Communication between tumor cells and their microenvironment. The activating role of fibroblasts in tumor growth and invasion.

Beside tumor cells cancers of solid tumors are built up by wide variety of stromal components, including fibroblasts, inflammatory and immune cells and blood vessels embedded into the extracellular matrix. Accepting the idea, that these non-tumorous components support the development and progression of cancer, our goal in the last 30 years was to study their implication in the development and progression of cancer. This initiated our last OTKA project as well. Here, two model systems were created. The reason why we created the **first one** was the fact that during pregnancy the development of placenta is a benign but invasive process providing opportunity for comparison its events with that of the invasion of cervical cancers. These studies resulted 3 publications and 1 PhD

This project focuses on two protein molecules, *TFPI-2 and syndecan-1*, both involved in the regulation of the pericellular microenvironment. In addition to inhibition of fibrinolysis, TFPI-2 is also responsible for regulation of invasive processes. The other protein is syndecan-1, which is able to link events in the pericellular space to intracellular processes, thereby also being involved in cell invasion. TFPI-2 is likely to bind to heparan sulfate chains, which enhances its activity. The role of the proteins in placenta and cervical cancer was studied in three model systems.

1. We found that TFPI-2 expression gradually increases every trimester in healthy pregnancy. In case of abnormal placentation such as in early preeclampsia with or without HELLP syndrome, measured in the third trimester, the expression of TFPI-2 is increased and this is in a good agreement with increased MVMP scores, and decreased birthweight and placental weight. These data provide evidences for the role of increased TFPI-2 levels in abnormal pregnancies. Changes in the serum TFPI-2 levels could be monitored and could therefore be used for diagnostic purposes. The inhibitory effect of TFPI-2 is likely to be enhanced by the heparane sulfate changes of syndecan-1 expressed in syncytiotrophoblasts**. PLACENTA 76: pp. 30-39., 2019**

2. MessengerRNA array, derived from primary tumor-associated fibroblast cultures of cervical cancer, showed decrease of *TFPI2* mRNA to 10% of normal fibroblasts. To clarify the cause of this phenomenon, we found that while *TFPI2* is inhibited in tumor cells by promoter methylation, the DNA of tumor-associated fibroblasts is not methylated, and its *TFPI2* is inactivated by *miR-23a*. Tumor cells stimulate upregulation of *miR-23a* in fibroblasts, however its mechanism requires further investigation. In contrast with normal cervix, human cervical cancer specimens fail to express TFPI2, indicating the tumor suppressor activity of the protein. **PLOS ONE 15: (6) e0234873, 2020**

3. Examining cervical cancer samples, we found that cell surface syndecan-1 expression in a 15-year follow-up proved to be a good prognostic factor for the first 7 years. In contrast, syndecan-1 expression of stromal fibroblasts, frequently observed in tumors, did not proved to be prognostic factor *in vivo*. In the meantime, in tissue culture, not only tumor-associated but also normal fibroblasts stimulated tumor cell proliferation, this was accompanied by induction of syndecan-1 on the surface of normal fibroblasts as well. This suggests that other factors also play a role in cancer aggression in vivo. **PATHOLOGY AND ONCOLOGY RESEARCH 26: (4) pp. 2255-2264., 2020**

The **second model** focused on proteoglycans and several matrix proteins such as thrombospondin, collagens TGF beta, integrins, etc on the events of cirrhosis induction, hepatocarcinogenesis and liver cancer.

1.Liver cirrhosis was induced in wild type and $SDC^{+/+}$ transgenic livers by thioacetamide. Development of liver cirrhosis was delayed by four months in transgenic animals as an effect of syndecan-1

shedding that removed TGF□1 and its activator thrombospondin from the liver circulation. *In vitro* experiments exposing the LX2 hepatic stellate cell line to conditioned media of wild type and syndecan-1 transfected Hep3B cell lines proved that medium with high syndecan-1 content inhibits TGFβ1-induced upregulation of SMA, TIEG, collagen type I and thrombospondin-1 expression**. MATRIX BIOLOGY 68-69: pp. 474-489., 2018**

2.Hepatocarcinogenesis was induced by DEN in wild type and $SDC^{+/+}$ transgenic livers. The events of carcinogenesis were followed for11 months. Final results revealed that SDC 1 overexpression delayed HCC development for six months. Mass spectrometry data provided evidences that the overexpressing proteoglycan interfered with the lipid metabolism by downregulating FASN and seven other proteins involved in lipid metabolism, inhibited the Akt-mTOR and beta catenin pathway know to be involved in development of non alcoholic fatty liver and subsequent cancer development. This calls attention for syndecan-1 beneficial potential against this human disease. **CANCERS 13: (7) 1548, 2021. 11. 30.**

3.Syndecan-1 expression in healthy and disesed human livers. This manuscript assessed the expression of the proteoglycan in healthy, and cirrhotic livers, as well as in primary and metastatic human liver cancers. Healthy liver has low amounts of syndecan-1 and the highest expression was found in HCV positive cancer. The explantion for the latter is, that syndecan-1 serves as HCV receptor**. PATHOLOGY AND ONCOLOGY RESEARCH 26: (2) pp. 813-819., 2020**

4. The deleterious effect of hyperglycemia on the biology of the liver is supported by clinical evidence. It can promote the development of fatty liver, liver fibrosis, even liver cancer. As liver fibrosis is the consequence of hepatic stellate cell (HSC) activation, the questions were addressed whether alterations induced by high glucose concentration are directly related to TGFB1 effect. High glucose concentration initiated profound alteration of LX-2 cells, different from those observed upon interaction with TGFB1. It resulted in decreased MMP2 activity, retardation of type I collagen in the endoplasmic reticulum, with decreased pS6 expression, pointing to development of endoplasmic stress and sequestration of $p21^{CIP1/WAF1}$ in the cytoplasm which can promote the proliferation of LX2 cells. **PATHOLOGY AND ONCOLOGY RESEARCH 2020 Jan;26(1):291-299**

5. In this study, we demonstrated that re-expression of full-length or ectodomain-deleted syndecan-1 in hepatocellular carcinoma cells downregulates phosphorylation of ERK1/2 and p38, with the truncated form exerting an even stronger effect. Furthermore, overexpression of syndecan-1 in hepatoma cells is associated with a shift of heparan sulfate structure toward a highly sulfated type, specific for normal liver. As a result, cell proliferation and proteolytic shedding of syndecan-1 from the cell surface are restrained, which facilitates differentiation of hepatoma cells to a more hepatocyte-like phenotype. Our results highlight the importance of syndecan-1 in the formation and maintenance of differentiated epithelial characteristics in hepatocytes partly via the HGF/ERK/Ets-1 signal transduction pathway. Reporter gene assay revealed the inhibition of Ets-1 as well as AP-1 transcription factor-induced promoter activation, presumably an effect of the heparan sulfate switch. **BIOMOLECULES 10: (10) 1356, 2020**

6. Decorin, a small leucine-rich proteoglycan of the extracellular matrix, represents a powerful tumor cell growth and migration inhibitor by hindering receptor tyrosine kinases and inducing $p21^{WAF1/CIP1}$ In this study, we tested decorin expression in HCCs utilizing *in silico* data, as well as formalin fixed paraffin embedded tissue samples of HCC in a tissue microarray (TMA). *In silico* data revealed that DCN/SMA mRNA ratio is decreased in HCC compared to normal tissues and follows the staging of the disease. Among TMA samples, 52% of HCCs were decorin negative, 33% exhibited low, and 15% high decorin levels corroborating *in silico* results. Decorin gene delivery reduced tumor formation, in

parallel with decreased pEGFR, increased pIGF1R levels, and with concomitant induction of pAkt (T308) and phospho-p53, suggesting a novel mechanism of action. Our results suggest the idea that decorin can be utilized as an anti-cancer agent. **FRONTIERS IN ONCOLOGY 10: 645, 2020**

7. Decorin is an extracellular matrix small leucine rich proteoglycan. Its cancer inhibitory potential is well established. This manusript addressed the question if this cancer inhibitory effect of decorin depends on the phenotype of liver cancer? Our results revealed that decorin is capable to exert its suppressor effect in hepatoma cells without respect to their phenotype and molecular background. **CELLULAR SIGNALLING 62: 109354, 2019**

8. We set out to investigate whether excess decorin may protect against the liver metastases of colon carcinoma. We also analyzed the effect of decorin in tissue microarrays of human colon carcinoma liver metastasis and examined whether the tumor cells can directly influence the decorin production of myofibroblasts. In humans, low levels of decorin in the liver facilitated the development of colon carcinoma metastases. In vitro, colon carcinoma cells inhibited decorin production in LX2 hepatic stellate cells. On contrary liver-targeted decorin delivery in mice effectively attenuated metastasis formation of colon cancer. Overexpressed decorin reduced the activity of multiple receptor tyrosine kinases (RTKs) including the epidermal growth factor receptor (EGFR), an important player in colorectal cancer (CRC) pathogenesis.In conclusion, decorin may effectively inhibit metastatic tumor formation in the liver. **BIOMOLECULES 10: (8) p. 1199., 2020**

9. As their name indicates, proteoglycans are molecules where the portein core carries one or more glycosaminoglycan sugar chain. The structure of these sugar chains can be modified by O and N sulfation which influences the behavior of the whole molecule, as an epigenetic modification. Thus, the effect of proteoglycans cannot be understanding without the analysis of their sugar chains. This is a difficult methodology, and a new approach is introduced in the MS Proteomics Research Group, Research Centre for Natural Sciences. They analyzed normal, cirrhotic and tumorous liver specimens, in cooperation with our research group. Although, our OTKA project was not mentioned in the paper, the specification of two others of the participant scientists are included. This cooperation is a terrific support for our research, which opens new possibilities for better understanding the functions and roles of proteoglycans in the development and progression of cancer. **J Chromatogr A . 2020 May 24;1619:460979**.

Submitted to Frontiers in Oncology: **Spock1 supports the development of hepatocellular cancer.** Fast growing number of publications indicate that Spock-1 plays an outstanding role in the development and progression of cancers. The aim of the present work was to provide mechanistic details as Spock1 facilitates cancer development. Its amounts keep increasing in the cytoplasm of hepatocytes starting with very low expression in the normal cells, and appears in much higher quantity in cells of cirrhotic and tumorous human livers. Similar phenomenon was observed in diethylnitrosamine induced mouse hepatocarcinogenesis. Cytoplasmic Spock1 colocalizes with mitochondrial markers, such as mitotrack and TOMM20, the latter is a characteristic protein of the outer membrane of mitochondrion. Spock1 downregulation inhibits cell proliferation, upregulates p21, p27 and caspase3, interferes with pAkt and CDK4 expression. Its inhibition alters the MAPK signaling and downregulates several members of Sarc familly. all related to the aggressivity of the hepatoma cell lines. Our experiments support the fact, that Spock1 enhancement in the liver is an active player in human and rodent hepatocarcinogenesis and cancer progresson.

Further analysis of the role of Spock-1 in the liver cancer will be supported by OTKA project 138593 ***A SPOCK1/Testican-1 onkogén hatásai a májban. A tumor-stróma interakció jelentősége***