Final report for OTKA-FWF 118-119: Cell contractility in mesothelioma progression

In this project we could demonstrate that contractility-driven aggregate formation is a characteristic growth process for MPM in vivo and in vitro. We hypothesize that this concept of contractility may enable tumor nodules disseminated on the pleural surface to somehow detach from the pleural layer into the surrounding fluid to support locoregional disease dissemination. We developed a novel preclinical model of 3D-printed culture chambers, which may represent a promising novel tool to more accurately illustrate tumor growth dynamics in vitro in MPM. Importantly, the concept of tumor nodule formation may also be translatable to different malignancies and could therefore open a promising novel field in cancer research. In fact, tumor cells growing in 3D multicellular aggregates seem to behave differently when compared to cells part of 2D monolayers. Thus, our preclinical 3D model may be able to better mimic the in vivo circumstances. Strikingly, we could identify TGF-beta signaling as major determinant for tumor nodule formation and thus interfering with TGF-beta signaling could decrease aggregate formation. Those results let us hypothesize that TGF-beta signaling represents a very promising novel therapeutic approach interfering with growth dynamics in MPM.

1. Our mesothelioma-related results are included in three publications:

1.1 Laszlo et al (2018) is a large, multi-institutional study about a promising novel drug treatment (Nintedanib) of this devastating disease. Nintedanib's target receptors were expressed in 20 human MPM (malignant pleural mesothelioma) cell lines. Nintedanib inhibited MPM cell growth in both short- and long-term viability assays. Reduced MPM cell proliferation and migration and the inhibition of Erk1/2 phosphorylation were also observed upon nintedanib treatment in vitro. In an orthotopic mouse model of human MPM, Nintedanib significantly reduced tumor burden and vascularization and prolonged the survival of mice when it was administered intraperitoneally. Our laboratory contributed with videomicroscopic and computational studies to the overall project.

1.2 Tarnoki-Zach et al (2020) demonstrates that macroscopic multicellular aggregates, reminiscent of the MPM nodules found in patients, develop when MPM cell lines are cultured at high density for several weeks. Surprisingly, the nodule-like aggregates do not arise by excessive local cell proliferation, but by myosin II-driven cell contractility. Accordingly, nodule formation can be prevented or reversed by pharmacological inhibitors of myosin II activity. Contractile nodules contain prominent actin cables that often span several cells. Such multicellular actin cables are also abundant in MPM surgical specimens. In vitro MPM nodule development can largely be explained by a computational model that assumes uniform and steady intercellular contractile forces within a monolayer of cells, and a mechanical load-dependent lifetime of cell-cell contacts. The model behaves as a self-tensioned Maxwell fluid and exhibits an instability that leads to reproducible pattern formation. The model predicts that nodules appear faster when cell contractility is increased or when cell-substrate adhesion stability is decreased. Furthermore, decreased stability of cell-substrate adhesions favors the formation of fewer but larger clusters. Altogether, our findings suggest that inhibition of the actomyosin system may provide a potentially useful therapeutic approach for treatment of MPM. Unfortunately, our animal studies remained inconclusive.

1.3 Stockhammer et al (in preparation) demonstrates that TGF-beta signaling enhances nodule formation, and conversely, TGF-beta signaling inhibition decreases nodule formation in several mesothelioma cell lines. High cell density cultures were generated

from nodule-forming and non-nodule-forming MPM cell lines and treated with either TGF-beta or TGF-beta receptor I (RI) kinase inhibitors. The effects on altered TGF-beta signaling were measured by immunoblot. Changes in gene expression patterns upon nodule formation and in a larger MPM cell line panel between nodule-forming and non-nodule-forming cell lines were evaluated by expression microarrays, gene set enrichment analyses (GSEA) and corresponding pathway analyses using the ingenuity software package. We found that TGF-beta enhanced nodule formation in all four nodule-forming lines and induced de novo nodule-like aggregates in two of the four non-nodule-forming models. Interestiungly, expression of major immunosuppressive signals including PD-L1 increased upon nodule formation but even to a greater extent upon TGF-beta treatment. Strikingly, TGF-beta signaling inhibition could decrease nodule and colony formation in three out of four nodule-forming lines. However, neither TGF-beta nor its inhibition did interfere with cell viability. In our large panel of 28 MPM lines, in vitro nodule formation capacity associated with the expression of certain extracellular TGF-beta family members including LTBP1, LTBP3 and GREM1. These findings provide a strong rationale for targeting TGF-beta in combination tumor therapies against mesothelioma. Currenly we wait for animal studies to conclude, which were substantially delayed due to the COVID-19 pandemic.

2*. Studies of cell contractility are reported in four publications:*

2.1 Priya et al (2017) proposes and analyzes a mathematical model representing core interactions involved in the spatial localization of junctional RhoA signaling. We demonstrate how the interplay between biochemical signaling through positive feedback, combined with diffusion on the cell membrane and mechanical forces generated in the cortex, can determine the spatial distribution of RhoA signaling at cell-cell junctions.

2.2 Czirok et al (2017) demonstrates that by appropriate computational analysis of optical flow data one can identify distinct contractile centers and distinguish active cell contractility from passive elastic tissue deformations. Our proposed convergence measure correlates with myosin IIa immuno-localization and is capable to resolve contractile waves and their synchronization within maturing, unlabeled induced pluripotent stem cell-derived cardiomyocyte cultures.

2.3 Mehes et al (2019a) desrcibes a novel computational bioassay to determine cell contractility, and characterizes the cellular function of an intrinsic contractillity regulator protein, S100A4. We demonstrated that the Matrigel patterning assay, widely used to characterize endothelial cells, is a highly sensitive tool to evaluate cell contractility within a soft extracellular matrix (ECM) environment. We proposed a computational model to explore how cell-exerted contractile forces can tear up the cell-Matrigel composite material and gradually remodel it into a network structure. We identified quantitative measures that are characteristic for cellular contractility and can be obtained from image analysis of the recorded patterning process. The assay was calibrated by inhibition of NMII activity in A431 epithelial carcinoma cells either directly with blebbistatin or indirectly with Y27632 Rho kinase inhibitor.

2.4 Oelz et al (2019) investigates how cell sheet migration depends on the mechanical properties of cells and their interactions. We developed a quantitative model for collective cell sheet motility in an epithelial monolayer to understand the interplay between cell polarity, active motility, and elastic forces due to the mechanical cell-cell interactions.

3. Collective cell behavior and multicellular pattern formation is reported in seven publications.

3.1 Pongor et al (2017) reports that short and long distance cell dispersal can have a marked effect on tumor structure. Cellular motility, in particular, could lead to faster cell mixing and lower intratumor heterogeneity when assayed by next generation sequencing.

3.2 Neufeld et al (2017) uses a reaction-diffusion equation based model of tumor growth to investigate how the invasion front is delayed by resection. We show that the delay time is highly sensitive to qualitative details of the proliferation dynamics of the cancer cell population. We find that in glioblastoma cell cultures the cell proliferation rate is an increasing function of the density at small cell densities. Our analysis suggests that cooperative behavior of cancer cells, analogous to the Allee effect in ecology, can play a critical role in determining the time until tumor recurrence.

3.3 Lakatos et al (2018) proposes computational models to shed light on how vascular patterning is guided by self-organized gradients of the VEGF/sVEGFR1 factors. We demonstrate that a diffusive inhibitor can generate structures with a dense branching morphology in models where the activator elicits directed growth. Inadequate presence of the inhibitor leads to compact growth, while excessive production of the inhibitor blocks expansion and stabilizes existing structures. Model predictions were compared with time-resolved experimental data obtained from endothelial sprout kinetics in fibrin gels. In the presence of inhibitory antibodies against VEGFR1 vascular sprout density increases while the speed of sprout expansion remains unchanged. Thus, the rate of secretion and stability of extracellular sVEGFR1 can modulate vascular sprout density.

3.4 Mehes et al (2019b) investigates differences in single cell motility, collective motility and patterning of endothelial cells, in two dimensional vs three dimensional culture environments -- in relation with a cytoskeletal adapter protein, Tks4.

3.5 Szeder et al (2020) reports Tks4-dependent differences in humon colon cancer cell motiliy. We demonstrated that collective motility of colon cancer cells requires cell adhesion, which is strongly regulated by the Tks4 cytoskeletal adaper protein.

3.6 Goering et al (2021) demonstrates that the cytoskeletal adapter protein SPECC1L modulates the collective motility of palate mesenchyme, and animal models indicate that deficiency in coordinated migration and contractility in SPECC1L mutants contribute to common birth defects such as cleft palate.

3.7 Khataee et al (2020) employs a quantitative simulation of collective cell motility to establish the role of cell contractility in coordinating cell movements. The model captures the propagation of the polarization wave and suggests that the cells cortex can regulate the migration modes: strongly contractile cells may depolarize the monolayer, whereas less contractile cells can form swirling movement. Cortical contractility is further found to limit the cells motility, which (i) decelerates the wave speed and the leading edge progression, and (ii) destabilizes the leading edge.

4. We have a report about novel technical aspects of our experimental work:

4.1 In Gulyas et al (2018) we describe a package of open source software tools that we developed specifically to meet bioprinting requirements: Machine movements can be (i) precisely specified using high level programming languages, and (ii) easily distributed

across a batch of tissue culture dishes. To demonstrate the utility of the reported technique, we present custom fabricated, biocompatible 3D-printed plastic structures that can control cell spreading area or medium volume, and exhibit excellent optical properties even at 50 ul sample volumes.

5. Finally, our focus on the COVID-19 epidemic resulted in a disease modeling study -- only very loosely associated with the current research project.

5.1 In Neufeld et al (2020) we set up a theoretical model to investigate how targeted isolation of the vulnerable sub-population may provide a more efficient and robust strategy at a lower economic and social cost than full-scale lockdowns.

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