PROTEIN KINASE WNK2 AS A TUMOUR SUPPRESSOR GENE IN MALIGNANT GLIOMAS.

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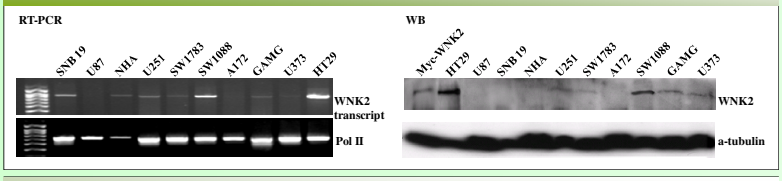
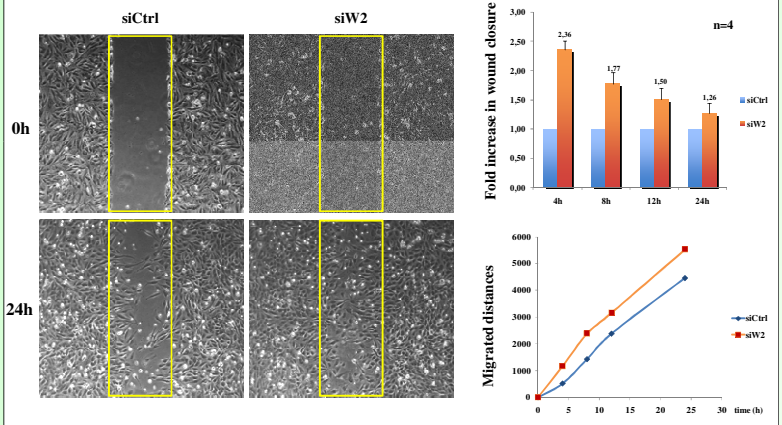
SUMMARY Malignant glioblastomas are the most common and lethal adult brain tumours, with patients dying within two years from diagnosis. Little is known about the molecular mechanisms underlying the formation and/or development of these tumours, which present a very invasive phenotype within the brain and are genetically heterogeneous and highly resistant to both chemo- and radio-therapies. Recently, the promoter region of the protein kinase WNK2 gene was found to be hypermethylated in 29 of 31 infiltrative gliomas and about 80% of meningiomas. We have previously described that the experimental depletion of WNK2 expression decreases RhoA activity whilst leading to increased Rac1 activity. Because RhoA/Rac1 activities are important for cell migration and glioblastomas are very invasive tumours, we tested the effects of WNK2 on wound-healing assays in glioma cell lines SW1088 and A172. SW1088 cells express endogenous WNK2 and we observed that wound closure was increased upon experimental depletion of endogenous WNK2. In contrast, A172 cells display complete promoter region methylation and WNK2 re-expression was found to decrease migration. Consistently, we observed an increase in Rac1 activity in SW1088 cells upon WNK2 down-regulation, but lower levels of active Rac1 in A172 cells stably expressing WNK2 cDNA when compared with an equivalent cell line stably transfected with the same empty vector. Our studies indicate that loss of WNK2 expression promotes Rac1 activation and may contribute to the highly invasive phenotype that glioblastomas present. We also observed that, in a panel of glioblastoma cell lines, WNK2 promoter methylation correlates with a marked deregulation in Akt, MEK1/2 and ERK1/2 activities, suggesting WNK2 may also be important for tumour cell survival and cell cycle progression.

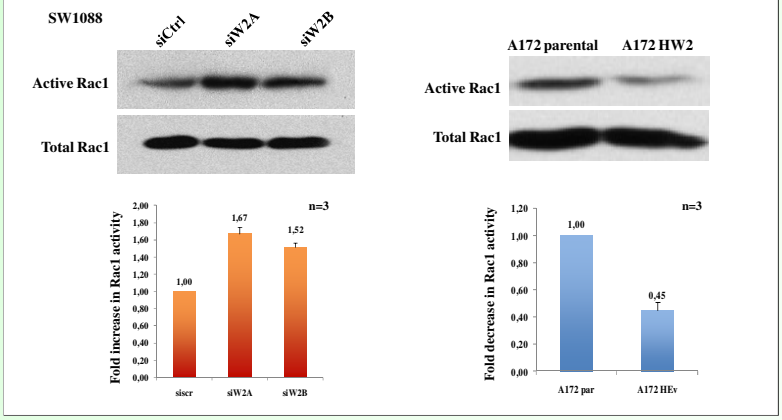
WNK2 expression in glioblastoma cell lines



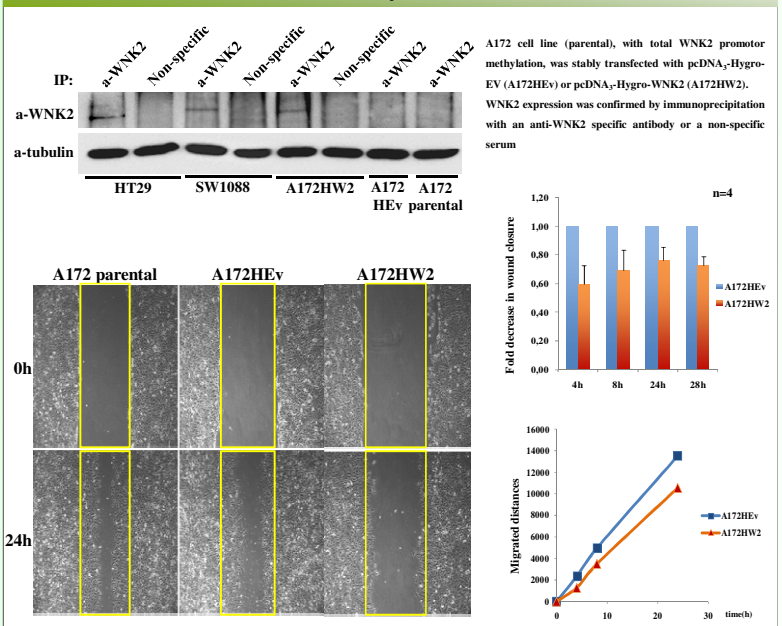
Depletion of endogenous WNK2 in SW1088 cells leads to increased wound closure in scratch assays



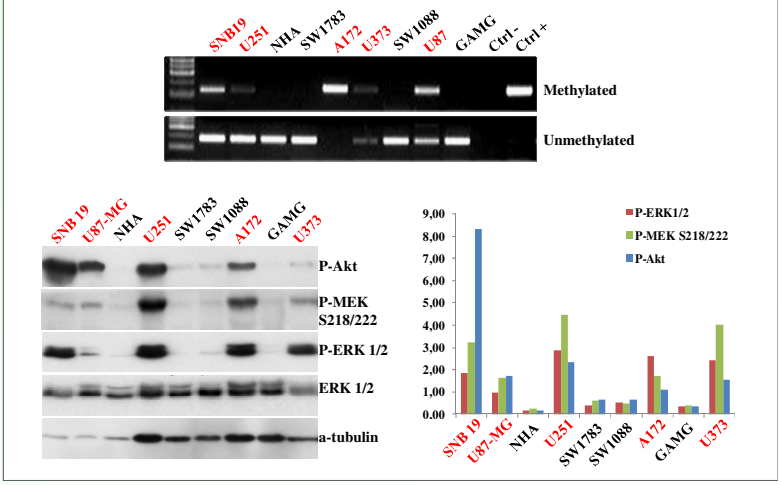
Endogenous WNK2 depletion results in increased Rac1 activity, while its re-expression reduces active Rac1



Stable WNK2 re-expression in A172 cells retards wound closure in scratch assays…



WNK2 promotor methylation correlates with increased Akt, MEK1 and ERK1/2 activities



Conclusions:

- WNK2 experimental depletion originates a phenotype of increased cell migration and Rac1 activity, supporting that it plays a role in regulating the characteristic invasiveness of these tumours.
- WNK2 epigenetic silencing correlates with the increased activity of key regulators of cell proliferation and survival, suggesting an important role for this protein kinase in glioblastoma development.