Pten Inhibition Promotes Neural Differentiation of Mouse Embryonic Stem Cells *In Vitro*

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Introduction and Objectives

Transplanting ESC-derived and iPSC-derived neurons into the lesioned brain could reduce neurological deficits and improve functional outcomes in Parkinson's disease mice (Barker, 2019). A major bottleneck in neuronal replacement therapy is the differentiation of precursor cells into neurons. Although many molecules had been implicated in neural differentiation from ESCs, the exact signalling pathways remain poorly understood. Wang and his colleagues suggested the tumour suppressor gene *Pten* played a significant role in embryonic development and ectodermal organogenesis (Wang et al., 2020). Pten inhibits the PI3K signalling pathway and subsequent AKT activity, and promotes ESC differentiation. *Pten* gene inactivation increases ectoderm marker expression, and had been implicated in promoting the renewal and differentiation of neural and glioma stem cells and the induction of glioblastoma multiforme (Benitez et al., 2017). This study aims to elucidate the role of *Pten* in regulating neural differentiation from ESCs.

Method

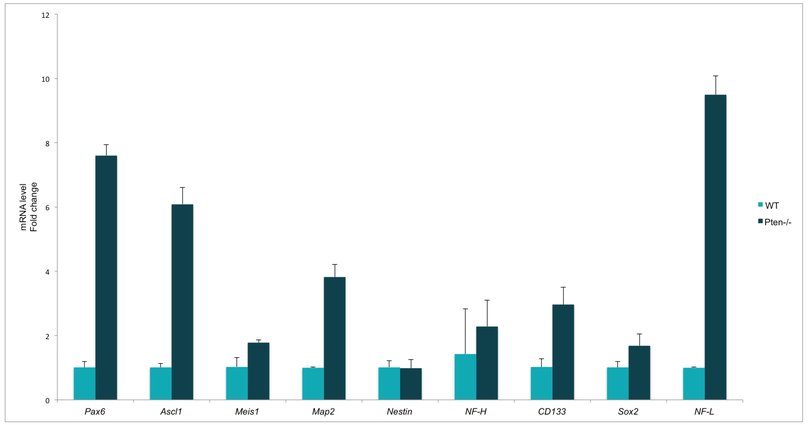
Pten-deficient mouse ESCs were generated using the CRISPR-Cas9 system. The potential of wild type ESCs and *Pten*$^{-/-}$ ESCs to differentiate into neural stem cells and neurons *in vitro* were compared. qRT-PCR and western blot experiments were performed to assess the ability of WT ESCs and *Pten*$^{-/-}$ ESCs to express neuronal and neural stem cell markers following differentiation.

Results

The results are consistent with the hypothesis that *Pten* knockdown facilitates neural stem cell proliferation. However, the expression of neuronal markers also increased, which suggested a bias of neuronal differentiation.

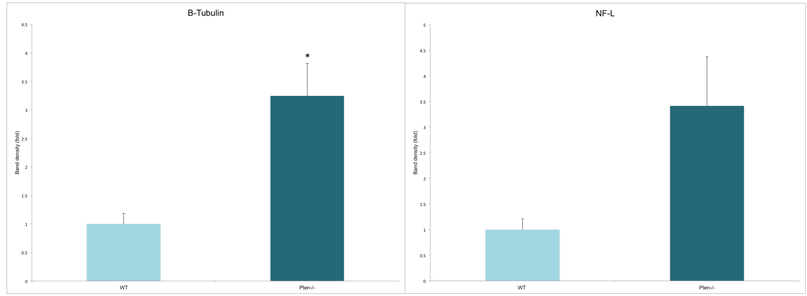
We observed significantly increased mRNA levels of 2 neuronal marker genes, *Map2* and *NF-L*, and 5 neural stem cell marker genes (*Ascl1*, *CD133*, *Msi1*, *Pax6*, and *Sox2*) in *Pten*$^{-/-}$ ESCs following differentiation for 15 days.

Fig. 1 Expression of neuronal and neural stem cell markers assessed by qRT-PCR in WT and *Pten*$^{-/-}$ embryoid bodies



We observed increased protein levels of 2 neuronal markers, NF-L and βIII-tubulin in *Pten*$^{-/-}$ ESCs following differentiation for 15 days.

Fig. 2 Western blot analyses of WT and *Pten*$^{-/-}$ ESCs showing the expression of beta-tubulin (βIII-tubulin) and neurofilament light polypeptide (NF-L)



These results suggest that *Pten* deletion plays a role in regulating early and late differentiation of neuronal lineages.

Conclusion

Pten deletion promotes ESC differentiation into neural stem cells and nervous tissue. Endogenous *Pten* inhibition plays a key role in promoting the differentiation of neuronal lineages during embryonic development.

References

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