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Nano-scale dynamics of cellular interactions

The most life-threatening aspect of cancer is metastasis; cancer patient mortality is mainly due to metastasis. Among all metastases, presence of brain metastasis is one with the poorest prognosis; the median survival time can be counted in months. Therefore, prevention or decreasing their incidence would be highly desired both by patients and physicians. Since the central nervous system (CNS) lacks classical lymphatic circulation, metastatic cells invading the CNS must breach the blood-brain barrier. The key step in this process is the establishment of firm adhesion between the cancer cell and the cerebral endothelial layer. Brain endothelial cells play important role in contact establishment; therefore we have tested their temporal stability regarding elastic parameters. Compared to epithelial cells the brain endothelial cells elasticity varies on time scale of minutes considerably. Even more, periodic oscillations can emerge. The results concerning temporal elasticity of endothelial cells were published in the journal of Biochemistry and Biophysics Reports 2016: **Varga et al. BBRep 2016, DOI:10.1016/j.bbrep.2016.06.015.**

Using the atomic force microscope, a high resolution force-spectrograph, our aim was to explore the connections among the cell morphology, cellular mechanics and biological function in the process of transendothelial migration of metastatic cancer cells. These experiments were based on single-cell force spectroscopy measurements of intercellular adhesion (single cell adhesion model). First the proper immobilization of the studied cancer cells to the cantilever was tested, and found to be firm enough for each studied cell type. After this preliminary immobilization tests, affinity of the cell-decorated probe to a confluent endothelial layer were performed. Intercellular adhesion was directly measured at quasi-physiological conditions. According to our previous results, ROCK inhibition induced an increase in the adhesion force between melanoma and brain endothelial cells. Plausible mesenchymal cell flattening would have been partly responsible for this phenomenon. We supposed that induction of the amoeboid phenotype using the Rac inhibitor does not have such a significant impact on the shape of tumor cells detached from the surface. Therefore, the area of contact between tumor cells and endothelial cells does not significantly differ between control and Rac inhibited tumor cells, resulting in similar adhesion forces in the presence or absence of EHT1864, as measured by single cell force spectroscopy. These results were published in the journal of **Cell Adhesion and Migration by Molnar et al. DOI: 10.1080/19336918.2015.1122156.**

Furthermore, spatial distribution of elasticity and detachment strength was characterized by adhesion and elasticity maps recorded with a melanoma cell decorated probe on a confluent layer of endothelial cells. Our aim was to explore the connections among the cell morphology, cellular mechanics and biological function in the process of transendothelial migration of metastatic cancer cells. By immobilization of a highly metastatic melanoma cell (A2058), binding characteristics to a confluent layer of brain

endothelial cells was directly measured by means of single cell force spectroscopy. Several nanomechanical parameters related to cell elasticity and intercellular adhesion were calculated and presented as pseudo colored three dimensional maps. The reconstructed maps reveal elastic, plastic and adhesive heterogeneity of the endothelial layer, but not directly linking these parameters. All these data point towards, that the invading melanoma cell might somehow "screen" for the best places prior to start the transmigration process over the endothelial layer. These results were published in the **Journal of Molecular Recognition DOI: 10.1002/jmr.2603**

In order to explore invasiveness related adhesion properties, we have compared the de-adhesion properties and dynamics of three melanoma cells types (WM35, A2058 and A375) from a confluent layer of brain micro-capillary endothelial cells. Cell type dependent adhesion characteristics were presented, pointing towards the existence of metastatic potential related nanomechanical aspects. Apparent mechanical properties such as elasticity, maximal adhesion force, number, size and distance of individual rupture events showed altered values pointing towards cell type dependent aspects. Cell type dependent adhesion characteristics of living melanoma cells to a confluent layer of endothelial cells has appeared in the journal of **BBA General Subjects; DOI: 10.1016/j.bbagen.2017.10.013.**

Not closely related to the subject of the present project another article was published describing the pattern recognition receptors (nod like receptors and toll like receptors) expression of control and immune stimulated brain pericytes and their activation on the non-canonical inflammasome pathway. These receptors and their activation plays important role in the immune response of the central nervous system, where the pericytes might play important regulating roles in neuroinflammation as well. These results were published in the journal of **Brain Behaviour and Immunity; DOI: 10.1016/j.bbi.2017.04.010**.

Although the lung is a frequent target for metastatic cells, the nanomechanics of the lung endothelium in presence of anti-cancer drugs is not entirely understood. Not closely related to the subject of the project the nanomechanical properties of lung derived endothelial cells was studied in the presence of several anti-cancer drugs dasatinib and imatinib. These results were published in the **Frontiers in Physiology; DOI: 10.3389/fphys.2018.00537.**

The bi-faceted role of the neurovascular unit in the process of metastasis formation hold several aspects which needs to be elucidated. Molecular pathways as well as emerging nano-mechanical aspects are summarized and discussed in the article published in the **Journal of Cerebral Blood Flow and Metabolism; DOI:10.1177/0271678X17732025**.

The characteristic hallmarks of the intercellular adhesion pattern have many crucial players at its origin. Starting from outside the glycocalyx layer, the surface adhesion molecules, the physical state of the membrane, the underlying cytoskeletal network and is anchoring points all have a contribution to the measured final adhesion build-up. It is a

rather challenging task to exactly quantify the contribution of all components, where one can hinder influences from others. This probably too ambitious goal was not entirely fulfilled during this last year of the project due to several on-road difficulties. The contribution of the membrane fluidity was extensively studied. In order to identify the origin of the observed individual rupture events, we have explored the importance of the membrane fluidity. By adding benzyl alcohol, a well know membrane fluidizing agent to the system, distribution of the observed rupture events was compared to pre-treatment state. The membrane fluidity is an important factor which severely effects the distribution of the rupture events. Moreover, two population of de-adhesion events were distinguished and determined their ratio to total linkage strength: one between cytoskeleton bonded and the other between freely moving adhesion molecules within cell membrane. Above our expectations, fluidizing the membrane introduces several effects which unfortunately produces blurred results instead to clarify. However, after successful data analysis for the most effective and straightforward visualization of the obtained results is close to be published in the near future.

The other important factor in the intercellular adhesion build up is the layer of the glycocalyx which might be present on both cell surfaces. According to literature data, long term presence of flavonoids might result a swollen layer, which in turn could directly affect metastasizing cells in their adhesive process. Extensive experiments were conducted to reveal the flavonoid related adhesion alterations between melanoma and endothelial cells. Results of these experiments are on the way to be visualized and structured to be published soon.

As it was planned, in order to ease the data handling and analysis a home-written custom routine was developed and tested to efficiently extract and visualize the parameters of interest from recorded force curves. The results obtained with this tool were presented in already published articles as well as incorporated in manuscripts pending to be submitted in the future.

Our results highlight the importance of cellular mechanics in brain metastasis formation and emphasize the enormous potential towards exploration of intercellular dynamics related processes. Based on our findings, it can be underlined the importance of mechanical details in case of intercellular interactions. Nevertheless, it suggests that in adequate circumstances elastic and adhesive characterizations might be used as biomarkers.

All the above described and mentioned results were presented in several domestic and international conferences and meetings as well.