The role of overexpressed decorin in primary and metastatic liver carcinoma

PhD thesis

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1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common primary liver malignancies and it is the fourth cancer-related death cause in the world. The risk of development of liver metastasis highly depends on the site of the primary tumor. Colorectal carcinoma (CRC) is one of the primary malignancies that most frequently invade the liver. Regarding liver tumors, there have been hardly any data on the role of decorin in the literature.

The extracellular matrix (ECM) is a highly dynamic structure that is present in all tissues and continuously undergoes controlled remodeling. ECM macromolecules exhibit important functional roles in the control of several cellular events such as adhesion, migration, proliferation, differentiation, and survival. The matrix is well known for its ability to provide structural and biochemical support for organs and tissues. The ECM is composed of collagens, elastin, proteoglycans (PGs), and non-collagenous glycoproteins. Matrix remodeling plays an important role in the development of HCC. This process involves quantitative and qualitative changes in the ECM. Tumor cells can manipulate their microenvironment to enhance their survival, thereby creating a positive tumorigenic feedback loop. Accordingly, during the last decades, extensive research activities focused on the better understanding of the cancer cell and stroma interactions.

Decorin is a member of the ECM small leucine-rich proteoglycan (SLRP) gene family containing a single chondroitin sulfate or dermatan sulfate chain and is expressed mainly by fibroblast and myofibroblasts. In a healthy liver, a small amount of decorin is deposited around the central veins and in the portal tracts. However, during fibrogenesis together with other matrix proteins the amount of decorin significantly increases in the connective tissue septa. Decorin has been described to be involved in many biological and physiological processes including growth regulation, cell differentiation, collagen fibrillogenesis, muscular development, wound healing, stem cell biology, kidney and liver fibrosis, angiogenesis, regulation of inflammation and autophagy.

Decorin represents a powerful tumor cell growth and migration inhibitor by interaction with matrix constituents and regulating several signaling pathways. The first growth factor discovered as a decorin interacting partner was the transforming growth factor-β (TGF-β). Binding of TGF-β by the proteoglycan attenuates the proliferation of tumor cells dependent on the growth factor. Previous studies have shown that decorin is an endogenous, soluble pan-receptor tyrosine kinase (RTK) inhibitor, known to interact with a variety of cell surface receptors including epidermal growth factor receptor (EGFR/ErbB1) as well as another member of the ErbB RTK family. Moreover, decorin negatively regulates insulin-like growth factor receptor I (IGF-IR), the hepatocyte growth factor receptor Met, vascular endothelial growth factor receptor-2 (VEGFR-2), and platelet-derived growth factor receptor (PDGFR). This pan-RTK blockage often leads to growth arrest and hinders tumor growth by keeping tumor cells in quiescence. It has been proven that a functional $p21^{WAF1/CIP1}$ that causes G1 phase arrest is indispensable for the tumor repressor action of decorin in most tumor cell lines. Besides initiating signaling, this decorin/RTK interaction can induce caveosomal internalization and receptor degradation.

Theoretically, utilization of decorin as a physiological tyrosine kinase receptor inhibitor, targeting multiple receptors, is possible and the idea is well-established. At present, Sorafenib (Nexavar) is applied as RTK inhibitor in chemotherapy of HCC, blocking the activity of VEGFR, PDGFR, and Raf kinase. All of these molecules represent targets of decorin.

In CRC, various treatment approaches such as surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy are applied. Patients with colorectal cancer were among the first major beneficiaries of the introduction of targeted therapy, mainly because of the well- known molecular mechanisms responsible for the malignant phenotype. At present, EGFR inhibitors such as cetuximab (Erbitux) and panitumumab (Vectibix) are widely used in the targeted therapy of CRC. Decorin is known to effectively block the activity of EGFR but also of

VEGFR, PDGFR, Met, and Raf kinases. Thus, decorin either alone or as an adjuvant could have a place in the clinical treatment of HCC and CRC.

To better understand the role of decorin in HCC and liver metastasis, this study aimed to examine the expression of decorin in liver tumor using *in silico* approaches as well as FFPE tissue microarray (TMA) samples of HCC and liver metastasis of CRC. Our previous studies showed that the lack of decorin favors primary hepatocarcinogenesis resulting in higher tumor incidence. Thus, to confirm the protective role of decorin in the other way around, we designed a model system to investigate the effects of overexpressed decorin in a mouse model of hepatocarcinogenesis induced by thioacetamide (TAA), as well as colonization model evoked by inoculating murine colon c38 cell line into the spleen.

2. AIMS

Our previous studies showed that the lack of decorin favors primary hepatocarcinogenesis, which results in higher tumor incidence. Thus, we hypothesized that overexpression of decorin in the liver may inhibit tumor formation in primary hepatocellular carcinoma and liver metastasis. To challenge our presumption a 3-step model system was designed.

- Tissue microarrays were assembled from human HCC and colon adenocarcinoma liver metastasis cases and immunostained for decorin, α-smooth muscle actin (SMA) and EGFR
- To test whether tumor cells are capable of directly influencing the decorin production of fibroblasts, LX2 stellate cells were exposed to hepatoma and colon adenocarcinoma conditioned media, then we measured the protein and mRNA level of decorin in LX2 cell media.
- For animal experiments, pLIVE vector coding human decorin cDNA were targeted to the liver by hydrodynamic gene delivery. We investigate the role of overexpressed decorin in hepatocarcinogenesis model, evoked by TAA; and mouse models of liver metastasis evoked by inoculating murine colon c38 cell line into the spleen.

Furthermore, other goals of my research included mapping the molecular background of the experimental systems presented above, detecting quantitative and qualitative changes in the participating molecules, and gaining a comprehensive picture of the role of decorin in primary and metastatic liver tumors.

3. METHODS

***In silico* gene expression datasets** for HCC and non-tumorous liver, samples were collected from the public microarray repository ArrayExpress database, provided by the European Bioinformatics Institute. Our datasets with accession E-MTAB-950 includes 36 normal, 112 tumors, and 10 pair of tumors–non-tumorous adjacent tissues (NATs). Most of the HCC patients have the underlying etiology of Hepatitis C Virus and Hepatitis B virus infection. All the raw data were processed using R-programming language due to its detailed clinicopathological data. To compensate for the variation of fibroblast content, decorin expressions were normalized to SMA content. SMA is a well-known marker for activated fibroblasts, which are the key producer of decorin.

For HCC-TMA experiments: We utilized FFPE tissue samples of HCC with and without cirrhosis. Biopsy samples of 29 HCCs (20 cirrhotic, 9 non-cirrhotic) and 9 control livers (hemangioma) were selected for TMA assembly. From each HCC, one core from the tumor and one from the NAT was selected. TMA block was sectioned, and slides were **immunostained for decorin and SMA.** Staining intensities were analyzed by Pannoramic Viewer software using a 12-score system and evaluated by two independent pathologists' visual scoring. Every sample was given a score according to the intensity of the decorin staining (no staining = 0, low decorin staining = 1–6, and high decorin staining = 7–12). The final label is determined by averaging two pathologists' scores. HCC samples were divided into decorin negative, low, and high decorin expressing categories. We normalized decorin expression to SMA content, as previously described.

Liver metastasis of colon adenocarcinoma TMA was assembled from tissue cores containing liver metastases and surrounding non- tumorous liver tissue, as well as the primary tumor and normal colon tissue of each patient. A growing pattern was categorized as desmoplastic or replacement type in liver metastases. The TMA block was sectioned, and slides were counterstained with hematoxylin and

immunostained for decorin. SMA staining of surrounding liver samples was performed to describe stroma abundance. Decorin and SMA staining intensities were analyzed and scored by the Pannoramic Viewer software TMA module by two independent pathologists. The final score was determined by averaging the scores.

In vitro LX2 human stellate cells were exposed to hepatoma (HLE, HepG2, Huh7, Hep3B) and colon adenocarcinoma (HT29) conditioned media.

***In vivo* studies:** In brief, we utilized 2-month-old male mice all in a C57Bl/6 background. Decorin cDNA was cloned into a pLIVE vector. Vectors were injected using hydrodynamic gene delivery method. Using TAA, we established a model of hepatocarcinogenesis and a colonization model by inoculating mouse c38 colorectal carcinoma cells into the spleen. Samples from animal experiments were used in molecular assays.

Protein activity, localization, and expression assays: Measuring the activity of tyrosine kinase receptors and phospho-kinases, relative phosphorylation was investigated by Proteome Profiler Phospho-Kinase Array Kit and Proteome Profiler Mouse Phospho-RTK Array Kit. To determine the localization of each target molecule ICC and IHC methods were applied with appropriate antibodies. **Western blot and dot blot** techniques were used for quantitative comparative assays for proteins. The tested target molecules were: decorin, SMA, EGFR, pEGFR (Tyr1068), pIGF-1R (Y1161), pAkt (Thr308), pAkt (S473), pERK1/2 és β-actin. Human decorin was quantified from mouse serum by **ELISA.**

Statistical Analysis: All statistical analyses were performed by Graphpad Prism 4.03 software. Data evaluation was performed using D'Agostino and Pearson's omnibus normality test and non-parametric tests (Mann-Whitney) or Students' t-tests depending on the distribution of the data. The difference between control and decorin treated groups in tumor prevalence was tested for significance by χ square test. $P < 0.05$ level was declared statistically significant.

4. RESULTS

Role of decorin in primary HCC

Downregulation of decorin in human HCC in silico experiments

Analysis of the ArrayExpress database's HCC cases revealed that tumor samples had significantly decreased decorin mRNA expression compared to normal liver and displayed moderate increases in NATs. When normalized to SMA content, decorin expression was significantly reduced in tumor samples compared to normal tissue and NAT sections. According to the *in silico* analysis, DCN/SMA content distinguishes between normal and cancerous samples and is even characteristic for very early-stage HCC. DCN/SMA ratio gradually decreases from very early to advanced HCC, while it is overexpressed in cirrhosis. In addition, decorin expression seems to follow the BCLC (Barcelona Clinic Liver Cancer) staging classification as significantly decreased decorin level was observed in every BCLC stage compared to normal liver, and its level gradually decreases from BCLC 0 to BCLC C.

Inhibited Decorin expression in human HCC

Next, we aimed to detect changes in decorin expression at the protein level. To this end, we utilized FFPE HCC tissue samples with or without cirrhosis. From each HCC sample, one core from the tumor and one from NAT was selected and immunostaining specific for decorin and SMA was performed. Immunohistochemical staining of SMA reflects on the number of activated hepatic stellate cells, the main source of decorin in the liver. In the normal human liver, SMA is localized in the perisinusoidal area as well as in the vascular walls of the portal tract and the central vein. In the control liver, weak immunopositivity of decorin was detected around the central veins and in the portal tracts. DAB positivity for SMA in cirrhotic and non-cirrhotic liver samples are strongly and diffusely located in the cytoplasm of fibroblasts in connective tissue septa and the perisinusoidal spaces of residual hepatic parenchyma. In the NAT of both cirrhotic and non-cirrhotic HCCs, a high number of SMA positive activated stellate cells were detected with

extremely strong decorin expression along the sinusoids, the portal tracts and around the central veins in the same tissue section. In contrast, a high number of SMA positive activated HSCs were detected in the tumor stroma, but there was hardly any or negative decorin expression in the same sample. This observation was detected in both cirrhotic and non-cirrhotic cases. When decorin normalized to SMA content, decorin expression both at protein and mRNA level was decreased in the tumor samples compared to their paired NAT. At the protein level, the difference was statistically significant. As decorin expression was normalized to the SMA level, differences were not caused by changes in the number of fibroblasts cells.

Immunohistochemical results were semi-quantified using a 12-score system and evaluated by visual scoring of two independent pathologists. Based on their intensity score, HCC samples were divided into decorin negative, low, and high expressing categories. Using this evaluation, 52% of HCCs were decorin negative, 33% showed low, and 15% high decorin expression. In addition, decorin amount negatively correlated with EGFR level, a receptor known to be downregulated by decorin.

Inhibited decorin production of LX2 stellate cells in vitro

To test whether tumor cells are capable of directly influencing the decorin production of fibroblasts, LX2 human stellate cells were exposed to conditioned media of different hepatoma cell lines (Hep3B, HLE, HepG2, and HuH7). Significantly less decorin was detected in the media of LX2 cells, when HLE, HepG2, and HuH7 conditioned media was applied. In the case of Hep3B cells, the observed effect did not reach statistical significance. These changes appeared at the transcriptional level, as decorin mRNA level was reduced in LX2 cells media when conditioned media of the hepatoma cells was applied. These results correlated well with our observations on human HCC tissue samples indicating that the presence of tumor cells reduces the expression of decorin highlighting its tumor suppressor effect in HCC.

In vivo primary hepatocarcinogenesis model

Our previous studies showed that the lack of decorin favors primary hepatocarcinogenesis resulting in higher tumor incidence. Based on these findings, we designed a new set of experiments to understand the implication of overexpressed decorin in our TAA-induced hepatocarcinogenesis model. For this experiment, human decorin cDNA was cloned into a pLIVE vector, where the expression is driven by a mouse AFP enhancer and albumin promoter. In addition, we applied a control vector coding serum alkaline phosphatase. When injected together with the human decorin-coding (pLIVE-DCN) or with the empty vector (pLIVE-0), the SEAP detected from blood provides indirect information about the activity of the pLIVE-DCN or pLIVE-0 vectors. Vectors were injected using the hydrodynamic gene delivery method.

The artificial decorin expression and localization were visualized by fluorescent immunostaining. Human decorin was successfully transfected and expressed in the livers. Control livers transfected with the empty vector (pLIVE-0) were completely negative for immunostaining of human decorin. Driven by albumin promoter, the human recombinant decorin produced by the transfected vector was mainly detected in hepatocytes around the central veins. As described, mice were injected with a plasmid encoding SEAP reporter gene. In most of the animals, the SEAP expression was high, measured from half-time TAA treated mice blood samples. Very low SEAP level was detected in three of the control (pLIVE-0), and four of the decorin treated (pLIVE-DCN) animals. The human recombinant decorin level was measured from the sera of mice by ELISA. The results correlated well with that of SEAP assay indicating that decorin delivery was successful and the proteoglycan production is active.

Depending on the transfection efficiency measured by SEAP assay, the decorin transfected group was subdivided into decorin negative, low, and high decorin expressing categories. Upon TAA-induced hepatocarcinogenesis, decorin transfection resulted in attenuated tumor formation in both low and high decorin expressing groups. The highest

tumor count was observed in mice with no decorin production. Decorin delivery decreased the number of tumors by 72 and 78% in low and high decorin expressing groups respectively, compared to decorin negative livers. Lower liver mass/body mass ratios of decorin treated animals corroborate the beneficial effect of excessive proteoglycan. Based on these results, we assume that decorin gene delivery has the potential to inhibit the development of HCC indicating that soluble decorin may act as a tumor suppressor.

As several publications reflected that soluble decorin acts as a pan-RTK inhibitor targeting a multitude of RTKs, their activity in our experimental hepatocarcinogenesis model was tested. We detected decreased levels of EGFR, PDGFRα, PDGFRβ, PDGFR/Flt-3, PDGFR/SCF, AXL, HGF/MSPR, MuSK, and Insulin receptor in pLIVE-DCN samples in TAA treated groups, relative to that of pLIVE-0. As an unexpected result, in contrast with other tyrosine kinases receptors, we observed striking activation in tyrosine phosphorylation of IGF-1R in decorin overexpressing TAA-driven tumors.

As Akt is a known downstream effector of IGF-1R, we tested whether the levels of phospho-Akt (S473) and phospho-Akt (T308) would be altered in our experimental animal model. In control lysates, hardly any phospho-Akt (S473) was detected, but phospho-Akt (T308) was ∼2.3-fold higher in pLIVE-0 mice than in pLIVE-DCN samples. Upon TAA exposure, their amount was raised, and no difference was observed in pAkt (S473) level between the transfected groups. In contrast, pAkt (T308) exhibited ∼a 1.5-fold increase in pLIVE-DCN mice compared to the pLIVE-0 group. In our experimental hepatocarcinogenesis model changes in p53 levels were identified by a phospho-array study. Three phosphorylated p53, namely phospho-p53(S392), phosphop53(S46), and phospho-p53(S15) exhibited significantly higher levels in response to the overexpressed of decorin. Notably, after TAA exposure, we found ∼2-fold, ∼1.6-fold, and ∼1.7-fold increase in phospho-p53(S392), phospho-p53(S46), and phospho-

p53(S15) in decorin transfected mice compared to that of null-vector, respectively.

In conclusion, all of these signaling pathways suggest that decorin plays a protective role in liver cancer.

Colon adenocarcinoma liver metastasis

Decorin expression decreased in liver metastases of human CRC

Human tissue microarrays were assembled from biopsy samples of normal colon tissue, primary tumor, liver metastasis, and surrounding liver tissue from each patient, and immunostaining for decorin was performed. The highest decorin levels were observed in the stroma of normal colon tissue. In the stroma of primary colon tumors, while decorin was still abundant, its expression was reduced compared to the normal tissue. The lowest amount of decorin, significantly less than in the normal colon and primary tumors, was detected in liver metastases. The level of decorin expression in liver metastases was similar to that seen in peritumoral liver tissue. Decorin level did not correlate with the type of desmoplastic reaction in primary tumors. However, we measure decreased decorin amounts in metastases with the more aggressive replacement growing pattern compared to desmoplastic ones. In line with that, grade III. metastases contained less decorin than those of grade II. tumors when measuring its absolute level, as well as its relative level compared to the primary tumor. Based on these observations, we speculate that decreased expression of decorin in liver metastases compared to the primary tumors may reflect the aggressiveness of the metastatic tumor.

Decorin expression of fibroblasts is inhibited in vitro

Based on the TMA studies of human liver metastasis and HCC studies, we can conclude that decorin expression decreases continuously during tumor progression. To prove this, we designed an *in vitro* system.

Significantly less decorin was observed in the media of LX2 cells when HT29- CM was applied to them. The downregulation occurred at

the transcriptional level, as the decorin mRNA level was significantly reduced in LX2 cells exposed to HT29- CM. These results correlated well with our observations on human CRC and HCC tissue samples, indicating that tumor cells reduce the production of decorin by fibroblasts in the liver, which supports a tumor- suppressive role for decorin.

Overexpressed decorin reduces tumor formation in vivo

For liver metastasis studies, we developed a colonization model, where c38 colon carcinoma cells were injected into the spleen and allowed to colonize to the liver. Targeted transfection of human decorin into the liver was conducted using the pLIVE vector and hydrodynamic gene delivery as described previously.

Elevated decorin expression following transfection was confirmed by immunohistochemistry and ELISA assay.

Three weeks after the inoculation of colon carcinoma cells, metastases appeared in the liver that maintained the phenotype of colon carcinoma. We observed a significant 63% reduction in the number of metastases in livers overexpressing decorin, in parallel with lower liver mass/body mass ratio, indicating decreased tumor burden of the organ.

We aimed to study the tumor-specific signaling pathways in our model induced by decorin transfection. After c38 tumor cell inoculation, significantly less active EGFR, PDGFRα, and HGF/MSPR receptors were detected in the livers of pLIVE-DCN animals compared to pLIVE-0 mice. Notably, we observed a 43%, 45%, and 63% reduction in phosphorylation of EGFR, PDGFRα, and HGF/MSPR in decorin overexpressed groups, respectively. Since in our earlier experimental primary hepatocarcinogenesis model decorin was shown to induce IGFR activity, changes in the level of phospho- IGF- 1R, along with pEGFR and pErk1/2, were assessed by Western blot analysis. In the tumor- free livers of sham- inoculated mice, the delivery of human decorin significantly reduced the level of pIGFR but caused no change in pEGFR or pErk1/2. In the metastasis- bearing livers of c38-inoculated mice, on

the other hand, transfection with pLIVE-DCN did not significantly affect pIGFR but markedly downregulated pEGFR and reduced pErk1/2 by 22% and 27%, respectively, compared to transfection with pLIVE-0.

Next, using a phosphokinase array we interrogated the decorin- induced changes in common signaling pathways that may have resulted in attenuated metastasis formation in the liver. In general, decorin delivery suppressed the activity of most major signaling pathways. Downstream of RTKs, decorin- overexpressing animals displayed inhibition of Ras/MAPK signaling (marked by decreased ERK1/2 and RSK1/2), and attenuation of the Akt/mTOR pathway indicated by decreased pAkt(T308) and phosphor-p70S6K. Concordant with decreased Akt activity, downregulation of WNK1 was also detected in the pLIVE-DCN group. The levels of most phosphorylated STAT proteins and c-Jun were lowered as well, along with reduced amounts of β-catenin, an important signaling protein in CRC, and a known target of decorin treatment. Conversely, increased activity of p38 MAPK and its downstream effectors such as MSK1/2 and CREB was detected upon decorin delivery. Overexpressed decorin raised the levels of phosphor-AMPK and phosphor-p53 as well.

Overall, these results suggest that the protective effect of decorin against CRC liver metastases may hinge on its blocking of RTKs.

5. CONCLUSIONS

Hepatocellular carcinoma (HCC) and colorectal (CRC) cancer represent the fourth and the second most common cause of cancer death worldwide. Regarding liver tumors, there was hardly any data on the role of decorin. To fill up this gap, we hypothesized that decorin may act as a tumor suppressor in HCC and liver metastasis. The tumor microenvironment plays a determining role in cancer development by regulating multiple processes between the extracellular matrix and tumor cells. Decorin, a prototype member of the SLRP family has gained recognition for its essential roles in several disorders including cancer. Studies on mice with ablated decorin gene revealed that the lack of decorin is permissive for tumor development. On the same note, reduced expression or abrogation of decorin was observed in several types of cancer, suggesting that decorin tends to act as a tumor suppressor in these contexts. Moreover, when applied as a therapeutic agent, decorin effectively inhibited tumor formation, progression, angiogenesis, and metastasis in a multitude of experimental models. In my dissertation, we report that decorin expression was significantly downregulated in most HCCs compared to NAT and normal liver. In addition, decorin content seems to follow the staging of HCC. Similarly, the liver metastasis of the same tumor often displayed reduced amounts of decorin, and to a greater extent in the more aggressive grade III tumors, and in metastases with replacement growing pattern. These observations suggest that the decreased decorin expression in liver metastasis of CRC may correlate with the aggressiveness of the tumor. Conditioned media from colon adenocarcinoma cells and hepatoma cells *in vitro* were able to attenuate decorin production of the LX2 stellate cell line of liver origin. Moreover, liver-targeted decorin delivery effectively inhibited hepatic tumor formation and metastasis spreading of colon cancer. As a mechanism of action, excessive decorin was able to reduce the activity of multiple RTKs including EGFR, an important player in HCC and CRC pathogenesis. Downstream of that, hindered signaling network was seen including ERK1/2, PLCγ, Akt/mTOR, STAT, and c-Jun pathways, while

p38 MAPK/MSK/CREB and AMPK displayed intensified action culminating in enhanced p53 function. Therefore, decorin as "a guardian from the matrix" may be an invaluable tool in combatting colorectal and hepatic cancer.

New findings regarding the relationship between decorin and liver cancer.

1. **The lack of decorin provides a survival advantage for tumor tissue.**
   a. *In silico* decorin expression was significantly downregulated in most HCCs compared to their NATs and the normal liver.
   b. mRNA expression analysis revealed that normalized decorin content seems to follow the staging of HCC.
   c. HCC tumor tissues have reduced or completely blocked decorin expression compared to their paired peritumoral liver areas.
   d. In our sample set, 52% of HCCs were decorin negative, 33% showed low, and 15% high decorin expression.
   e. The highest decorin expression was found in normal colon, but decorin was also abundant in the primary tumor stroma. However, the liver metastasis of the same tumor often displayed reduced amounts of decorin, and to a greater extent in the more aggressive grade III tumors, and in metastases with replacement growing pattern. These observations suggest that the decreased decorin expression in liver metastasis of CRC may correlate with the aggressiveness of the tumor.
   f. Significantly, less decorin was detected in the media of LX2 cells when hepatoma or colon adenocarcinoma cell lines conditioned media was applied.

2. **Liver- targeted decorin delivery effectively inhibited hepatocarcinogenesis and metastasis formation of colon cancer.**
   a. In our, i*n vivo* hepatocarcinogenesis model decorin inhibited several RTK activities characterized by decreased MAPK/ERK

function. In contrast, the induction of IGF-1R was observed with increased Akt/mTOR activity. Elevated phospho-p53 levels are thought to be responsible for the inhibited tumorigenesis.

b. In our, *in vivo* colonization model liver- specific transfection with the decorin gene inhibited several RTKs with a concomitant decrease in the activity of the MAPK/ERK and Akt/mTOR/p70S6K pathways. Increased AMPK (possibly via LKB1) and p38/MSK/CREB signaling resulted in elevated phospho- p53 levels; stabilization of this key tumor suppressor may result in a cell cycle blockade.

6. PUBLICATIONS

Publications in context of the thesis:

1. **Reszegi, A**., Horváth, Z., Fehér, H., Wichmann, B., Tátrai, P., Kovalszky, I., & Baghy, K. (2020). Protective Role of Decorin in Primary Hepatocellular Carcinoma. *Frontiers in oncology*, *10*, 645. https://doi.org/10.3389/fonc.2020.00645
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Book chapter

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