EphB1RECEPTOR AS TARGET TO DEVELOP INNOVATIVE THERAPEUTICS TO COUNTERACT GLIOBLASTOMA CELL PROLIFERATION AND MIGRATION

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Introduction: Eph receptors, the largest family of receptor tyrosine kinases (RTKs), and their ephrin ligands are membrane-anchored molecules that regulate cell-cell interactions. Increasing evidences show that Eph/ephrin signalling regulates cell migration, proliferation, differentiaton, adhesion, morphological changes and survival through cell–cell communication. Moreover, Eph/ephrin signaling seems to be involved in tumorigenesis, metastasis, and angiogenesis. Every member of this molecular system may act as tumor promoter or tumor suppressor, dependent on cellular context and type of cancer. Abnormal expression of EphB1receptor was detected in different brain tumors. As regards glioblastoma, one of the most aggressive brain tumors with an exceedingly poor prognosis, it was shown that EphB1may act as a tumor suppressor and the loss of its expression may associate with aggressive cancer phenotypes.
Moving from these considerations, we aimed at characterizing the role played by EphB1receptor on human glioblastoma U87cell proliferation and migration, both under basal conditions and after exposure to the endogenous EphB1receptor agonist or novel antagonists.

Methods: EphB1mRNA and protein levels were measured in U87cell line via real-time PCR and western blotting, respectively. [^{3}H]thymidine incorporation and wound healing assays were employed to evaluate cell proliferation and migration, respectively. U87-MG cells were exposed to EphrinB1-Fc (1μg/ml), an EphB1receptor soluble agonist, or to different EphB1receptor peptide antagonists, that we previously identified. Alternatively, U87-MG cells were transfected with p-CMV6-EphB1plasmid, to overexpress EphB1receptor, and then exposed to EphrinB1-Fc or peptides. Both native and transfected cells were assayed at different time points (0-48h).

Results: As regard EphB1basal expression, we found high mRNA but low levels of the corresponding protein. Interestingly, EphrinB1-Fc administration significantly increased EphB1mRNA and protein levels in native U87-MG cells. This treatment led to a significant decrease in glioblastoma cell proliferation (48h) and migration (18-24h). Conversely, the administration of EphB1peptide antagonists further increased cell migration at 24h, albeit decreasing the cell proliferation rate at 48h. Following transfection with EphB1plasmid, EphB1receptor expression was effectively increased in U87-MG cells, thus determining a remarkable decrease in cell proliferation at 24-48h and migration at 18-24h. Cells transfected with EphB1plasmid were also treated with EphrinB1-Fc or EphB1peptide antagonists: we found that EphrinB1-Fc administration further reduced cell migration, as compared to the EphB1receptor overexpression alone. Conversely, administration of EphB1peptide antagonists, significantly raised U87-MG cell migration as compared to EphB1overexpressing cells, but did not significantly influence cell proliferation rate.

Discussion and conclusion: Taken together, these findings confirm that EphB1receptor may act as a tumor suppressor in U87-MG human glioblastoma cells, as its overexpression significantly reduce cell proliferation and migration. Consistently, we found that administering the EphB1receptor agonist or antagonists further reduces or increases cancer cell aggressiveness respectively; thus, highlighting EphB1as a novel interesting pharmacological target for glioblastoma treatment. Moreover, the discrepancy between EphB1mRNA and protein levels discovered in this research suggests that the loss of EphB1receptor expression in glioblastoma cells could be related to some post-transciptional events. Studies are currently ongoing to unravel this mechanism and will be presented at the conference. Efforts aimed at elucidating the pathway leading to EphB1protein degradation and at increasing its expression in glioblastoma cells may lead to develop innovative pharmacological approaches to treat this aggressive malignant brain tumor.