

## Characterization of WNK2 Role on Glioma Cells Vesicular Traffic

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**Abstract :** Autophagy is a recycling and degradative system suggested to be a major cell death pathway in cancer cells. Autophagy pathway is interconnected with the endocytosis pathways sharing the same ultimate lysosomal destination. Lysosomes are crucial regulators of cell homeostasis, responsible to downregulate receptor signalling and turnover. It seems highly likely that derailed endocytosis can make major contributions to several hallmarks of cancer. WNK2, a member of the WNK (with-no-lysine [K]) subfamily of protein kinases, had been found downregulated by its promoter hypermethylation, and has been 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 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). Moreover, our results also suggest that WNK2 cells promotes 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 increase AVOs formation. This results also suggest the role of WNK2 in growth factor receptor turnover related to cell growth and homeostasis and associates one more time, WNK2 silencing contribution in genesis of gliomas.

**Keywords :** autophagy, endocytosis, glioma, WNK2

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