Project title: Combi-molecule, ZRBA1, as a novel molecular targeted therapy integrated with high dose rate ionizing radiation to treat malignant glioma tumors

Project timing: July 2013 – July 2015

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The most common and deadliest primary brain tumor derives from the cells that support neurons in the brain and is called glioblastoma multiforme (GBM). Although no contemporary GBM treatments are completely curative, development of targeted drugs and their combination with radiation has opened new treatment possibilities. In my project, as a fellow of the William Donald Nash Fellowship, I aim to evaluate the radiosensitizing abilities of the ZRBA1 "combi molecule" in the glioblastoma multiforme model. ZRBA1 was designed to block the EGFR signaling pathway while additionally inducing significant levels of DNA damage. Therefore, in the first part of my project, I have evaluated its cell killing ability as well as its inhibitory activity on the EGFR signaling pathway. By using three different GBM cell lines (with low, normal and high EGFR expression levels), I have been able to demonstrate that ZRBA1 is able to efficiently inhibit the phosphorylation of EGFR and its downstream protein substrates, even in the case of EGFR radiation induced activity. Furthermore, ZRBA1 significantly potentiated the radiation response in all of the tested cell lines with the dose enhancement factors at a surviving fraction of 0.1 ranging from 1.4 to 1.75. Importantly, the radio-sensitizing ability of the ZRBA1 was also visible in three dimensional spheroid models, which due to cell-to-cell interactions are known to better mimic tumor environment than monolayer cultures. Interestingly, in contrast to Temozolomide which enhances radiation response most effectively in MGMT (O^6-methylguanine DNA methyltransferase) - negative cells, the radiosensitizing properties of ZRBA1 does not depend on GBM MGMT methylation status. Moreover, the data so far obtained shows that the combination of radiation and ZRBA1 caused strong and long lasting G2/M cell cycle arrest. Additionally, as a measure of DNA double strand breaks, the number of γH2AX foci per cell was significantly greater up to 72h after the combined modality compared with individual treatment with radiation, Temozolomide or ZRBA1 alone. Overall, the results obtained so far have demonstrated increased levels of DNA damage when ZRBA1 was combined with radiation and suggests that the radiosensitizing properties of the "combi molecule" rely on inhibition of DNA repair and simultaneous increase in cell death. Presently I am focusing on testing ZRBA1 in combination with radiation in patient derived brain tumor stem cells (BTSC) as well as establishing an *in vivo* mouse model.

All of these results were selected for presentation at the 4th Quadrennial Meeting of the World Federation of Neuro-Oncology in San Francisco, California (November 2013) and will be presented at the 16th Biennial Canadian Neuro-Oncology Meeting in Halifax, Nova Scotia (June 2014). We are also in the process of preparing a manuscript for publication in Neuro-Oncology journal.