FUNCTIONAL ROLE AND CLINICAL IMPACT OF KIT GNNK+ AND GNNK- ISOFORMS IN GLIOBLASTOMA

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BACKGROUND: Glioblastoma is the most common adult brain tumor and one of the deadliest human malignancies. The reason for this dark scenario, which has not changed significantly in the last 3 decades, is inherent to the biological properties of glioblastoma. Therefore, it is urgent to understand its molecular players in order to develop novel and targeted therapeutic strategies. KIT, a member of the receptor tyrosine kinase (RTK) family III, is involved with the tumorigenesis of some tumors and the existence of specific small molecule inhibitors for KIT made this protein a key molecular therapeutic target in cancer. We and others have performed an extensive analysis of KIT alterations in malignant gliomas, and showed the absence of KIT activating mutations and instead the presence of KIT gene amplification. Importantly, due to mRNA alternative splicing, KIT is expressed by two different functional isoforms, which are characterized by the presence (+) or absence (-) of a tetrapeptide sequence (GNNK) in the extracellular juxtamembrane region. They were shown to display distinct intracellular signaling features and also different tumorigenic transforming activities in mouse fibroblasts.
JUSTIFICATIVE: Our preliminary results have showed that GNNK isoform are frequently co-expressed in both glioblastoma cell lines and normal tissues, with GNNK- being the prevalent form in glioblastoma cell lines, whereas in normal brain tissues there is predominance of GNNK+ isoform, suggesting that GNNK- isoform could play a role in glioblastoma tumorigenesis. Hitherto, there are no reports assessing KIT GNNK isoforms functional role in both normal and tumor brain tissues.
AIM: Thus, in the present project we aim to shed light on the functional and biological roles of KIT GNNK isoforms in glioblastomas and to assess the tumorigenic role of each KIT isoform. We believe that the outcomes of this project will be of the upmost relevance to clarify the role of KIT oncogene in glioblastoma biology and treatment and, importantly, they could lead to the identification of predictive biomarkers of anti-KIT therapy response.
MATERIALS AND METHODS: The mRNA of 117 frozen tissue and 19 FFPE (paraffin tissue) glioblastoma tissues was evaluated for expression of GNNK isoforms and also evaluated the KIT expression by immunohistochemistry in a series of 92 paraffin embedded tissues. Additionally, cellular assays were performed (*in vitro* and *in vivo*) in glioblastoma cell lines transfected with GNNK isoforms in order to observe whether the isoforms confer distinct cellular signaling and tumorigenesis role.
RESULTS: We observe the GNNK+ isoform predominance in tumor tissues, differences in survival between patients that express the mRNA of those who do not express, these have a better survival; in cellular assays, we noticed greater viability, potential invasion and tumor growth of cells transfected with the isoform GNNK-.
CONCLUSIONS: Although we didn't find statistically significant differences between the expression of isoforms and clinico-pathological information, through cellular assays, we can notice that there are differences in the KIT activation and tumorigenic role between isoforms, and the cells transfected with GNNK- have a more aggressive potencial.