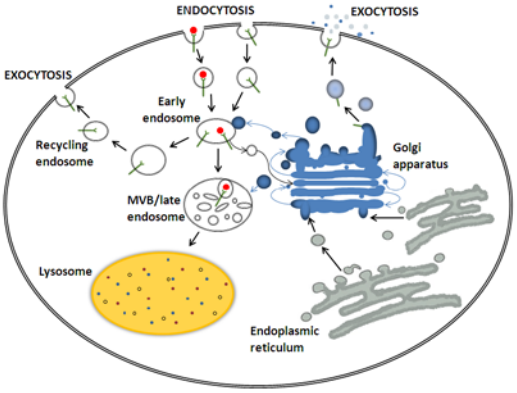
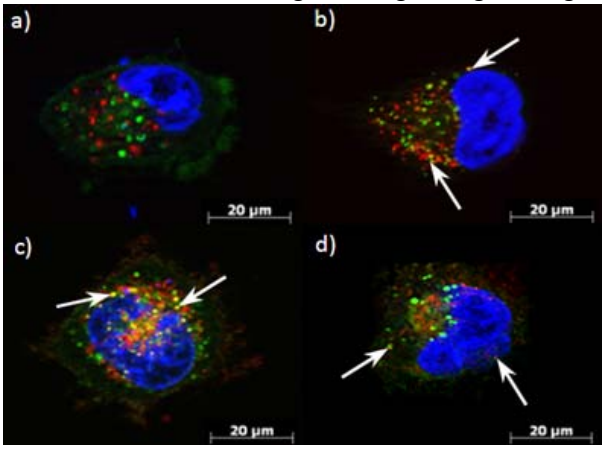
Receptor tyrosine kinase trafficking in human glioma cells

Extracellular signals activate transmembrane-receptor-triggered intracellular signal transduction pathways which in turn modulate the level of cell activation. For example, receptor tyrosine kinases (RTKs) initiate a cascade of events that modulate the level of growth factor stimulation. RTKs are activated by ligand binding, autophosphorylated and ubiquitinated, resulting in internalization of the receptor followed by its recycling or degradation via the endosomal/lysosomal pathway which finally terminates signaling. Trafficking is essential for normal cell functioning and de-regulation can result in diseases such as cancer. Inhibition of RTKs reduces the proliferative signal and has been demonstrated to be an effective therapy in tumors. Fibroblast growth factor receptors (FGFRs) are one type of RTKs which are commonly over-expressed in many types of cancer including glioblastoma multiforme. Glial cells express fibroblast growth factor receptors 1-4, FGFR1 being most abundant in the nervous system. Ligand binding leads to FGFR1 dimerization and autophosphorylation of the cytoplasmatic domains. Activation of the receptor recruites a number of signaling molecules relevant for cell proliferation and angiogenesis.



FGFR1 activation is followed by rapid endocytosis and degradation of both, the receptor and the ligand. Sorting of the receptor at the level of early endosomes into intraluminal vesicles of multivesicular endosomes is essential for subsequent delivery to lysosomes followed by degradation via lysosomal enzymes. Most of the knowledge about tyrosine kinase receptor degradation has been obtained from EGFR and, in spite of the large amount of work performed in the FGF field, little is known about the intracellular trafficking of FGFRs. Haugsten et al. showed that FGFR1, R2 and R3 are mainly sorted for lysosomal degradation but that FGFR4 escapes into a recycling pathway. FGFR1 revealed the highest levels of ubiquitination and the fastest degradation indicating that different levels of ubiquitination of FGFRs determine their intracellular sorting. Receptor trafficking becomes an emerging focus of interest since sorting and signaling are tightly interconnected.



Images of human glioma cells U373 transfected with the FGFR1–eGFP (green), red: LysoTracker (a+b), Lamp1-DsRed (c), Transferrin (d), blue: Hoechst (nucleus), yellow: indicating colocalization (arrows). a) control cells, b) FGF-2-stimulated cells, c) Leupeptin-stimulated cells, d) internalization of transferrin at 37°C

The primary goal of my project is to explore the internalization and degradation of the receptor tyrosine kinase, FGFR1, in a human glioma cell line (U373). For this purpose, we analyze the expression of FGFR1 in these cells by RT-PCR, Western blotting and immunocytochemistry. Transfection of cultured tumor cells with FGFR1 constructs fused to fluorescent marker proteins allows the study of internalization and degradation of the receptor in response to the ligand applying confocal imaging of cells in vitro. For the imaging experiments the Leica TCS SP5 (Biocenter) and the Zeiss AxioObserver with ApoTom are used. The lysosomal inhibitor leupeptin is being applied to study the influence of the protein degrading machineries on the level of membrane receptor fluorescence. The kinetics of receptor internalization and degradation are determined from the receptor trafficking data and correlated with the ability of the transfected cells to incorporate bromodeoxyuridine (BrdU) indicating the proliferative status of the cells. The analysis of FGFR1 trafficking may lead to the discovery of novel therapeutic targets which enhance inhibition or degradation of receptor tyrosine kinases in glioma cells.