenerative foci (Figure 4B).

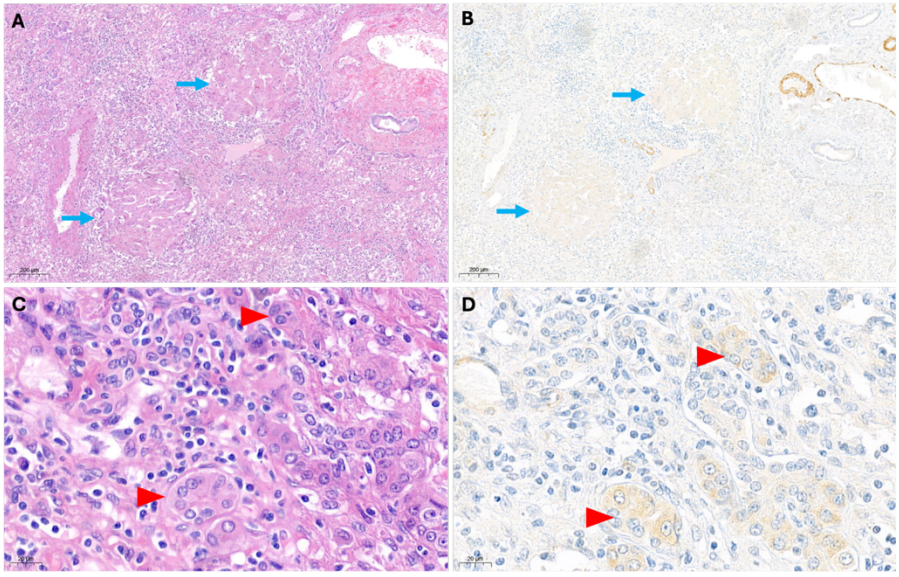

Figure 4. SPOCK1 IHC in regeneration group from progenitor cells. (A) HE staining of regenerative foci (blue arrow). (B) These showed low SPOCK1 expression on IHC. (C) Higher magnification image showed the ductular proliferation. (D) Larger cells in certain ductules (red arrowhead) resembling hepatocytes showed positivity on SPOCK1 IHC staining (D). Scale bar (A)(C) 200μm, (B)(D) 20μm.

4.4. SPOCK1 expression in human liver cirrhosis and HCC

We collected 58 human liver samples and prepared tissue microarray from patients diagnosed with liver cirrhosis and HCC of various etiology, and hemangioma surrounding samples as control. To evaluate the SPOCK1 expression we performed IHC.

The normal control livers (Figure 5A) showed positive staining in hepatocytes surrounding the central and portal veins as described above. Increased expression of SPOCK1 was observed in the cirrhotic livers (Figure 5B) where the cirrhotic nodules showed intense granular staining pattern. Apart from the blood vessels, the connective tissue was mostly negative. Similar staining pattern was observed in HCC as well (Figure 5C).

We quantified the expression level of SPOCK1 by densitometry and calculated the H-scores (Figure 5D). This revealed significantly higher expression in HCV-associated cirrhotic and HCC samples as well as in samples of unknown etiology when compared to control livers. Cirrhotic and HCC samples with alcoholic etiology showed elevated SPOCK1 expression, however this increase was not statistically significant. Cirrhotic and HCC samples of the same etiology showed similar expression level in all cases.

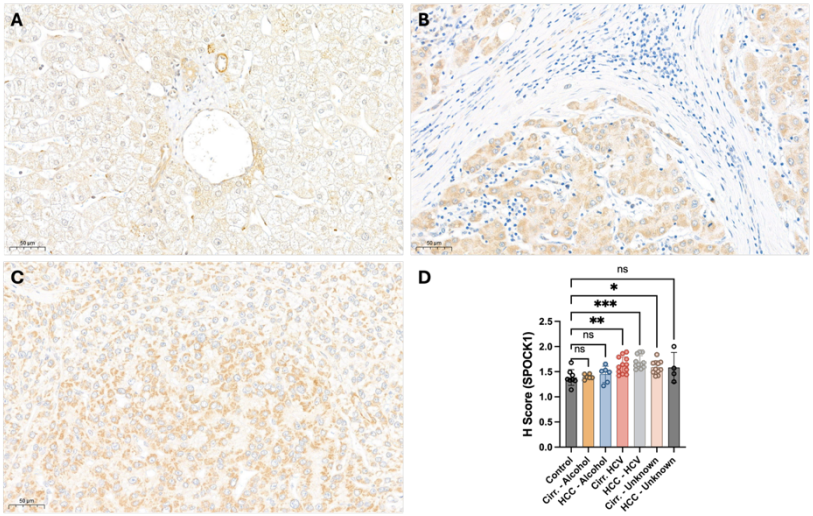

Figure 5. Representative images of SPOCK1 IHC on control, cirrhotic and HCC liver samples. (A) In control samples periportal hepatocytes showed intense positivity on SPOCK1 IHC. Increased expression was also observed in cirrhotic livers (B) and in HCC (C). Quantification of SPOCK1 expression by densitometry showed significant increase in case of HCV related cirrhosis and HCC also in case of cirrhosis of unknown etiology. Data is presented as mean ± SD, alcohol-related cirrhosis n=6; alcohol-related HCC n=6; HCV-related cirrhosis n=12; HCV-related HCC n=11; cirrhosis of unknown etiology n=11; HCC of unknown etiology n=4; control samples-hemangioma surrounding area n=8. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test.

We validated our results by performing RNA-seq based analysis using TNMplot publicly available tool (www.tnmplot.com) to compare the *SPOCK1* expression in HCC and normal control samples or in HCC and paired surrounding liver tissues. This tool uses datasets collected from GEO, GTEx, TCGA, and TARGET databases. Statistical analysis revealed significant increase in *SPOCK1* gene expression in HCC samples compared to normal livers. Moreover, the expression of *SPOCK1* was also elevated in HCC samples compared to the surrounding normal area (data not shown here). High *SPOCK1* gene expression correlated with significantly shorter overall survival in HCC patients (hazard ratio 1.57 and 95% confidence interval 1.05-2.32).

Syndecan-1 is the major proteoglycan of the liver, and our previous studies showed that overexpression of the human syndecan-1 protects against DEN induced hepatocarcinogenesis in mice. Thus, we aimed to compare the expression level of syndecan-1 and SPOCK1 in human liver cirrhosis and HCC. Syndecan-1 showed strong membranous staining pattern on hepatocytes in the cirrhotic nodules. The SPOCK1 was strongly expressed in the smooth muscle cells of the arterial wall, while faint positivity was observed in the hepatocytes (Figure 6A). The micro-metastases of HCC in the connective tissue showed high SPOCK1 expression whereas the expression of the syndecan-1 decreased (Figure 6B).

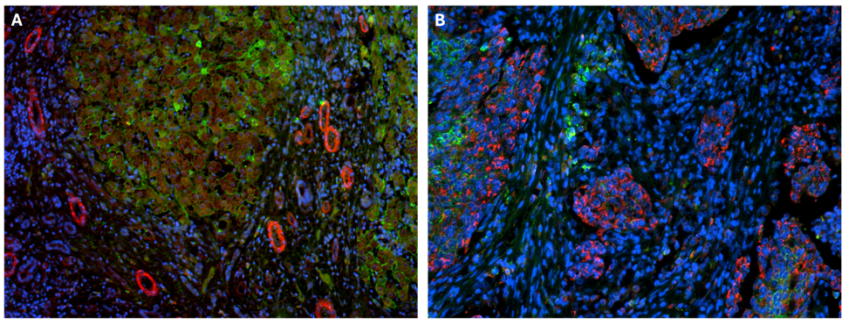

Figure 6. Expression of syndecan-1 and SPOCK1 in cirrhosis and in micro-metastases of the HCC. (A) Representative image of double fluorescent immunostaining in human cirrhotic liver. Intense membranous staining of syndecan-1 (green) was observed on the hepatocytes in the cirrhotic nodules, however these hepatocytes showed weak SPOCK1 positivity (red). The arterial wall showed intense positive reaction. (B) The micro-metastases in the connective tissue showed high SPOCK1 (red) expression whereas decreased syndecan-1 (green) expression was observed in these cells. DAPI – blue, (A) 10X, (B) 20X magnification (94).

4.5. SPOCK1 expression during DEN hepatocarcinogenesis in mice

We designed an experimental mouse model to analyze the alteration in SPOCK1 expression during the DEN hepatocarcinogenesis. This is a well described model where focal hepatic lesions are induced by 9 months. First basophilic foci appear on the HE staining, these are composed of altered hepatocytes (because of high cytoplasmic RNA content), with high nuclear to cytoplasmic ratio and crowded appearance. These lesions progress to trabecular hepatocellular carcinoma. The arterial wall, pericentral and periportal hepatocytes showed high expression of SPOCK1 in the control livers, similar staining pattern observed in normal human livers (Figure 7A). Increased SPOCK1 expression was detected 9 months after the DEN exposure in the foci (Figure 7B). Intense positive reaction of the CHD1L (Figure 7C), the known transcription factor of the SPOCK1 on these samples further strengthens our results.

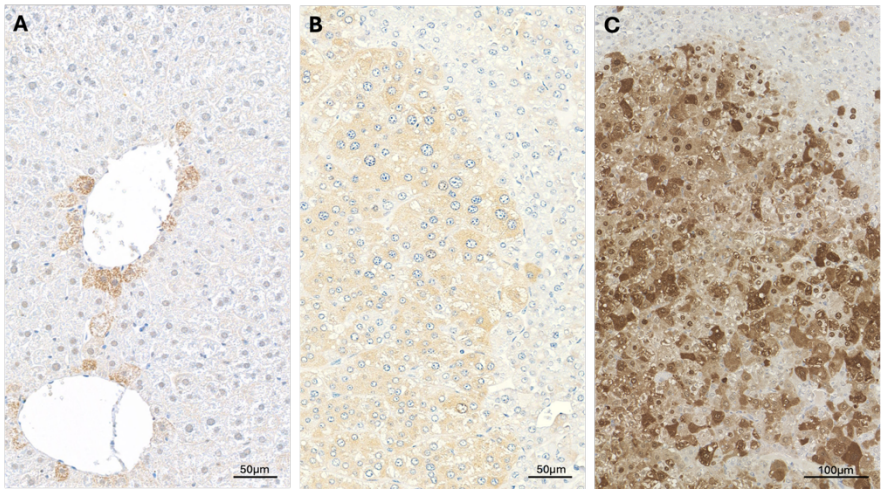

Figure 7. Expression of SPOCK1 in DEN hepatocarcinogenesis mouse model. (A) In control mouse livers SPOCK1 showed intense positive reaction on IHC in the pericentral hepatocytes. (B) Nine months after the DEN exposure SPOCK1 expression was detected in foci. (C) CHD1L IHC showed increased expression and nuclear localization at 9-month time point. Scale bar (A), (B) 50 µm, (C) 100 µm.

4.6. Detection of secreted SPOCK1 in human HCC cell lines and serum from HCC patients

SPOCK1 carries a signal sequence characteristic of extracellular proteins, and we investigated the localization of the SPOCK1 in cellular and extracellular compartments in vitro. HLE, Huh7 and HepG2 human HCC cell lines were cultured and SPOCK1 was detected by WES capillary

electrophoresis in the cellular fraction and secreted in the conditioned medium (Figure 8A). Interestingly, protein electrophoresis showed strong bands of different molecular weight, suggesting a different glycosylation pattern of SPOCK1 in the three cell lines. In HLE and HepG2 the dominant form was around 66 kDa, while in Huh7 it was at 116 kDa.

Our results, showing that HCC cell lines secrete high levels of SPOCK1 in culture media prompted us to study its concentration in human serum samples. Serum samples were collected from patients with HCC and from those without HCC and analyzed by ELISA. In HCC patients the serum level of SPOCK1 increased 2.2 folds, however this change was not significant (Figure 8B).

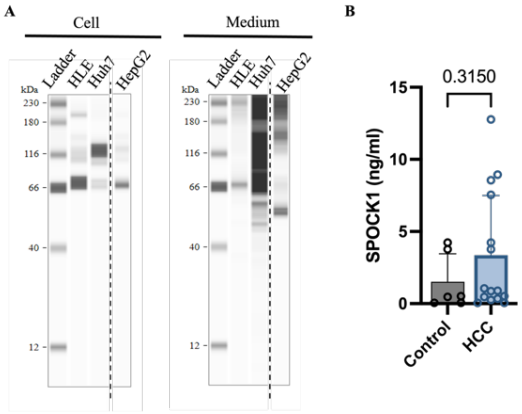

Figure 8. SPOCK1 detection in the culture medium of the HCC cell lines and in human serum samples. (A) SPOCK1 was present in the cells and also in the conditioned medium detected with capillary electrophoresis of the three HCC cell line. Dashed line: the microcapillary electrophoresis was performed separately. (B) ELISA revealed elevated levels of SPOCK1 in the serum of HCC patients compared to control patients without HCC. Data is presented as the mean ± SD, control group n=6, HCC group n=15. Statistical analysis was performed using unpaired t-test.

4.7. Effect of SPOCK1 on the proliferation of the human HCC cell lines

We used siRNA to silence SPOCK1 expression in human HLE and Huh7 HCC cell lines. The *SPOCK1* mRNA expression decreased by 0.07- and 0.292-fold in HLE and Huh7 cell lines respectively, tested by RT-qPCR. Interestingly, the intracellular level of SPOCK1 protein remained constant after silencing with siRNA, as measured by WES capillary electrophoresis. In contrast, secreted levels of SPOCK1 protein decreased in HLE and Huh7 cell lines (Figure 9).

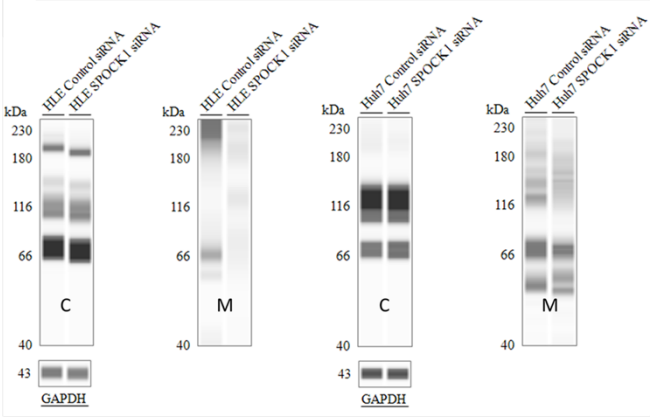

Figure 9. Silencing SPOCK1 in HLE and Huh7 cell lines decreased the level of secreted SPOCK1. Expression of SPOCK1 measured by WES capillary electrophoresis showed similar level of intracellular SPOCK1 ("C") after silencing using siRNA. Interestingly the secreted SPOCK1 was reduced in the conditioned medium ("M").

To study the role of SPOCK1 in proliferation, BrdU incorporation assay was performed in silenced cell lines (Figure 10A). *SPOCK1* silencing decreased the ratio of the positive nuclei from 39.18% to 11.52% in HLE and from 18.67% to 10.28% in Huh7 cell lines compared to the control siRNA transfected cells (Figure 10B).

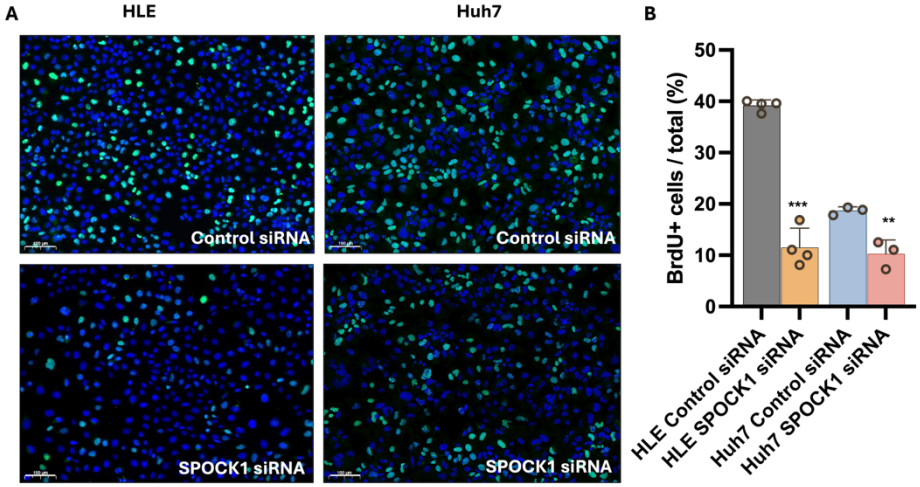

Figure 10. SPOCK1 silencing reduced the BrdU labeling index in human HLE and Huh7 cell lines. (A) Representative images of BrdU incorporation assay showed reduced number of labeled nuclei after SPOCK1 silencing. Blue: DAPI, green: BrdU (B) The BrdU labeling index was significantly reduced in SPOCK1 silenced cells. Data is presented as the mean ± SD, n>3/group. Statistical analysis was performed using unpaired t-test.

To further investigate the effect of SPOCK1 on HCC cells, a *SPOCK1* expression vector was transfected in human HepG2 cell line, resulting in a 30.517-fold increase in *SPOCK1* mRNA levels. The intracellular protein level didn't change after the transfection, but increased levels of SPOCK1 were observed in the conditioned media (Figure 11A). We performed BrdU incorporation assay and overexpression of the *SPOCK1* significantly increased the proportion of positive nuclei from 33.52% to 43.73% (Figure 11B, 11C).

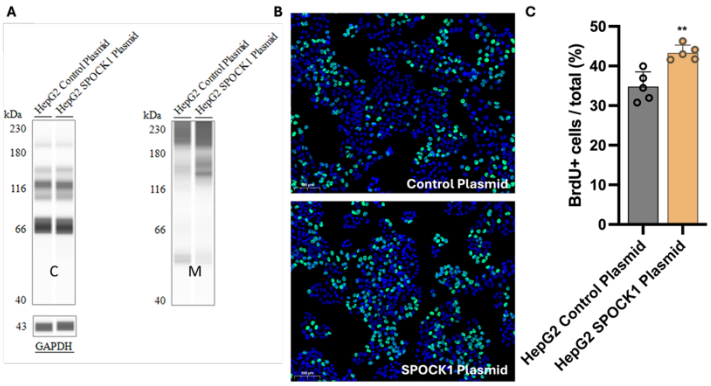

Figure 11. Overexpression of SPOCK1 in the human HepG2 cell line increased the level of secreted SPOCK1 and promoted cell proliferation. (A) SPOCK1 levels in intracellular and extracellular compartments measured by WES capillary electrophoresis. Overexpression of SPOCK1 resulted in an increase in the secreted level ("M") but not in the intracellular SPOCK1 level ("C"). (B)(C) Overexpression of SPOCK1 significantly increased the BrdU labeling index of the HepG2 cells as assessed by BrdU assay. Blue: DAPI, green: BrdU. Data is presented as the mean ± SD, n=5/group. Statistical analysis was performed using unpaired t-test.

4.8. Effect of *SPOCK1* silencing on the signaling pathways

We used phospho-kinase array to analyze the changes in the activation/inhibition of signaling pathways following *SPOCK1* silencing in HLE and Huh7 cell lines.

In both cell lines, significant decreases in phosphorylated EGFR, ERK1/2, TOR(S2448) and Yes levels were detected. Phosphorylated MSK1/2, CREB, Src, Lyn, Fyn, Hck and Fgr levels were also decreased.

5. Conclusions

Our aim was to evaluate the role of the SPOCK1 in normal liver, regenerating liver, cirrhosis, and HCC. For this we evaluated the SPOCK1 expression in human and mouse liver samples and measured its level in human serum samples. In addition, we assessed the effect of SPOCK1 overexpression and silencing on human HCC cell lines and analyzed the signaling pathways involved.

Based on our study, we conclude the following new findings:

1. In normal human liver, SPOCK1 is expressed in periportal and pericentral hepatocytes and in the smooth muscle cells of the arterial wall.
2. We identified the co-localization of SPOCK1 with mitochondria in cultured cells.
3. In human liver SPOCK1 shows a distinct expression pattern depending on the type of liver regeneration:
    a. It is highly expressed in the dedifferentiated hepatocytes.
    b. It is expressed in the differentiating ductular cells with hepatocyte morphology derived from progenitor cells, however it is weakly expressed in the regenerative foci.
4. A significantly higher SPOCK1 expression was observed in HCV associated human cirrhotic livers and HCC.
5. Higher SPOCK1 levels were detected in the serum samples of HCC patients.
6. Elevated SPOCK1 expression was observed in transformed foci in DEN-induced mouse hepatocarcinogenesis model.
7. Silencing SPOCK1 reduced the BrdU labeling index of HLE and Huh7 cell lines and SPOCK1 overexpression increased the BrdU labeling index of the HepG2 cell line.
8. Silencing SPOCK1 reduced the levels of Src family kinases phosphoproteins.

6. Bibliography of the candidate's publications

6.1. Related to the thesis

1. **Váncza, L.**; Tátrai, P.; Reszegi, A.; Baghy, K.; Kovalszky, I., SPOCK1 with unexpected function. The start of a new career, AMERICAN JOURNAL OF PHYSIOLOGY: CELL PHYSIOLOGY 322: 4 pp. C688 C693. (2022)

2. **Váncza, L.**; Karászi, K.; Péterfia, B.; Turiák, L.; Dezső, K.; Sebestyén, A.; Reszegi, A.; Petővári, G.; Kiss, A.; Schaff, Zs.; Baghy, K.; Kovalszky, I., SPOCK1 Promotes the Development of Hepatocellular Carcinoma, FRONTIERS IN ONCOLOGY 12 Paper: 819883 , 18 p. (2022)

3. **Váncza, L.**; Karászi, K.; Péterfia, B.; Turiák, L.; Dezső, K.; Sebestyén, A.; Reszegi, A.; Petővári, G.; Kiss, A.; Schaff, Zs.; Baghy, K.; Kovalszky, I. Corrigendum: SPOCK1 promotes the development of hepatocellular carcinoma FRONTIERS IN ONCOLOGY 13 Paper: 1203745 , 1 p. (2023)

6.2. Related to the topic

1. **Váncza, L.**; Horváth, A.*; Seungyeon, L.*; Rókusz, A.; Dezső, K.; Reszegi, A.; Petővári, G.; Götte, M.; Kovalszky, I.; Baghy, K., SPOCK1 Overexpression Suggests Poor Prognosis of Ovarian Cancer, CANCERS 15 : 7 Paper: 2037 , 19 p. (2023)

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