GENETIC, EPIGENETIC, TRANSCRIPTIONAL AND FUNCTIONAL COMPARISON OF TUMOR-ASSOCIATED AND ADJACENT NORMAL MYOFIBROBLASTS

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Summary of the Ph.D. Thesis

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Introduction

Although the number of available cancer therapeutic agents has been increased in the past few years, the rate of cancer related mortality is still over 30,000 cases a year in Hungary. The explanation for the contradiction, at least in part, is the fact that most of the cancer therapeutic agents target the neoplastic cells, but do not affect the microenvironment of the tumor. The tumor microenvironment (TME) is established by tumor cells and the host tissues, and as a result of rather intensive reciprocal interactions between these cells it changes rapidly and evolves dynamically during tumor progression, thereby contributing actively to cancer progression and disease outcome. In TME, cancer cells recruit various cell types of mesenchymal origin to form a tumor stroma rich in fibroblasts, various immune cells, endothelial cells and pericytes. These cells are responsible for substantially reorganizing the extracellular matrix to form a fibrotic and rigid structure which, in turn, facilitates the survival and the proliferation of tumor cells, and enhances their metastatic capacity. Moreover, remodeling of the tumor matrix and the consequentially increased interstitial fluid pressure might seriously hinder the delivery of pharmaceutical agents into the tumor tissue. Fibloblast-like cells are the most abundant cell types in the

TME, they are commonly referred to as cancer-associated fibroblasts (CAF). CAFs are considered as a rather heterogeneous population of cells and myofibroblasts form the most prominent subset. Myofibroblasts, introduced sometimes as activated fibroblasts, share characteristics of fibroblasts and smooth muscle cells and while their function in wound healing was recognized earlier, their role as an active component of tumor progression is a relatively recent recognition. By histological examination of various solid tumors, myofibroblasts were identified in increased number and in an altered arrangement compared to that of normal tissues, which indicated a potential functional alteration of myofibroblast recruited to TME. Myofibroblasts express growth factors, cytokines and chemokines, synthesize ECM components, and secrete tissue proteases, such as matrix metalloproteinases (MMPs). All these properties of myofibroblasts may profoundly contribute to a tumor-promoting and invasion-assisting activity within the TME, however, the molecular background behind the development of such a metastasis-stimulating myofibroblast phenotype has not been elucidated yet.

Aims

Matching pairs of primary myofibroblast cultures isolated from the tumor tissues and from the adjacent normal tissues of gastrointestinal (GI) cancer patients were involved in our experiments. Our aim was to characterize the tumor-promoting phenotype of stromal myofibroblast cells in order to understand the possible molecular mechanisms responsible for the morphological and functional changes in tumor-derived myofibroblasts.

Methods

- α-smooth muscle actin, vimentin and cytokeratin expression was analyzed by RT-PCR to validate the purity of the myofibroblast primary cultures.

- Genomic DNA was isolated and targeted next-generation sequencing (NGS) was performed to identify cancer-related variants in tumor-associated myofibroblasts.

- Immunocytochemical staining and western blot analysis were carried out to reveal changes in the level of various histone posttranslational modifications.

- RNA was isolated and TaqMan low density gene expression arrays were accomplished to detect tumor-related gene expressional changes in myofibroblasts derived from the tumor tissues.

- Quantitative polymerase chain reactions (qPCR) were performed to examine the mRNA expression levels of numerous ECM proteoglycans and glycoproteins, as well as of matrix metalloproteinases.

- The MMP3 and MMP10 protein amounts of the cultured myofibroblasts were assessed by western blot analysis and the gelatinase activity of MMP2 was detected by gelatin zymography.

- The migration capacity of myofibroblasts was determined by *in vitro* scratch assay.

Major findings

1, The number of mutations is not elevated in tumor-associated myofibroblasts.

255 kbp genomic sequences corresponding to cancer-related genes and single nucleotide polymorphisms (SNPs) were analyzed by targeted NGS. In adjacent normal myofibroblasts only a few somatic variants could be identified, and the number of somatic mutations observed in tumor tissue-derived myofibroblasts were nearly the same.

2, Significantly decreased global levels of H3K9me3 and H4K16ac were identified in myofibroblasts derived from tumor tissues.

Posttranslational modifications of H3 and H4 histones were examined by immunocytochemistry and western blot technique, and the results indicated lower levels of H3K9me3 and H4K16ac modifications in tumor-derived myofibroblasts compared to normal counterparts.

3, Tumor-associated myofibroblasts showed altered gene expressional profile

Expression of 190 selected, tumor-related genes were simultaneously quantified by TaqMan low density gene expressional arrays. The results of the arrays revealed that several genes involved in the invasion-metastasis process, in the formation and the remodeling of the ECM, in TGF-ß and Wnt signaling pathways and in the regulation of cell cycle exhibited altered expression in myofibroblasts isolated from tumor tissues.

4, Lower expression of ECM proteoglycans and glycoproteins could be detected in tumor-derived myofibroblasts.

mRNA levels of decorin, fibromodulin, nidogen 1, perlecan and TGF-ß receptor 3 were measured by RT-qPCR. Significantly lower levels of mRNAs corresponding to these ECM components were detected in tumor-associated myofibroblast cells.

5, Tumor-derived myofibroblasts exhibited altered MMP expressional pattern

Using RT-qPCR the expression of MMP1, 2, 3, 10 and 12 was quantified. The results showed that at least one of the examined MMP genes showed elevated expression in tumor-associated myofibroblasts compared to their normal myofibroblast pairs. In addition, elevated levels of mRNAs corresponding to MMP3 and MMP10 could be detected in most of the tumor-derived myofibroblasts involved in this study. Western blot experiments verified the qPCR results, since increased levels of MMP3 and 10 proteins were detected in myofibroblast cells obtained from the tumor tissues. The gelatinase activity of intracellular MMP2 showed no significant differences between tumor- and normal tissue-derived myofibroblasts, but elevated activity of secreted MMP2 could be detected in the conditioned media of certain tumor-tissue myofibroblasts.

6, Significantly higher migration capacity was detected for tumor-associated myofibroblasts.

In scratch assays myofibroblasts isolated from GI tumor tissues exhibited elevated motility than myofibroblasts derived from normal tissues. The average migration capacity was 1,56

times higher in tumor-associated myofibroblasts compared to normal tissue derived counterparts.

Summary

The results of our work indicate that the gene expressional and functional changes identified in tumor-associated myofibroblasts contribute to the tumor-promoting phenotype of these cells. Based on the lack of notable genetic alterations, epigenetic mechanisms could be responsible for the observed differences between tumor- and normal tissue derived myofibroblasts.

List of publications

MTMT ID: 10029158

1. Publications that fulfil the requirements of the doctoral procedure:

1.1. Publication related to the thesis:

Ildikó Huliák, László Bodai, Mátyás Czepán, Dávid Kovács, Anikó Szabó, László Tiszlavicz, György Lázár, Zoltán Rakonczay jr, Péter Hegyi, Imre M Boros, Monika Kiricsi: *Genetic, epigenetic and transcriptional comparison of esophagus tumor-associated and adjacent normal myofibroblasts*. Oncology Reports 2019; 41(2): 839-852. https://doi.org/10.3892/or.2018.6909.
Q1; IF: 3.417

1.2. Publication not related to the thesis:

Ildikó Huliák, Ádám Sike, Sevil Zencir, Imre M. Boros: *The objectivity of reporters: interference between physically unlinked promoters affects gene reporter expression in transient transfection expreriments.* DNA and Cell Biology 2012; 31(11):1580-4. https://doi.org/10.1089/dna.2012.1711.
Q2; IF: 2,344

2. Other publications:

Barbara N Borsos, **Ildikó Huliák,** Hajnalka Majoros, Zsuzsanna Újfaludi, Ákos Gyenis, Péter Pukler, Imre M Boros, Tibor Pankotai: *Human p53 interacts with the elongating RNAPII complex and is required for the release of actinomycin D induced transcription blockage*. Scientific Reports 2017; 7: 40960. https://doi.org/10.1038/srep40960.
Q1; IF: 4.122

Péter Bencsik, Krisztina Kupai, Zoltán Giricz, Anikó Görbe, **Ildikó Huliák**, Susanna Fürst, László Dux, Tamás Csont, Gábor Jancsó, Péter Ferdinandy: *Cardiac capsaicin-sensitive sensory nerves regulate myocardial relaxation via S-nitrosylation of SERCA: role of peroxynitrite*. British Journal of Pharmacology 2008; 153 (3): 488-96. https://doi.org/10.1038/sj.bjp.0707599.
Q1; IF: 4.902

Attila Kiss, László Juhász, **Ildikó Huliák**, Ágnes Végh: *Intracoronary infusion of peroxynitrite protects against ischaemia and reperfusion-induced arrhythmias in anaesthetised dogs without affecting mitochondrial KATP channels.* British Journal of Pharmacology 2008; 155(7): 1015-24. https://doi.org/10.1038/bjp.2008.344.
Q1; IF: 4.902

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