Role of WNK2 on glioma cells endocytosis pathway.

Viviane A O Silva (Barretos Cancer Hospital, Barretos, S. Paulo, Brazil), Angela M Costa (Health Sciences Research Institute, University of Minho, Braga, Portugal and ICVS/3B's - Braga/Guimarães, Portugal) Glaucia N. M.Hajj (A. C.Camargo Cancer Center São Paulo, Brazil), Ana Preto (Center of Molecular and Environmental Biology, Braga, Portugal), Aline Tansini (Barretos Cancer Hospital, Barretos, S. Paulo, Brazil), Martin Roffé (A. C.Camargo Cancer Center São Paulo, Brazil), Peter Jordan (National Institute of Health Doutor Ricardo Jorge, Lisbon Portugal and BioFig-Center of Biodiversity, Lisbon, Portugal), Rui M. Reis (Barretos Cancer Hospital, Barretos, S. Paulo, Brazil and Health Sciences Research Institute, University of Minho, Braga, Portugal).

BACKGROUND: WNK2, a member of the WNK (with-no-lysine [K]) subfamily of protein kinases,was found downregulated by its promoter hypermethylation, and it was proposed to act as a specific tumour-suppressor gene in brain tumors. Although some contradictory studies indicated WNK2 as an autophagy modulator, its role in cancer cell death is largely unknown. There is also growing evidence for additional roles of WNK kinases in vesicular traffic.
AIM: To evaluate the role of WNK2 in autophagy and endocytosis on glioma context.
METHODS: Wild-type (wt) A172 cells (WNK2 promoter-methylated), and A172 transfected either with an empty vector (Ev) or with a WNK2 expression vector, were used to assess the cellular basal capacities to promote autophagy, through western blot and flow-cytometry analysis. Additionally, we evaluated the effect of WNK2 on general endocytosis trafficking routes by immunofluorescence.
RESULTS: The re-expression of ectopic WNK2 did not interfere with autophagy-related protein light chain 3 (LC3-II) expression levels as well as it did not promote mTOR signaling pathway alteration when compared with Ev or wt A172 cells. However, the restoration of WNK2 resulted in a marked increase (8 to 92.4%) of Acidic Vesicular Organelles formation (AVOs). Preliminary results also suggest that WNK2 cells promotes acceleration of EGFR degradation compared to Ev or wt A172 cells and shows the greater delay in uptake and internalization rate of cholera toxin B and transferrin ligands.
CONCLUSIONS: The restoration of WNK2 interferes in vesicular traffic during endocytosis pathway and also increase AVOs formation. This results also suggest the role of WNK2 in growth factor receptor turnover related to cell growth and homeostasis and associates once more, WNK2 silencing contribution in genesis of gliomas.