Epigenetic Regulation of Neural Development and its Use in Peripheral Nerve Regeneration

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INTRODUCTION

Peripheral nerve injury remains a challenging clinical problem. We have previously found trauma-induced mesenchymal progenitor cells (MPCs) at these injury sites, and demonstrated their ability to secrete neurotrophic factors.

The repressor element-1 silencing transcription factor (REST) is a nuclear factor that acts as a master regulator of neurogenesis by repressing terminal neuronal differentiation.

Previous reports have found that REST was decreased following central nervous system insult, but the role that REST plays in peripheral nerve injury and the associated pathways are not well described.

BACKGROUND

C-terminal domain small phosphatase-1 (CTDSP-1) dephosphorylates and stabilizes REST at a specific regulatory site (see Figure 1). Using a novel peptidomimetic decoy to bind CTDSP-1 at this binding site limits its phosphorylation ability. Without the CTDSP-1 blockade on REST, neurogenic gene expression and increased neurotrophic factor expression can facilitate neuron axonal growth after nerve injury. (see Figure 2) It is not known if this peptidomimetic is able to access CTDSP-1 at its site of action, the nucleus.

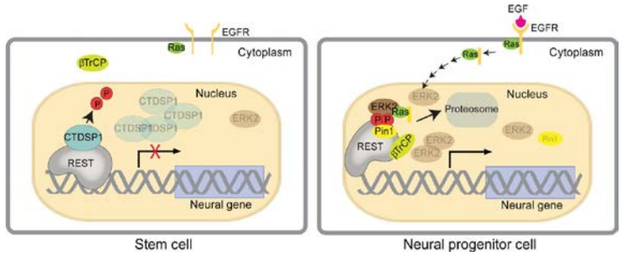

Figure 1 REST gene regulation. REST sits on the chromatin and represses neuronal gene expression (*from Nesti, Neurogenesis 2015*)

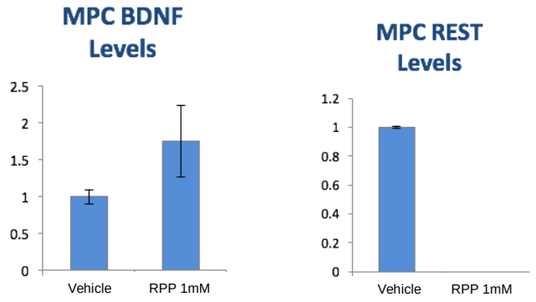

Figure 2. QPCR analysis . Mesenchymal stem cells were treated with 1 mM RPP or vehicle control for 24 h. The bar graphs show QPCR analysis of BDNF (left) or REST (right). mRNA levels were normalized to HPRT1 (SD, n=3).

HYPOTHESIS

We believe that we will be able to visualize the intracellular localization of the CTDSP-1 binding peptidomimetic.

METHODS

MPCs were seeded onto coverslips on a 24-well plate. The next day, the cells were treated with the CTDSP-1-binding peptidomimetic, containing a FLAG-tag. Cells were harvested at 1 day after treatment, and fixed for immunocytochemistry with FLAG antibody for the peptidomimetic and Hoechst 33342 for the nucleus. Secondary antibodies 488 anti-mouse (as FLAG antibody) and 594 phalloidin (for cell morphology) were used for fluorescent visualization. Images were taken using confocal laser scanning microscopy. REST expression after peptide treatment was analyzed by Western blot, using a wild-type / mutant construct.

RESULTS

The fluorescent tag for the CTDSP-1-binding peptidomimetic was collocated with fluorescent stain that binds DNA. That is, the peptidomimetic gained access to the nucleus in trauma-induced mesenchymal progenitor cells. In Figure 3, the left-most panel demonstrates the FLAG antibody, associated with the peptide (in green). The second panel shows the Hoechst staining (in blue) of the nucleus, and the third panel demonstrates the co-localization (composite image). The right-most composite image includes phalloidin staining for cell morphology. This localization of the CTDSP-1-binding peptidomimetic to the nucleus demonstrates the feasibility of the peptide to affect neuronal gene transcription, by destabilizing REST and lifting its blockage. In the mesenchymal progenitor cells, the peptide is present one day after treatment, and up to at least 6 days from time of treatment (Figure 4). This stability in the cell suggests that, used as a therapeutic, the drug would not need to be re-dosed as the affected cells support neural regeneration in the first week of healing. Figure 5 is the quantification of the intensity of the peptide in the cytoplasm and nucleus, demonstrating the the peptide reached peak intensity in the nucleus at 6 days after administration. Western blot demonstrated that MPC's treated with RPP at 100nM concentration, had a 58% decrease in REST expression (Figure 5). Cells with mutant-type REST that had an altered phosphorylation site, which did not allow peptide binding were used as a negative control.

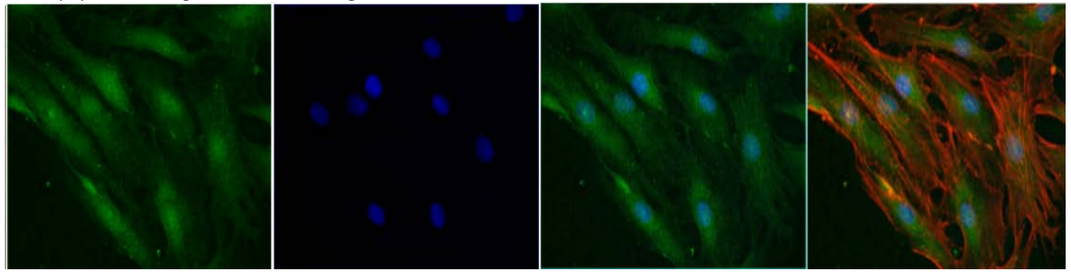

Figure 3. Immunofluorescence. Confocal microscopy of MPCs treated at 100ng/mL peptidomimetic harvested at 1 day

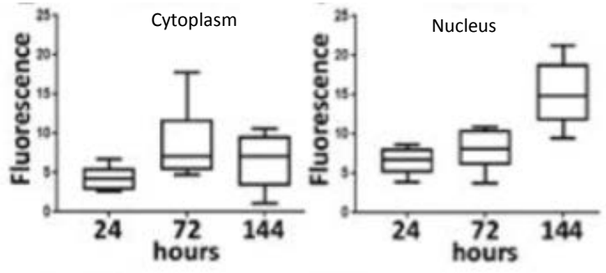

Image 5. Fluorescence Intensity. Histogram of average Flag (green) fluorescence intensity in the cytoplasm over 6 days.

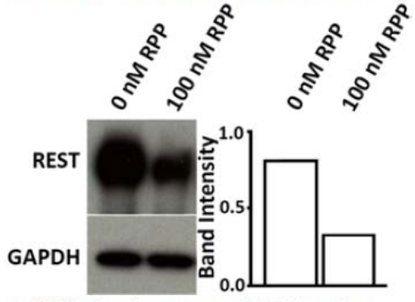

Figure 6. Left Western blot analysis. REST and GAPDH antibody applied. Right Bar graft of the blot, measuring band intensity calculated by measuring the area of gel peaks using imageJ.

RESULTS (con't)

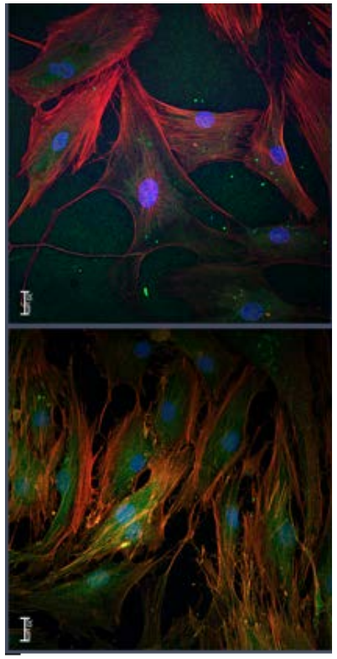

Figure 4. Immunofluorescence. Confocal microscopy of MPCs with peptidomimetic, 40x, 1 day (top) & 6 days (bottom). Both at 1000ng/mL.

CONCLUSIONS

- **REST is a regulator of neural differentiation, and it is controlled by CTDSP-1**
- **The peptidomimetic, with affinity for CTDSP-1, can translocate to the nucleus, which strengthens the feasibility of modulating the epigenetic control of REST**
- **In addition to the binding CTDSP-1, the peptide also lowers the expression of REST itself**

REFERENCES

- Bulken-Hoover, et al. Inducible expression of neurotrophic factors by mesenchymal progenitor cells derived from traumatically injured human muscle. Mol Biotech (51) 2:128-136; 2012
- Nesti, E. Harnessing the master transcriptional repressor REST to reciprocally regulate neurogenesis. Neurogenesis 2:1, e1055419; Jan-Dec2015

DISCLAIMER