Project 6: **Crosstalk between melanoma cells and the blood-brain barrier: impact on coagulation and brain metastasis to identify new anti-metastatic targets.**

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Short Summary

Human malignant melanoma is a highly metastatic tumor, and especially metastatic lesions in the brain are associated with poor prognosis. To metastasize to the brain, cancer cells must interact with cerebral endothelial cells (ECs) and migrate through the blood-brain barrier (BBB). The vascular endothelium is activated by tumor cells, which is followed by the release of inflammatory cytokines and the procoagulatory protein von Willebrand factor (VWF) known to promote tumor progression. Although treatment with heparin, a known anti-coagulant, revealed a therapeutic effect in experimental models, the underlying mechanisms are poorly understood. To investigate the impact of the coagulation system on tumor spreading, we will analyze the molecular pathways of melanoma-induced EC activation using an *in vitro* model of the BBB, and we will address the effect of melanoma-derived factors on EC permeability. Furthermore, taking advantage of a novel *in vivo* multiphoton microscopy model allowing real-time imaging of brain metastases, we will investigate how the coagulation pathway influences melanoma cell arrest and extravasation. Finally, we will characterize the ret-transgenic mouse melanoma model to evaluate therapeutic effects of anti-coagulants *in vivo*.

3 State of the Art

3.1 State of knowledge in the field

Melanoma has the highest propensity to metastasize to the brain, and brain metastases are a major cause of mortality. Although little is known about the interaction between melanoma cells and brain microvascular endothelial cells (BMECs), malignant cells need to overcome the BBB to form brain metastasis. We hypothesize that this interaction is a multimodal process that includes melanoma cell-induced EC activation followed by the development of a proinflammatory and procoagulatory EC surface that facilitates melanoma cell adhesion. The bidirectional melanoma-EC interaction leads to an increase in BBB permeability followed by melanoma cell transmigration and metastases formation. This hypothesis is supported by clinical and experimental reports showing that tumor-mediated activation of the coagulation system enhances the risk of thromboembolism and promotes tumor cell spreading in patients. Patients treated with heparins showed a better outcome and heparins reduced the formation of metastasis in animal models.

3.2 Preliminary work by the participants

In previous studies we could show that melanoma-derived MMP-1 activates ECs followed by the release of proinflammatory and procoagulatory factors. Recently, we described two additional pathways that enable melanoma cells to stimulate ECs. First, a tissue factor (TF)-thrombin-PAR1 dependent pathway was discovered. Second, we identified melanoma-derived VEGF acting via VEGF-R2 as the main direct activator of ECs. This melanoma-induced EC activation was attenuated by heparins. All these pathways induce an acute Weibel-Palade body (WPB) exocytosis and the formation of ultralarge VWF fibers at the luminal surface of ECs, directly mediating platelet adhesion. Moreover, we could show that VWF supports leucocyte extravasation by increasing endothelial permeability, a process that may exhibit similarities with tumor cell extravasation. Moreover, our data show that melanoma cell-induced EC activation depends on the type of melanoma cells and ECs. However, studies on the molecular mechanisms of melanoma cell interaction with the endothelium of the BBB are lacking. The Winkler lab has established novel applications of *in vivo* multiphoton microscopy (MPLSM), where brain endothelial cells, blood perfusion, single cancer cells in subcellular resolution, and the single steps of brain metastasis formation of melanoma cells can be imaged through a cranial window in real time over months.

Application of this technology allows the study the interaction of melanoma cells with brain ECs in the physiological microenvironment. This unique experimental platform is available to other projects of this proposal to investigate the role of molecular pathways and the effect of therapeutic intervention on distinct steps of the metastatic cascade. All in all, new mechanistic insights into the crosstalk between melanoma and BMECs may have important consequences for diagnostic and therapeutic strategies in patients suffering from malignant melanoma.

4 Project Plan

4.1 Specific Aims

1. Impact of coagulation on tumor spreading
2. Mechanisms of melanoma - blood-brain barrier interaction
3. Impact of anticoagulants on brain metastasis in an animal model

4.2 Experimental program

1. The molecular mechanisms of melanoma-mediated activation of BMECs *in vitro* and on the integrity of the EC monolayer will be analyzed. In previous work we have found that some melanoma cell lines such as A2058 can form parenchymal brain metastases, whereas other lines (i.e. B16F10) do not. The metastatic potential is reflected by a distinct ability of EC activation. In order to clarify the molecular mechanisms of melanoma-derived mediators that activate ECs, different melanoma cell lines will be compared by gene expression analysis and proteome profiling.
2. a) The impact of melanoma-mediated EC activation on expression of adhesion molecules and melanoma cell adhesion will be assessed using microfluidic devices established by the Schneider lab. b) *In vivo* MPLSM imaging of VWF and adhesion molecules, and their co-localization with arrest and extravasation of melanoma cells in the brain will be investigated.
3. Finally, we will study the relevance of our results using the ret-transgenic mouse melanoma model. To this end, VWF-selective changes in BBB integrity will be evaluated by analysis of candidate molecules for vascular permeability, by application of heparins, new anticoagulants and knockdown of adhesion factors in melanoma cells in our *in vivo* MPLSM imaging model. We will thereby determine their role in the metastatic cascade, which should lead to rapid identification of most promising therapeutic targets.

4.3 Collaborations with other Projects in the RTG

Cell-based assays and in-vivo stemness reporter systems to identify the role of melanoma stem cells will be performed with Projects 1 and 2. Angiopoetin-2 and its impact on BBB permeability will be analyzed with Project 8. Analysis of brain metastases in the ret mouse model will be characterized together with Project 13.

5 References

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