Role of WNK2 on glioma cell death mechanisms

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Introduction and objectives: WNK2, a member of the WNK (with-no-lysine [K]) subfamily of protein kinases, has been found downregulated by its promoter hypermethylation, and has been proposed to act as a specific tumour-suppressor gene in brain tumors. The role of WNK2 in cancer cell death is largely unknown, and recent contradictory studies addressed WNK2 as an autophagy suppressor. Autophagy been suggested to be a major cell death pathway in cancer cells, and associated with anticancer agents response. In this report, we aimed to evaluate the role of WNK2 in cell death on glioma context. Material and Methods: We used wild-type A172 cells (WNK2 promoter-methylated), and A172 transfected either with an empty vector (Ev) or with a WNK2 expression vector (W2), previously established by our team, and analyzed the three cell lines by Western blotting and flow-cytometry for their basal capabilities to promote autophagy. Additionally, the cell lines were treated with temozolomide (TMZ) (pro-autophagic and late apoptotic effect in glioma), and their viability was analyzed by the MTS assay. Apoptosis and cell cycle was quantified by flow-cytometry. Results: The re-expression of ectopic WNK2 did not increase the expression of the autophagy-associated protein LC3-II when compared with Ev or wt A172 cells, but resulted in a marked increase (92.4%) of Acidic Vesicular Organelles formation (AVOs) detected by Acridine Orange. In addition, neither the treatment of three cell lines with TMZ altered the response to TMZ when the respective IC⁵₀ values were compared, nor changes in cell cycle or apoptosis were observed. Conclusions: The restoration of WNK2 in A172 cells was not able to interfere with TMZ sensitivity. However, these preliminary results clearly suggest that WNK2 restoration promotes increase in AVOs formation and may be related in some way to vesicles formation during autophagy pathway.

Keywords: Glioma, cell death, autophagy.

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