Project 7: **Liver-specific endothelial mechanisms of melanoma metastasis**

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Short Summary

Hematogenous metastasis is remarkably organ-specific. The liver with its unique sinusoidal vascular system is one of the preferred sites of malignant melanoma (MM) metastasis. Liver-specific, endothelial-dependent mechanisms of MM cell dissemination will be analyzed by scrutinizing liver sinusoidal endothelial cell (LSEC)-specific candidate molecules such as Stabilin-1 and Stabilin-2 identified by us as well as other scavenger and lectin-like receptors. Their role in tumor cell adhesion and transmigration will be investigated in a microfluidic chamber model *in vitro* using over-expression in human umbilical vein endothelial cells (HUVEC) as well as by using LSEC from the respective KO animals. A special focus will be on hyaluronan (HA)-mediated interactions. Results will be confirmed *in vivo* using the ret model as well as a B16 luciferase model of MM metastasis to the liver in WT and KO animals. The final goal of this project is to develop novel strategies to treat metastasis in this devastating disease.

3 State of the Art

3.1 State of knowledge in the field

Metastatic spread to distant organs in general comprises a series of steps in which endothelial cells (EC) are intricately involved such as tumor cell adhesion and transmigration. In many cancers, including MM, however, cancer cell dissemination is remarkably organ-specific. Organ-specific metastasis is likely caused by tumor cell heterogeneity as well as by organ-specific stromal factors. Among these, EC heterogeneity may impact on tumor-EC interactions by modulating the well-known general adhesive mechanisms or by providing organ-specific pathways. Besides the lungs and the brain, the liver is a preferred site for distant metastasis in MM, and the primary site for metastasis in uveal melanoma. LSEC are a prime example of organ-specific EC differentiation. LSEC selectively express several scavenger and lectin-like receptors such as stabilin-1/2, LYVE-1, MRC1, CD32B, CLEC-1B, -4G, and -4M. Two of these molecules, stabilin-2 and LYVE-1 are known HA receptors. In addition, the HA receptor CD44 is also expressed by LSEC. Furthermore, HA-dependent mechanisms have been shown to be of high importance for melanoma metastasis in general and especially in the liver. HA is generated by HA synthases on the surface of MM cells and could contribute to liver metastasis by binding to stabilin-2, LYVE-1 or to CD44. Conversely, CD44 has also been found on many types of tumor cells including MM. Therefore, MM cells could also adhere to HA bound by the three HA receptors on the surface of LSEC. In addition, stabilin-1 and Clec-4G/LSECtin have already been demonstrated to mediate binding of tumor cells other than MM cells to LSEC. In summary, the analysis of this set of candidate LSEC adhesion molecules may open new avenues to target EC-dependent, organ-specific MM metastasis to the liver.

3.2 Previous work by the participants

Our group has a longstanding track record in analyzing the specific molecular repertoire and functions of LSEC including identification of the scavenger receptors stabilin-1 and -2. Stabilin-2 KO mice, although displaying no obvious phenotype, show highly increased plasma levels of HA proving that stabilin-2 is the major receptor for HA turnover. Due to impaired clearance of other noxious blood factors, stabilin-1/2-/- double deficient mice have a reduced lifespan and develop severe glomerulosclerosis indicating the importance of stabilin function for the whole organism. LSEC specifically produce wnt2 that acts as an autocrine growth factor by cross-stimulating the VEGF pathway. Comprehensive gene expression analysis revealed a LSEC-specific, hepatic microenvironment-dependent differentiation program comprising distinct sets of growth and transcription factors as well as of adhesion- and endocytosis-associated molecules including the novel junctional protein Leda-1.

4 Project Plan

4.1 Specific Aims

The general aim of the project is to analyze the organ-specific, endothelial-dependent mechanisms of MM cell dissemination to the liver. For this purpose, we will thoroughly study MM-LSEC interactions *in vitro* and *in vivo*. *In vitro*, we will analyze (1) MM cell-LSEC adhesion and transmigration in a microfluidic chamber model using HUVEC retrovirally transfected with LSEC-specific candidate adhesion molecules, as well as LSEC isolated from the respective knockout mice. Special attention will be given to HA-mediated mechanisms. *In vivo*, (2) LSEC-dependent MM metastasis to the liver will be investigated using a B16 luciferase model, the ret MM model, and a human xenotransplant model of MM metastasis.

4.2 Experimental program

1. LSEC-specific candidate molecules for liver-specific melanoma-EC adhesion and transmigration, i.e. stab1, stab2, Lyve-1, MRC1, CD32b, CLEC-1B, -4G, and -4M, will be retrovirally transfected into HUVEC. Using transwell migration assays and a microfluidic chamber device that simulates organ-specific flow conditions, MM cell adhesion and transmigration will be studied using transfected HUVEC as well as murine LSEC and – as a control – murine lung microvascular endothelial cells (LMEC) from wild-type and knock out animals (stab1-/-, stab2-/-, Lyve-1-/-, CD44-/-). In these assays, the relevance of HA-dependent mechanisms will be analysed by pre-incubation of either MM or endothelial cells with HA to block HA binding proteins, by hyaluronidase treatment and by inhibition of HA synthases. The function of CD44 expressed by MM cells as a ligand for HA deposited on LSEC will be investigated by CD44 knock-down in MM cells.
2. *In vivo*, organ-specific MM cell-EC adhesion and metastasis will be analysed by injecting luciferase-expressing B16F10 mouse MM cells into the spleen (liver metastasis) or tail vein (lung metastasis) of wild-type and knockout animals (stab1-/-, stab2-/-, Lyve-1-/-, CD44-/-). HA dependent mechanisms will be studied in vivo using i.p. injection of hyaluronidase and p.o. administration of HA synthase inhibitors. Development of metastases will be traced and quantified by bioluminescence in vivo imaging. Adhesion molecule-deficient animals will be back-crossed with ret MM mice and analysed for spontaneous liver metastases. Adhesion molecules that can be shown to be involved in MM adhesion and metastasis in those murine models will be further scrutinized in a xeno-transplant model of human melanoma metastasis.

4.3 Collaborations with other Projects in the RTG

Melanoma cell-LSEC adhesion will be analyzed with Project 6 (microfluidic chamber) and Project 1 (CD44-mediated adhesion). The ret model of MM and the human MM mouse xeno-transplant model will be provided by Projects 13 and 12, respectively.

5 References

1. Schledzewski K*, **Geraud C***, Arnold B, Wang S, Grone HJ, Kempf T, Wollert KC, Straub BK, Schirmacher P, Demory A, Schonhaber H, Gratchev A, Dietz L, Thierse HJ, Kzhyshkowska J, **Goerdt S.** 2011. Deficiency of liver sinusoidal scavenger receptors stabilin-1 and -2 in mice causes glomerulofibrotic nephropathy via impaired hepatic clearance of noxious blood factors. *J Clin Invest* 121:703-14.
2. **Geraud C***, Schledzewski K*, Demory A, Klein D, Kaus M, Peyre F, Sticht C, Evdokimov K, Lu S, Schmieder A, Goerdt S. 2010. Liver sinusoidal endothelium: a microenvironment-dependent differentiation program in rat including the novel junctional protein liver endothelial differentiation-associated protein-1. *Hepatology* 52:313-26.
3. Klein D, Demory A, Peyre F, Kroll J, Augustin HG, Helfrich W, Kzhyshkowska J, Schledzewski K, Arnold B, **Goerdt S.** 2008. Wnt2 acts as a cell type-specific, autocrine growth factor in rat hepatic sinusoidal endothelial cells cross-stimulating the VEGF pathway. *Hepatology* 47:1018-31.