A Road Map for Melanoma – Anatomic and molecular mapping of the intravasation niche

MMD internship project: January – July 2017
Department: Cell Biology (Cell Dynamics group)
Supervisor: Prof. Dr. Peter Friedl
Daily supervisor: Sarah Weischer (Sarah.Weischer@radboudumc.nl)

Background

Metastasis is the main factor that causes poor prognosis in cancer patients. An early and very important step to metastasis formation in distant organs is the entry of tumor cells into blood vessels (intravasation), which releases circulating tumor cells (CTC) detectable in patients' blood. However, the cellular and molecular mechanisms of intravasation remain unknown because commonly used microscopy approaches cannot optically reach and visualize deep events in established tumor lesions.

In this project, we aim to resolve the process of tumor cell intravasation and define the niches that promote this critical step towards metastasis. We predict that intravasation will occur in permissive regions ('hot spots") guided by particular anatomic and molecular properties (e.g. hypoxic and necrotic regions, tumor-promoting stromal cell infiltrates). We are currently developing a microendoscopic imaging tool for investigating deep tumor regions in a murine B16F10 melanoma model. In this model we can readily detect single CTCs as well as circulating microemboli.

Aim of the study

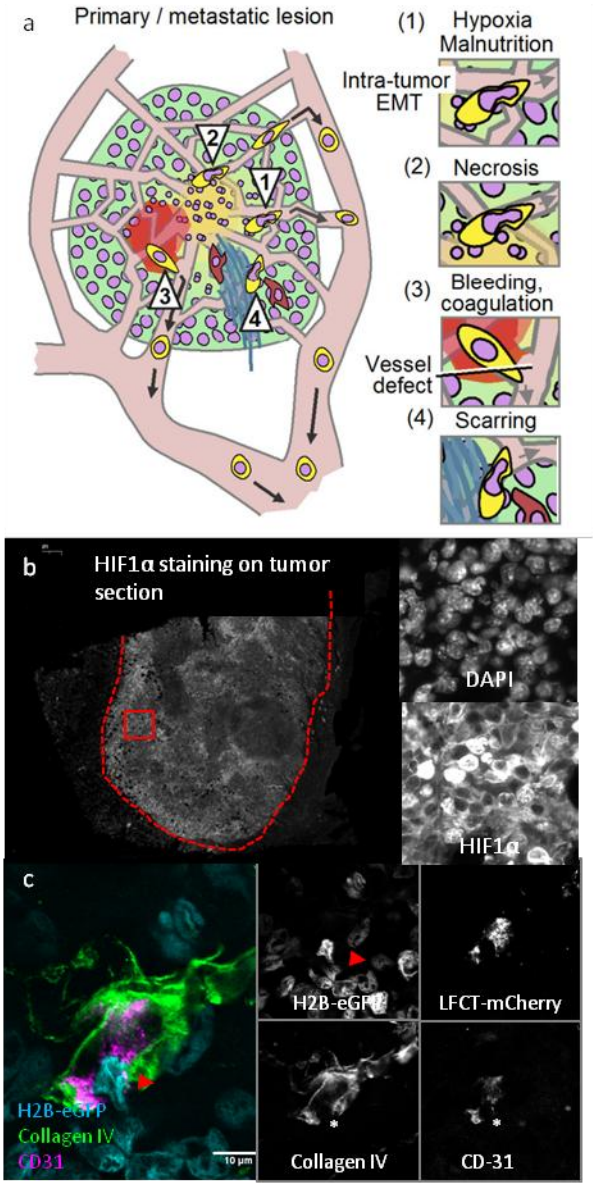
Using this model, we will create a spatial-temporal map of triggers that have been hypothesized to facilitate metastases inside deep tumor regions. This map will be created by combining advanced technologies: (1) intravital imaging, (2) multiparametric immunofluorescence on *ex vivo* 2D and 3D tissue sections and patient material, (3) identification of intravascular tumor cells in tissue slices, (4) analysis of CTC in mouse blood and (5) distant metastases formation as read-outs for intravasation. Since hypoxia has been described to facilitate metastases formation, we hypothesize that cells in a hypoxic response state, characterized by increased levels of hypoxia-inducible factor 1α (HIF1α), will frequently intravasate and thereby increase metastases formation. Therefore, the aim of this project will be to experimentally generate a strategy for high intravasation, by HIF1α overexpression in melanoma cells, and characterize these cells on a molecular and functional level *in vitro* and *in vivo*. Cells after HIF1 overexpression will be first characterized molecularly, tested for growth and invasion in advanced 3D tissue culture, and the effect of (chronic) hypoxia *in vivo* will be verified in proof-of-principle experiments using intravital micro(endo)scopy, CTC detection and metastases screens.

Objectives:

1) Generation of HIF1α over-expressing melanoma cells, *in vitro* biochemical and functional characterization, proof-concept-experiments *in vivo*
2) Multiparametric tumor mapping
   Detection of molecular-metabolic fingerprint (e.g. blood vessel network, hypoxia, necrosis, stromal cell infiltration, protease activity) in vivo and on ex vivo 2D and 3D tissue sections

Techniques:

Tumor tissue preparation/fixation/cutting;
Immunohistochemistry/fluorescence on tissue sections, multi-color image acquisition, advanced image analysis; cell culture, viral transfection/transduction; Western blot; advanced 3D tumor cell culture; basic small animal handlings (e.g. animal fixation, i.p. injections, dissection of organs); first insights into intravital microscopy + animal experiments



a. Intratumor intravasation. The tumor core is a possible region where intravasation takes place due to hypoxia and malnutrition (1), necrosis (2), bleeding (3) and scarring of tissue (3). b. Using overviews of a paraffin-embedded tumor sections hypoxic regions can be identified throughout the whole tumor (dashed line). Zooms show typical nuclear HIF1α location as indicator for hypoxia. c) On 3D tumor sections a deformed nucleus (red arrow, nucleus: H2B-GFP, actin: lifeact-mCherry) was identified in the blood vessel wall between basement membrane (CollagenIV) and endothelium (CD31). The blood vessel staining shows a gap the location of the tumor cell with a possible migration channel (asterisks).