**‘The role of CXCR4 receptor in Ewing sarcoma angiogenesis: a single-molecule and super-resolution microscopy study’**

The main goal of this project was to identify the regulatory mechanisms by which the G-protein coupled receptor CXCR4 initiates chemotaxis upon stimulation with its chemokine SDF1applying super-resolution single-molecule microscopy. Many vital but also various aberrant cellular processes require the establishment of cell polarity ultimately leading to directed cell migration. These cellular processes are summarized by the term 'chemotaxis'. Extracellular gradients of low molecular-weight proteins, the chemokines, are faithfully detected by cells even in shallow gradients of few percent across the cell body. Any malfunction during this complex process can lead to severe diseases. Only precise knowledge of the molecular mechanisms will allow for the development of new drugs and specific treatment of patients.

I investigated the molecular mechanisms following stimulation in a model cell line of mouse 3T3 fibroblasts. To that end, 3T3 cells, that do not express CXCR4 endogenously, were transiently transfected with CXCR4-eYFP. First, I characterized the localization of CXCR4-eYFP in 3T3 cells with confocal imaging. I found that the receptor was homogenously distributed in the cell membrane. Thus, no *a priori* structuring was detected. To justify the choice of 3T3 cells as model cells, I designed a motility assay to assess the impact of CXCR4/SDF on the mobility of the whole cell. In this experiment a confluent cell layer was grown on glass. Subsequently, a line of cells was scratched away (width ~ 2mm) and the filling of this scratch was recorded in phase contrast time lapse microscopy. I compared the ‘area velocity’ (area covered with cells/time) of wild type (wt) 3T3 cells and of cells transfected with CXCR4 in the presence of 12.5 nM SDF1. I found that the +CXCR4 cells showed accelerated scratch filling in comparison to wt cells by a factor two. This speed-up proved that although wt 3T3 cells are not able to process SDF-signalling, they can be turned into chemotaxis-potent cells by providing them with the CXCR4 receptor.

Further, I applied single-molecule fluorescence microscopy, to study the dynamics of individual CXCR4-eYFP receptors on millisecond timescales with a localization precision of 30 nm as limited by the autofluorescent background of the cells. Particle Image Correlation Spectroscopy analysis (PICS) was used to determine mobile fractions, diffusion constants and confinement zones under various conditions. I characterized the resting state as well as the stimulated state and investigated the effect of actin depolimerization on both states. Combination of this information, allowed me to develop a model of the molecular mechanism following stimulation of CXCR4 with its ligand SDF. In the resting state only 60% of the receptors are mobile and excitable. There is a 40% large fraction of immobile receptors recycling between the membrane and the cell’s interior, that potentially is localized in clathrin-coated pits. Upon stimulation, most of the mobile receptors slow down and form lager complexes, while a small fraction associates with the actin cytoskeleton and immobilizes. While ligand-dependent formation of signalling clusters has already been detected with complementary techniques [Angers et al. Annu. Rev. Pharmacol. Toxicol. 2002], the functional role of actin association that has to involve additional cytosolic proteins remains unclear and needs to be further investigated.

Since, it had been shown recently, that expression of CXCR4 in Ewing’s sarcoma tumor cells correlated with an increased rate of metastasis in patients (Bennani-Baiti et al. Clin. Canc. Res. 2010), I am currently performing the experimental routines established for 3T3 cells in the Ewing’s sarcoma cell line TC32.

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