



Ruprecht-Karls-Universität Heidelberg  
Medizinische Fakultät Mannheim  
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## The role of the EphB4 Receptor in Tumor Biology

Autor: Julija Aranza-Helm  
Institut / Klinik: Neurochirurgische Klinik  
Doktorvater: Prof. Dr. med. Peter Vajkoczy

Malignant primary brain tumors are invariably fatal, recurring near the resection margin in almost all cases. The most frequent and the most malignant histological type is glioblastoma. The median survival is 15 months. The invasion of glioma cells into surrounding healthy brain tissue is a pathological hallmark of malignant gliomas and is not seen in any solid tumor that metastasizes into the brain. This study analyses the role of this EphB4 receptor tyrosine kinase in glioma migration, invasion and angiogenesis. The EphB4 receptor is found to be overexpressed in breast, small-lung and colon carcinomas, mesotheliomas, gliomas and in endometrial hyperplasias and carcinomas. An intriguing feature of the Eph receptors is their involvement in bi-directional signaling. Signaling can be propagated not only downstream of the Eph receptors but also downstream of the ephrin ligands. In order to distinct the role of the forward and the reverse signaling in the EphB4 receptor signaling pathway, human glioma cell lines over-expressing EphB4 full-length or mutated receptor (lacking the tyrosine kinase activity) were used in this study. The results of the Migration Assays performed on Matrigel, laminin, collagen IV and tenascin-C coating showed that EphB4 receptor in the extracellular matrix environment has a pro-migratory role. The read out of the Migration Assays was the migration area (surface covered with migrating cells), which was calculated from the photomicrographs of the tumor spheroids with tumor cells spreading from their periphery. The migration experiments also showed that the pro-migratory effect of the EphB4 receptor is dependent on the tyrosine kinase domain of the EphB4 receptor in the case of plastic and tenascin-C and Matrigel coated surfaces, but independent of the tyrosine kinase domain in the case of laminin and collagen IV. Furthermore, this study provided evidence that stimulation of the EphB4 receptor in tumor cells with its ligand ephrinB2 leads to inhibition of migration. Since on one hand, activation of EphB4 receptor with its ligand ephrinB2 has anti-migratory effect, and on the other hand, laminin and other tested ECM components influence EphB4 to exhibit pro-migratory effect, it can be speculated that the EphB4 receptor overexpressed in human glioma cells has a pro-migratory effect in a ligand-free environment. An intriguing explanation would be that ECM components have a high affinity for the EphB4 receptor, and cause activation of distinct intracellular signaling pathways than the ones activated after ephrinB2-ligand binding. As a model for testing the invasion capability of tumor cells into the brain, the *ex vivo* Confrontation Assay was performed, which consists of confronting tumor spheroids with fetal rat brain aggregates and following the invasion process daily under the inverted microscope. Analysis of the invasion process followed under the confocal scanning laser microscope. In order to study the invasive behavior of the EphB4 manipulated cells *in vivo*, a subcutaneous implantation model was used. The *ex vivo* and *in vivo* invasion experiments in this study are comparable to the results of the *in vitro* migration experiments and provide evidence that EphB4 receptor has an important role in tumor invasion. To determine whether over-expression of EphB4 receptor in human glioma cells affects tumor vascularization, a CD31 (a marker for endothelial cells) staining of the subcutaneous tumor sections was performed. The counting of the blood vessels which had the blood vessel diameter bigger than 50  $\mu\text{m}$  (relative to the total number of the blood vessels counted) supported our observation that the vascular diameters in the peripheral areas of EphB4 over-expressing-tumors were markedly bigger than in the control tumors. These results provide evidence that EphB4 over-expression in tumor cells has influence also on tumor angiogenesis by increasing the tumor blood vessel diameters. In conclusion, the work presented in this thesis demonstrates that EphB4 receptor plays an important role in glioma cell migration, invasion and angiogenesis. This makes EphB4 receptor stand out as attractive candidate as target for future therapeutic strategies in gliomas.