FINAL REPORT

Therapeutic utilization of decorin as a receptor tyrosine kinase inhibitor in primary and metastatic liver cancers (OTKA-PD-105763)

Duration 2013/01/01-2015/12/31 – 36 months

Kornélia Baghy, PhD
1st Department of Pathology and Experimental Cancer Research

CONTENT

INTRODUCTION AND AIMS OF THE PROJECT 3
RESULTS 4
I. HEPATOCELLULAR CARCINOMA 4
1. *In vitro* experiments with hepatoma cell lines 4
2. Experimental hepatocarcinogenesis *in vivo* 5
3. Decorin in human hepatocellular carcinoma 7
II. LIVER METASTASES OF COLON CARCINOMA 9
1. *In vitro* experiments 9
2. *In vivo* colon carcinoma-liver metastasis model 10
3. Decorin in liver metastases of human colorectal cancer 10
PUBLICATIONS in connection with the recent project 11
REFERENCES 12

INTRODUCTION AND AIMS OF THE PROJECT

Hepatocellular carcinoma (HCC) represents the most frequent type of primary liver tumors, and it is the third most common fatal malignancy disease worldwide [1]. The highest HCC incidence and mortality is observed in Eastern Asia and central Africa, but its frequency has been rapidly increasing in Europe and in the United States in the last decades.

During malignant transformation, the tumorous extracellular matrix (ECM) undergoes qualitative and quantitative changes. As a result, the matrix is capable to provide the proper environment for tumor progression. Accordingly, in the last decade scientific activities have been directed towards the better understanding of the relationship between the tumor and its matrix.

Decorin is a small leucine-rich proteoglycan of the extracellular matrix, and primarily produced by fibroblasts and myofibroblasts [2, 3]. It is involved in several physiological and pathological processes such as collagen fibrillogenesis [4-6], muscular development [7], wound healing [8], myocardial infarction [9], kidney [10] and liver fibrosis [11], tumor growth [12, 13]. Decorin is typically located around proliferating tumor cells so-called tumor microenvironment [14]. It represents a powerful tumor cell growth and migration inhibitor by both modulating tumor stroma deposition and cell signaling pathways. Decorin causes growth arrest by inducing $p21^{Waf1/Cip1}$ tumor suppressor in most tumor cell lines [15].

In addition to above all, decorin is able to directly bind to and block the action of several tyrosine-kinase receptors, such as epidermal growth factor receptor (EGFR) as well as other members of the ErbB receptor tyrosine kinase family [16-19]. These receptors are overexpressed and/or mutated in many types of cancers accelerating tumor progression. Moreover, decorin targets EGFR to degradation by caveola-mediated endocytosis [20]. Decorin also negatively regulates hepatocyte growth factor receptor Met [21], vascular endothelial growth factor receptor (VEGFR) [22], insulin-like growth factor-1 receptor (IGF-1R) [23].

Beside the accumulated evidences that decorin is a potent inhibitor of tumor cell growth of various histological backgrounds *in vitro*, *in vivo* experiments has recently also been applied to test the antitumor effect of decorin [24-28]. Although these therapeutic approaches are promising, but generally utilize tumor cell lines injected into SCID mice and follow the growth rate of these tumors. These cells are either transfected with decorin or the molecule is delivered by adenovirus via intravenous or intratumoral injection. We must note that the aforementioned *in vivo* systems are not capable of modeling the human diseases closely, in particular the process of primary carcinogenesis, or the implication of the immune system.

Regarding liver tumors, there have been hardly any data on the role of decorin in the literature [29-31]. To fill up this hiatus, we designed a model system, in which we intended to examine the role of decorin both in the process of primary hepatocarcinogenesis and liver metastasis formation of colon carcinoma by utilizing decorin knockout (Dcn-/-) and wild type mice. This system, in contrast to the ones mentioned above, is suitable to observe changes happening in human pathological processes, and allows us to avoid using immunocompromised animals. In addition, we intend to explore whether ectopic delivery of decorin to knockout animals or artificial elevation of its level in wild type livers is capable to hinder or even to stop the process of hepatocarcinogenesis. 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 liver cancer, blocking the activity of VEGFR, PDGFR and Raf kinase. All of these molecules represent targets of decorin. In addition, decorin targets Met and EGFR both known to have an important role in the development of liver cancers, and are not suppressed by Sorafenib. Thus, decorin either alone or as a neoadjuvant could take place in the clinical treatment of these cancers.

Our ***hypothesis*** was that decorin acts as a tumor suppressor in primary and metastatic liver cancers. If the hypothesis is correct, the ***key question*** is whether decorin is applicable as a physiological receptor tyrosine kinase inhibitor in liver cancers.

To answer these questions we aimed:

1. To test the effect of decorin exposure on hepatoma and colon carcinoma cell lines *in vitro*.
2. To investigate the protective role of decorin in primary liver cancers and in liver metastases of colon carcinoma cells *in vivo*.
3. To determine the changes in hepatocarcinogenesis and development of metastases upon exogenous decorin delivery.
4. To examine the effect of decorin receptor tyrosine kinase activities both *in vitro* and *in vivo*.
5. To evaluate decorin expression and distribution in human HCCs and metastases of colon carcinoma, and its correlation with RTK activity and prognosis.
6. To find out if the GAG side chain takes part in the action of decorin.

RESULTS

I. HEPATOCELLULAR CARCINOMA

1. *In vitro* experiments with hepatoma cell lines

Human recombinant decorin utilized in the experiments was produced by CHO (Chinese hamster ovary) cells and purified by column chromatography.

In our *in vitro* system, we proved that exposure to exogenous decorin significantly decreases Hep3B hepatoma cell proliferation in a dose dependent manner. In parallel with proliferation blockade, expression of $p21^{Waf1/Cip1}$ cyclin dependent kinase was significantly elevated suggesting that decorin is able to arrest the cell cycle. This suggestion was further corroborated by the significantly lower mRNA levels of cyclin-dependent kinase 2 and 4 (CDK2, CDK4) and cell division cycle 25A (cdc25A) detected in cells exposed to decorin. Among intracellular signal molecules a significant decrease in Myc expression was seen in decorin-treated cells compared to that of control. Besides the cell cycle blockade, human recombinant decorin was able to enhance apoptosis of hepatoma cells (measured by FACS analysis), which was accompanied by elevated BAX2 proapoptotic gene expression and active caspase-3 levels.

Next, we studied further hepatoma cell lines to see whether the inhibitory effect of decorin is a universal phenomenon. Cell lines Hep3B, HepG2, HLE and HuH7 were utilized in further experiments. Addition of decorin to culture media significantly inhibited the proliferation of all cell lines. An important fact is that these cell lines utilize different signaling pathways for maintaining

their continuous division, and decorin was able to exert its blocking effect in all cases. Among others, Myc, β-catenin, Akt and ERK1/2 are all influenced by decorin. In those cells having intact restriction point regulation decorin acts via inducing $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. Hep3B cell line however is retinoblastoma and p53 deleterious mutant, thus cell cycle passes the G1 phase. The post-G1 inhibitory effect of decorin has been unknown yet; the exact mechanism is under investigation.

Regarding receptor tyrosine kinases, decorin exposure significantly decreased the level of phospho-EGFR in HepG2, HuH7 and Hep3B cell lines. Also, active insulin-receptor and IGF-1R were blocked in a dose dependent manner in HuH7 cells.

These effects were not linked to the present of glycosaminoglycan chains as expected from literature, thus we conducted further experiments with glycosylated decorin.

***Conclusion**: Addition of exogenous decorin inhibits proliferation of various hepatoma cell lines, and able to interfere with EGFR, IGF-1R and insulin receptor.*

2. Experimental hepatocarcinogenesis *in vivo*

a. Primary hepatocarcinogenesis in decorin-null and wild type mice

In the 1st round of primary hepatocarcinogenesis experiments, decorin-null and wild type C57Bl6 mice were exposed to two carcinogens: thioacetamide (TAA) and diethyl nitrosamine (DEN) for 7 and 9 months respectively. Decorin ablation resulted in enhanced tumor prevalence and larger tumor volumes in both chemicals, where statistically significant differences were observed when TAA was applied. In TAA-exposed livers, decorin was observed to deposit along the cirrhotic septa in large quantities. Connective tissue capsule of tumors also showed strong decorin immunopositivity. In addition, decorin of TAA-induced tumors is detected at higher molecular weight by Western blot analysis reflecting on changes in its GAG chains.

In decorin-null tumors, expression of $^{p21Waf1/Cip1}$ was significantly lower than that of wild type ones both at mRNA and protein levels (90% and 50% decrease respectively). In wild type livers $p21^{Waf1/Cip1}$ is produced by both hepatocytes and connective tissue cells. Within the tumors, tumors cells as well as stromal cells exhibit $p21^{Waf1/Cip1}$ immunopositivity. In contrast, only connective tissue and tumor stroma cells express $p21^{Waf1/Cip1}$ in livers lacking decorin gene. These observations suggest that the lack of decorin leads to enhanced tumor cell proliferation as failure of $p21^{Waf1/Cip1}$ induction promotes G1 to S transition in the cell cycle.

Four tyrosine kinase receptors exhibiting significantly higher activity were identified in decorin knock out tumors compared to wild type, namely epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor-α (PDGFRα), insulin-like growth factor-1 receptor (IGF-1R), and macrophage stimulating protein receptor (MSPR, alias RON). The enhanced RTK activity signals through Akt and Ras-Mapk pathways. Elevated phosphorylation of Akt inactivates GSK3 which in turn will be unable to phosphorylate c-myc and β-catenin. Failure in these phosphorylation events results in hindered degradation of c-myc and β-catenin allowing their nuclear translocation and enhanced action. Within the nucleus, c-myc induces AP4 transcription factor a known repressor of $p21^{Waf1/Cip1}$. Low levels of $p21^{Waf1/Cip1}$ leads to increased CDk4 activity and enhanced retinoblastoma phosphorylation.

As no report on a relationship between decorin and PDGFRα has been published before, we continued to explore the mechanism underlying their connection. Decorin knockout livers contain twice as much PDGFRα as wild type. In the normal liver, the receptor is found in the membrane of

non-parenchymal cells, primarily found in the periportal area. Scattered immunostaining was observed in sinusoids as well. However, hepatocytes showed no immunoreaction in their membranes. No difference between decorin-null and wild type normal livers was detected. In cirrhosis and hepatocellular carcinoma, PDGFRα level increases. The tumor stroma was positive mainly in Dcn-/- tumors. To determine whether decorin directly binds to PDGFRα, we performed double immunostaining and confocal laser microscopy analysis. The proteoglycan did not show colocalization with the receptor suggesting and indirect relationship and inhibition. Next, we tested whether decorin can directly bind to the ligand PDGF. Based on the results of these binding assays, we propose that decorin directly binds to the ligand PDGF, thus inhibits its association to the PDGFRα blocking downstream signaling pathways.

***Conclusions**: The lack of decorin sensitizes livers for tumor formation. The increased susceptibility is accompanied with higher RTK activities and accelerated cell cycle.*

b. Primary hepatocarcinogenesis with decorin administration

Based on the experiences of the 1st primary hepatocarcinogenesis experiments, we planned a new set of investigations, where a group of mice received decorin treatment. As significant increase in the prevalence and tumor volume upon decorin ablation was only seen TAA-exposed mice, we chose this model for further investigations. The method of decorin administration proposed in the grant has been changed based on the opinion of the reviewer 4. According to the reviewer, decorin injected i.p. might induce immune response in the animals, thus the effect of decorin would not be seen. To avoid the aforementioned problem we have set up and *in vivo* transfection system. Decorin cDNA was cloned into a pLIVE vector (Mirus Bio LLC), 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 injecting together with the decorin-coding or with the empty vector, the serum alkaline phosphatase detected from blood provides indirect information about the activity of the Dcn-pLIVE or Empty-pLIVE. Vectors were injected using hydrodynamic gene delivery method. The following groups were set for carcinogenesis experiments (Table 1.).

***Table 1**. Experimental set-up for hepatocarcinogenesis with decorin delivery.*

Group	Genotype	TAA	Vector	SEAP
1.	wild type	-	empty-pLIVE	yes
2.	wild type	-	Dcn-pLIVE	yes
3.	Dcn-/-	-	empty-pLIVE	yes
4.	Dcn-/-	-	Dcn-pLIVE	yes
5.	wild type	yes	empty-pLIVE	yes
6.	wild type	yes	Dcn-pLIVE	yes
7.	Dcn-/-	yes	empty-pLIVE	yes
8.	Dcn-/-	yes	Dcn-pLIVE	yes

Artificial decorin expression was tested by immunostaining. Decorin is expressed in the transfected livers. The excessive proteoglycan amount is mainly localized in the sinusoidal region. The serum alkaline phosphatase is easily detected from blood samples by a chemiluminescent assay. Depending on the transfection efficiency measured by SEAP assay, wild type experimental groups were subdivided into decorin negative, low decorin expression and high decorin expression categories (Figure 1A). Upon thioacetamide exposure the highest tumor number was observed in animals with no excessive decorin production. *In vivo* transfection of decorin-null animals is not as

efficient as seen in wild type mice, thus results are provided separately. As seen in Figure 1B, decorin expression was able to inhibit tumor formation in decorin knockout mice as well.

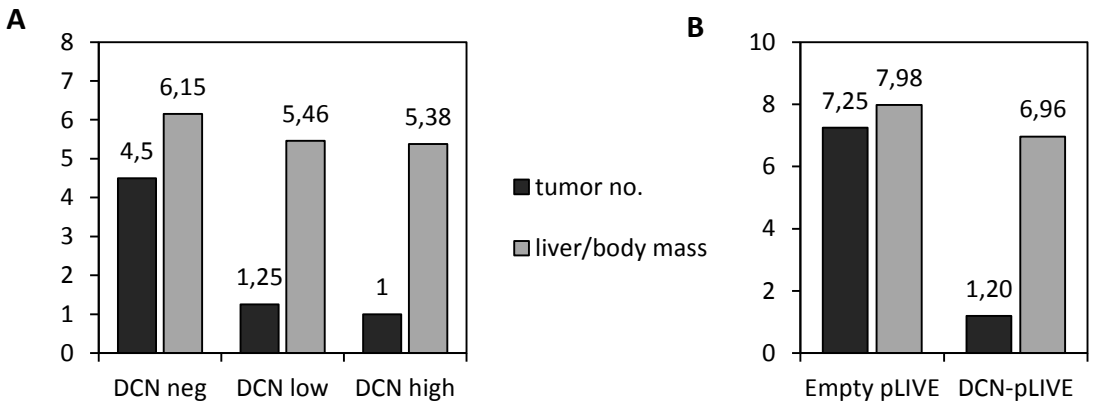

Figure 1*. Prevention of tumor formation by decorin gene delivery in TAA-induced hepatocarcinogenesis. Results of TAA-induced hepatocarcinogenesis in wild type (**A**) and in decorin knockout (**B**) animals.*

Conclusion*: Decorin delivery is able to effectively inhibit tumorigenesis evoked by thioacetamide.*

3. Decorin in human hepatocellular carcinoma

a. Tissue microarray results

To reveal changes in the expression of decorin in human hepatocellular carcinoma we utilized FFPE tissue samples of HCC with or without cirrhosis as well as *in silico* approaches. Biopsy samples of 29 HCCs (20 cirrhotic, 9 non-cirrhotic) and 9 control livers (haemangioma) were selected for tissue microarray (TMA) assembly. From each HCC, one core from the tumor and one from non-tumorous adjacent tissue (NAT) was selected. TMA block was sectioned and slides were immunostained for decorin, smooth muscle actin (SMA) and tyrosine-kinase receptors. Staining intensities were analyzed by Pannoramic Viewer software using a 12-score system, or DensitoQuant module. Decorin and SMA mRNA levels were determined by quantitative real-time RT-PCR analyses. To avoid distortion of results by the different number of decorin producing myofibroblasts (MFs), decorin expressions both at mRNA and protein level were normalized to smooth muscle actin (SMA) content.

In general, HCC tumor samples have decreased or blocked decorin expression compared to non-tumorous adjacent tissues (NAT), which was not due to changes in myofibroblast number, as results are normalized to SMA content. In our sample collection 52% of HCCs were decorin negative, 33% exhibited low, and 15% high decorin levels. Negativity and low expression was more emphasized in HCCs without cirrhosis. In addition, SMA corrected DCN amount negatively correlates with EGFR level, a receptor known to be downregulated by decorin (Figure 2).

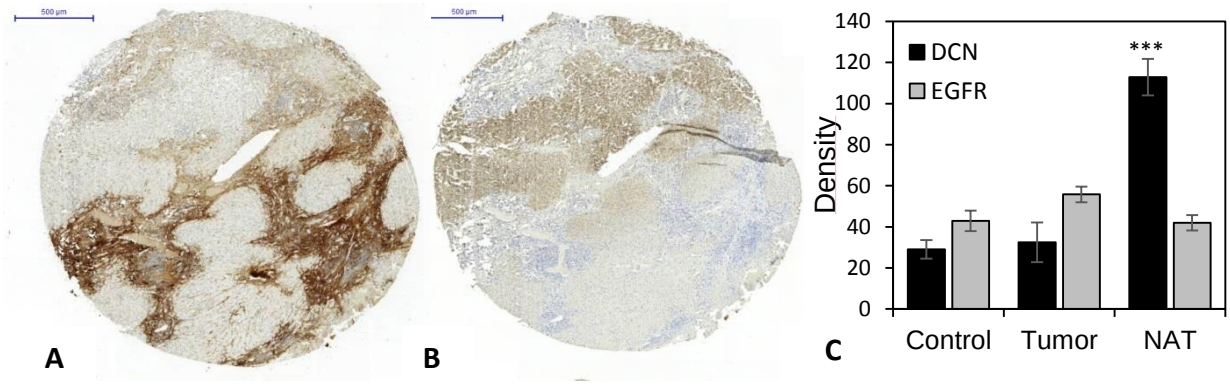

Figure 2**. Alternating intensity of decorin and EGFR. Quantification of decorin (**A**) and EGFR (**B**) immunostainings and their representative TMA cores (**C**). # p<0.5 to tumor.p<0.001 to control.*

b. In silico results

To enlarge our sample collection we also analyzed Dataset E-MTAB-950 (containing 34 normal, 112 tumor, and 5 pair of tumor–non-tumorous adjacent tissue (NAT)) from ArrayExpress database by R programming language due to its detailed clinicopathological data. *In silico* mRNA expression analysis revealed that DCN/SMA content distinguishes between normal and tumorous samples, and even characteristic for very early stage HCC. Decorin mRNA levels normalized to SMA gradually decrease from very early to advanced HCC, while it is overexressed in cirrhosis. DCN expression seems to follow the BCLC staging classification as well (Figure 3).

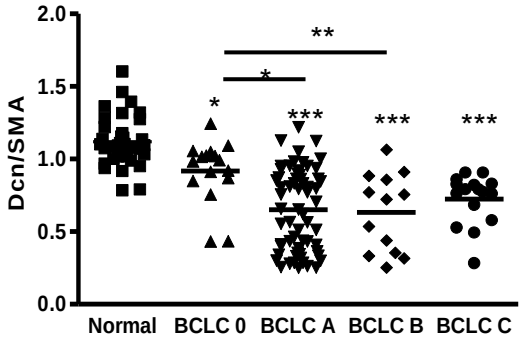

***Figure 3**. Relationship between decorin expression and BCLC classification of HCC.*

The most prominent downregulation of decorin mRNA expression was seen in tumors with β-catenin mutations, and a considerable decrease was observed in TP53 mutated tumors as well. Normalized DCN expression did not correlate with pRPS6, pAkt immunopositivity, with age or with gender. However, it seems that a certain DCN/SMA ratio (0.7) divides tumor samples into two well defined clusters regardless to the feature examined. Tumors with <0.7 DCN/SMA ratio were characterized by β-catenin mutations and low level of inflammation, whereas in those with >0.7 DCN/SMA β-catenin mutations were less frequent and inflammation was a typical feature. By comparing <0.7 tumors with >0.7 ones, EFEMP1 (EGF-containing fibulin-like extracellular matrix protein-1, alias fibulin-3), ASPN (asporin) and THBS2 (thrombospondin-2) were found to show the strongest correlation with DCN expression.

The fact that myofibroblasts reduce their decorin mRNA level in the tumor stroma compared to non-tumorous area of the same liver raises the possibility of epigenetic alterations such as promoter methylation or microRNA regulation. To explore these options Datasets GSE 7958 for

decorin expression and methylation, GSE1384 for miR181b expression were analyzed by GEO R[2]. Decorin expression was downregulated in most HCCs compared to their non-tumorous tissues similarly as seen previously. However, promoter methylation of decorin from the same samples does not seem to correlate with corresponding mRNA levels. Decorin is a known target of miR-181b [32]. However, its expression in HCC compared to the surrounding tissue does not show consequent changes suggesting other regulation mechanisms for decorin expression blockade.

***Conclusion**: decorin may act as a tumor suppressor in liver cancer as its expression is reduced or completely blocked in HCC. In addition, decorin level negatively correlates with EGFR.*

c. In vitro confirmation of human HCC results

To test whether tumor cells are capable of directly influence the decorin production of myofibroblasts, LX2 human stellate cells were exposed to hepatoma conditioned media. Significantly less decorin was detected in the media of LX2 cells when HLE, HepG2 or Huh7 conditioned media was applied. These changes appeared at transcription level, as decorin mRNA level was reduced in LX2 cells exposed to hepatoma media (Figure 4).

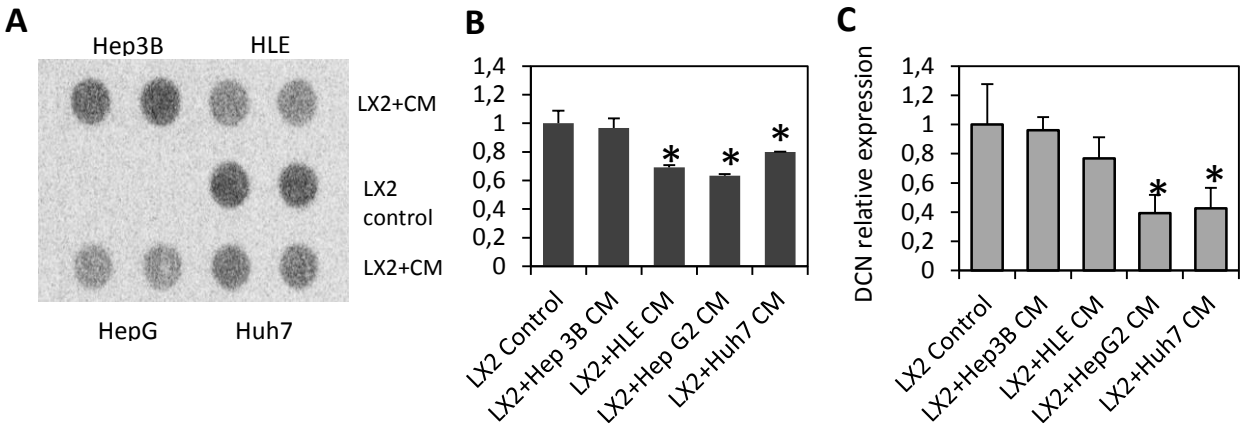

***Figure 4**. Decorin production in LX2 stellate cells upon exposure to hepatoma cell medium. Dot blot analysis of decorin content in LX2 cell media (**A**) and its quantification (**B**). Determination of decorin mRNA levels (**C**). CM=conditioned medium. *p<0,05.*

***Conclusion**: These results corroborate our observations in human HCC tissue samples. In addition, they indicate that the presence of tumor cells reduces the expression of decorin highlighting its tumor suppressor effect in hepatocellular carcinoma.*

II. LIVER METASTASES OF COLON CARCINOMA

1. *In vitro* experiments

C38 mouse colon carcinoma cells are normally maintained subcutaneously in C57Bl6 mice. Our attempts to transfer the cell line into cultures have failed so far. Thus no *in vitro* experiments could have been performed. The effect of decorin on colon cancer cell lines has already been published [33, 34].

2. *In vivo* colon carcinoma-liver metastasis model

In this model system, colon carcinoma cells are injected into the spleen followed by their colonization to the liver. Our first attempts revealed that C38 cells inoculated into decorin-null animals often grew locally, thus their colonization to the liver is dubious and cannot be compared to wild type animals. However, these primary tumors formed in Dcn-/- spleens are larger, and occur more often than observed in wild type. The cause of this phenomenon remains unknown yet. Based on these observations, our experiments have been modified utilizing only wild type animals. Decorin delivery was conducted using the same pLIVE vectors as described previously. In livers overexpressing decorin a 63% reduction in the number of liver metastases was observed in parallel with lower liver mass/body mass ratio reflecting on the tumor content of the organ as well (Figure 5A).

Receptor tyrosine kinase activities were measured in the experiments described above. Excessive decorin level in the liver reduced the levels of active EGFR, MSPR and PDGFRα, similarly as seen in primary liver cancers (Figure 5B).

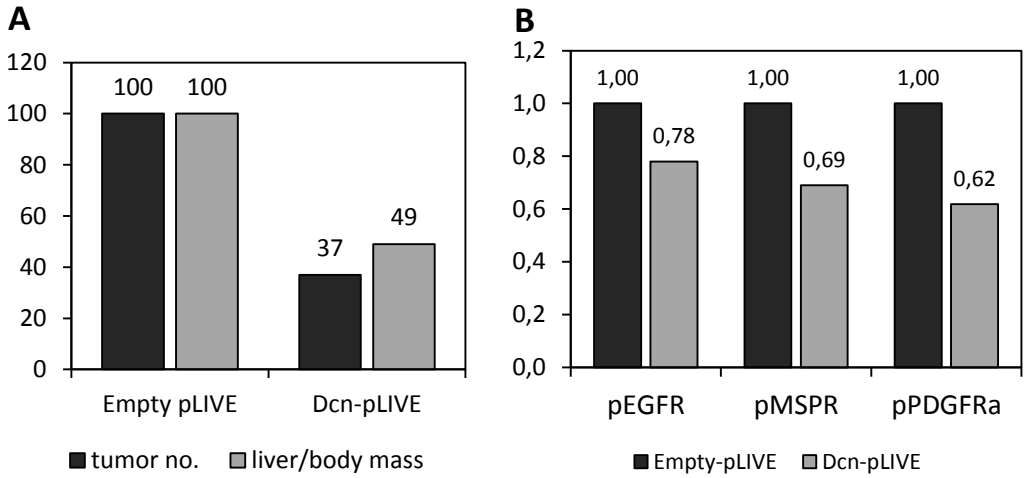

***Figure 5**. Effects of decorin delivery on tumor formation (**A**) and RTK activities (**B**) in metastatic liver cancer.*

***Conclusions**: These results suggests the protective role of decorin against liver metastases of colorectal cancer, and its blocking effect on tyrosine kinase receptors.*

3. Decorin in liver metastases of human colorectal cancer

In cooperation with the 2nd Department of Pathology, we have collected archive biopsy samples of patients with liver metastases of colorectal cancer. Not only liver metastasis, but surrounding non-tumorous liver, the primary tumor and normal colon of the same patients have all been used to build tissue microarrays. Up to now, 23 cases are included in our analysis. Similarly to HCC TMA slides, immunostaining specific for decorin and smooth muscle actin was performed. Decorin expression in primary tumor stroma is usually high (Figure 6A), however, liver metastasis of the same tumor often displays reduced amount of the proteoglycan (Figure 6C). Statistical analyses of immunostainings and their relationship with clinicopathological data are under evaluation.

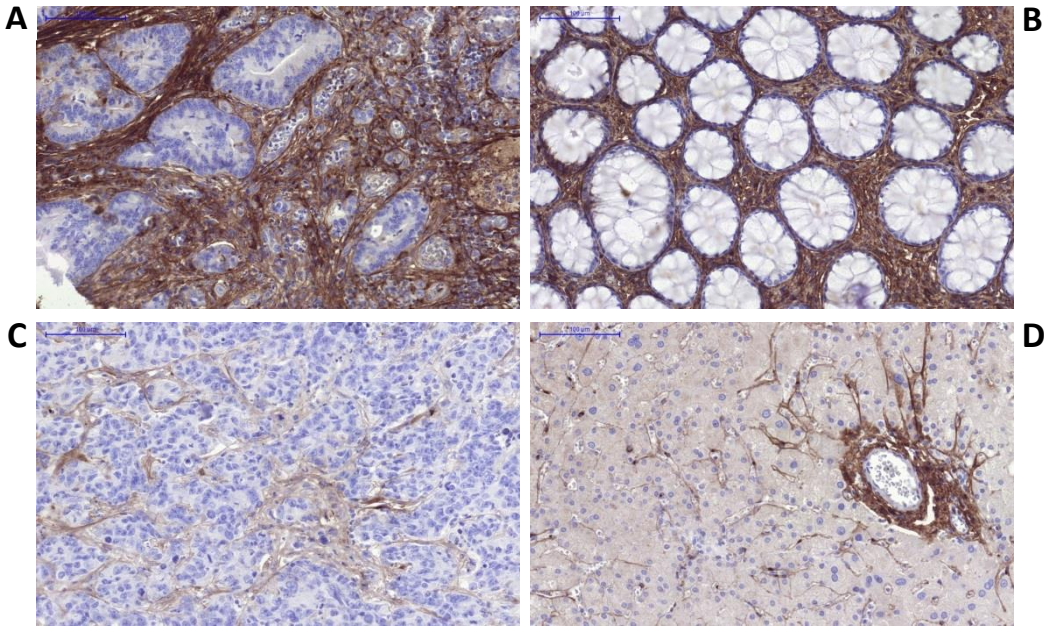

***Figure 6**. Representative images of decorin immunostaining of colorectal cancer (**A**), normal colon (**B**), liver metastasis (**C**) and surrounding liver (**D**)*

***Conclusion**: Decreased decorin expression in the liver metastasis compared to the primary tumor may reflect on the aggressiveness of the colorectal cancer.*

PUBLICATIONS in connection with the recent project

Peer reviewed articles *(with the acknowledgement of the OTKA-PD-105763)*

1. Proteoglycans in liver cancer. **Baghy K**, Tátrai P, Regős E, Kovalszky I. World J Gastroenterol. (2016) 7;22(1):379-93. *IF: 2,369 (2014)*

2. Remodeling of extracellular matrix by normal and tumor-associated fibroblasts promotes cervical cancer progression. Fullár A, Dudás J, Oláh L, Hollósi P, Papp Z, Sobel G, Karászi K, Paku S, **Baghy K**, Kovalszky I. BMC Cancer. (2015) 11;15:256. *IF: 3,362 (2014)*

3. Elevated miR-33a and miR-224 in steatotic chronic hepatitis C liver biopsies. Lendvai G, Jármay K, Karácsony G, Halász T, Kovalszky I, **Baghy K**, Wittmann T, Schaff Z, Kiss A. World J Gastroenterol. (2014) 7;20(41):15343-50. *IF: 2,369*

4. Decorin deficiency promotes hepatic carcinogenesis. Horváth Z, Kovalszky I, Fullár A, Kiss K, Schaff Z, Iozzo RV, **Baghy K**. Matrix Biol. (2014) 35:194-205. *IF: 5,074*

5. Decorin interferes with platelet-derived growth factor receptor signaling in experimental hepatocarcinogenesis. **Baghy K**, Horváth Z, Regős E, Kiss K, Schaff Z, Iozzo RV, Kovalszky I. FEBS J. (2013) 280(10):2150-64. *IF: 3,986*

REFERENCES

1. El-Serag, H.B. and K.L. Rudolph, *Hepatocellular carcinoma: epidemiology and molecular carcinogenesis.* Gastroenterology, 2007. **132**(7): p. 2557-76.
2. Ferdous, Z., et al., *Decorin-transforming growth factor- interaction regulates matrix organization and mechanical characteristics of three-dimensional collagen matrices.* J Biol Chem, 2007. **282**(49): p. 35887-98.
3. Hocking, A.M., T. Shinomura, and D.J. McQuillan, *Leucine-rich repeat glycoproteins of the extracellular matrix.* Matrix Biol, 1998. **17**(1): p. 1-19.
4. Danielson, K.G., et al., *Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility.* J Cell Biol, 1997. **136**(3): p. 729-43.
5. Ferdous, Z., et al., *Influence of cyclic strain and decorin deficiency on 3D cellularized collagen matrices.* Biomaterials, 2008. **29**(18): p. 2740-8.
6. Ruhland, C., et al., *The glycosaminoglycan chain of decorin plays an important role in collagen fibril formation at the early stages of fibrillogenesis.* FEBS J, 2007. **274**(16): p. 4246-55.
7. Brandan, E., C. Cabello-Verrugio, and C. Vial, *Novel regulatory mechanisms for the proteoglycans decorin and biglycan during muscle formation and muscular dystrophy.* Matrix Biol, 2008. **27**(8): p. 700-8.
8. Jarvelainen, H., et al., *A role for decorin in cutaneous wound healing and angiogenesis.* Wound Repair Regen, 2006. **14**(4): p. 443-52.
9. Weis, S.M., et al., *A role for decorin in the remodeling of myocardial infarction.* Matrix Biol, 2005. **24**(4): p. 313-24.
10. Schaefer, L., et al., *Absence of decorin adversely influences tubulointerstitial fibrosis of the obstructed kidney by enhanced apoptosis and increased inflammatory reaction.* Am J Pathol, 2002. **160**(3): p. 1181-91.
11. Breitkopf, K., et al., *Expression patterns of PDGF-A, -B, -C and -D and the PDGF-receptors alpha and beta in activated rat hepatic stellate cells (HSC).* Cytokine, 2005. **31**(5): p. 349-57.
12. Iozzo, R.V., *The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth.* Crit Rev Biochem Mol Biol, 1997. **32**(2): p. 141-74.
13. Iozzo, R.V., *The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins.* J Biol Chem, 1999. **274**(27): p. 18843-6.
14. Iozzo, R.V., *Tumor stroma as a regulator of neoplastic behavior. Agonistic and antagonistic elements embedded in the same connective tissue.* Lab Invest, 1995. **73**(2): p. 157-60.
15. Santra, M., et al., *Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous p21, an inhibitor of cyclin-dependent kinases.* J Clin Invest, 1997. **100**(1): p. 149-57.
16. Iozzo, R.V., et al., *Decorin is a biological ligand for the epidermal growth factor receptor.* J Biol Chem, 1999. **274**(8): p. 4489-92.
17. Csordas, G., et al., *Sustained down-regulation of the epidermal growth factor receptor by decorin. A mechanism for controlling tumor growth in vivo.* J Biol Chem, 2000. **275**(42): p. 32879-87.
18. Santra, M., I. Eichstetter, and R.V. Iozzo, *An anti-oncogenic role for decorin. Down-regulation of ErbB2 leads to growth suppression and cytodifferentiation of mammary carcinoma cells.* J Biol Chem, 2000. **275**(45): p. 35153-61.
19. Santra, M., C.C. Reed, and R.V. Iozzo, *Decorin binds to a narrow region of the epidermal growth factor (EGF) receptor, partially overlapping but distinct from the EGF-binding epitope.* J Biol Chem, 2002. **277**(38): p. 35671-81.
20. Zhu, J.X., et al., *Decorin evokes protracted internalization and degradation of the epidermal growth factor receptor via caveolar endocytosis.* J Biol Chem, 2005. **280**(37): p. 32468-79.

21. Goldoni, S., et al., *Decorin is a novel antagonistic ligand of the Met receptor.* J Cell Biol, 2009. **185**(4): p. 743-54.
22. Khan, G.A., et al., *Decorin is a novel VEGFR-2-binding antagonist for the human extravillous trophoblast.* Mol Endocrinol. **25**(8): p. 1431-43.
23. Schonherr, E., et al., *Decorin, a novel player in the insulin-like growth factor system.* J Biol Chem, 2005. **280**(16): p. 15767-72.
24. Reed, C.C., J. Gauldie, and R.V. Iozzo, *Suppression of tumorigenicity by adenovirus-mediated gene transfer of decorin.* Oncogene, 2002. **21**(23): p. 3688-95.
25. Tralhao, J.G., et al., *In vivo selective and distant killing of cancer cells using adenovirus-mediated decorin gene transfer.* Faseb J, 2003. **17**(3): p. 464-6.
26. Reed, C.C., et al., *Decorin prevents metastatic spreading of breast cancer.* Oncogene, 2005. **24**(6): p. 1104-10.
27. Biglari, A., et al., *Effects of ectopic decorin in modulating intracranial glioma progression in vivo, in a rat syngeneic model.* Cancer Gene Ther, 2004. **11**(11): p. 721-32.
28. Shintani, K., et al., *Decorin suppresses lung metastases of murine osteosarcoma.* Oncol Rep, 2008. **19**(6): p. 1533-9.
29. Shangguan, J.Y., et al., *[Effects and mechanism of decorin on the proliferation of HuH7 hepatoma carcinoma cells in vitro].* Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2009. **25**(9): p. 780-2.
30. Chung, E.J., et al., *Gene expression profile analysis in human hepatocellular carcinoma by cDNA microarray.* Mol Cells, 2002. **14**(3): p. 382-7.
31. Miyasaka, Y., et al., *Analysis of differentially expressed genes in human hepatocellular carcinoma using suppression subtractive hybridization.* Br J Cancer, 2001. **85**(2): p. 228-34.
32. Kwan, P., J. Ding, and E.E. Tredget, *MicroRNA 181b regulates decorin production by dermal fibroblasts and may be a potential therapy for hypertrophic scar.* PLoS One, 2015. **10**(4): p. e0123054.
33. Bi, X., et al., *Decorin-mediated inhibition of colorectal cancer growth and migration is associated with E-cadherin in vitro and in mice.* Carcinogenesis, 2012. **33**(2): p. 326-30.
34. Nyman, M.C., et al., *Decorin in Human Colon Cancer: Localization In Vivo and Effect on Cancer Cell Behavior In Vitro.* J Histochem Cytochem, 2015. **63**(9): p. 710-20.