Glutathione depletion overcomes chemotherapy resistance in aggressive medulloblastoma *stem-like* cells

B. D. Barbieri[1], M. Marçola[1], C. R. R. Rocha[2], O. K. Okamoto[1]
[1]Laboratory of Translational Genomics, IB, USP, [2]Laboratory of DNA Repair, ICB, USP

Introduction: Medulloblastoma (MB) is the most common pediatric brain tumor [1]. It is a highly aggressive cancer that affects the cerebellum, being classified as a grade IV tumor by the World Health Organization (WHO) [2]. Drug resistance is a major limiting step for the successful treatment of most cancers [3]. Additionally, the overexpression of pluripotency factors in cancer cells favors the process of treatment resistance, tumor recurrence and spreading [4]. Glutathione (GSH) is a major antioxidant intracellular molecule and its levels are directly correlated with tumor resistance [5,6]. Therefore, the purpose of this study was to verify whether GSH depletion can potentiate the effect of two chemotherapeutic drugs, cisplatin (Cis) and temozolomide (TMZ), in the treatment of aggressive medulloblastoma *stem-like* cells.

Materials and Methods: Daoy cells expressing normal (control tumor cells) or high levels of the pluripotency factor *OCT4A (stem-like* cells*)* were treated with a combination of BSO (glutathione inhibitor), cisplatin and TMZ. The effect of different drug combinations on cell viability was assessed through MTT and apoptosis assays. Additionally, we analyzed possible treatment side effects on healthy neuron cells by differentiating neural progenitor cells into neurons and proceeding with MTT assay as well. Lastly, we started analyzing treatment efficacy *in vivo* through an orthotopic and metastatic model of medulloblastoma, that consists in the intracranial surgery of tumor cells into the third ventricle of the brain.

Results: We found that both Daoy and Daoy-*OCT4A* cells were sensitive to cisplatin but resistant to TMZ. The highest dose of TMZ used in the study reduced the viability of Daoy cells to 56%, but only to 80% in Daoy-*OCT4A* cells. When combining either BSO+Cis or BSO+TMZ, Daoy-*OCT4A* cells did not respond to the treatment with TMZ+BSO, reinforcing its chemoresistant phenotype. Next, the ideal combination of the three compounds was defined. Daoy-*OCT4A* cells required a higher dose of TMZ than control Daoy cells to attain similar death rates, however the combined treatment was effective for both: a synergistic effect was observed for Daoy-*OCT4A* cells, reducing cell viability to ~17%, in comparison with cisplatin+TMZ alone, and as for Daoy cells, an additive effect was observed, and viability was reduced to ~15%. Preliminary results in the *in vivo* model indicated that BSO or chemotherapy regimen alone is well tolerated by the organism. The combination of the three compounds *in vivo* is being performed. Lastly, analysis of the treatment effect in healthy neurons showed no significant toxicity, revealing its potential clinical application.

Discussion: The aggressive tumor cell initially seemed to be TMZ-resistant, however, the combined treatment was able to overcome it. This synergistic effect has been observed in other brain tumors, such as glioma, and possible explanations include an increase in oxidative stress due to GSH depletion, which can lead to apoptosis, as well as the capacity of cisplatin to reduce the activity and expression of an enzyme involved in the DNA repair process of TMZ-induced damage [6].

Conclusion: The present results indicate that the use of a glutathione inhibitor in combination with cisplatin and TMZ may be useful in the treatment of medulloblastoma, even in the case of aggressive tumors. Similarly, this approach may also be useful in the development of therapeutic strategies for other chemoresistant tumors.

References: [1] doi:10.1038/nrclinonc.2014.181; [2] doi:10.1007/s00401-007-0243-4; [3] doi:10.1038/nrc2167; [4] doi:10.1089/scd.2015.0052; [5] doi.org/10.1016/j.semcdb.2012.03.017; [6] doi:10.1038/cddis.2014.465.