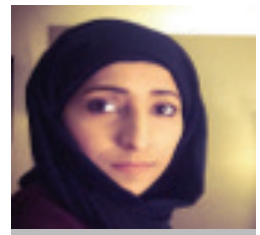

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Dissertation

Title *THE EXPRESSION PATTERN OF DEATH ASSOCIATED PROTEIN KINASE 1 (DAPK1) IN NORMAL DORSAL ROOT GANGLION NEURONS AND FOLLOWING PERIPHERAL NERVE INJURY*

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Defense Date 12 January 2015

Abstract

Death-associated protein kinase1 (DAPK1) is a calcium/calmodulin (Ca2+/CaM) regulated ser-ine/threonine kinase. Increasing body of evidence supports the significance of DAPK1 protein in cancer and CNS diseases. The role of DAPK1 in peripheral nerve regeneration and neuropathic pain remains com-pletely unexplored. We aimed to investigate DAPK1 expression pattern along with key pro- and anti-apoptotic cell signaling molecules (p53, Bax, AKT, ERK5, P38) and to verify the possibilities of DAPK1-NMDA NR2B relationship in dorsal root ganglion neurons (DRG) at 2 hours, 7 days and 14 days following sciatic nerve injury. ATF3 was used as neuronal injury marker. Using gene expression analysis and im-munohistochemistry assessed the effects of nerve injury. The results showed that DAPK1 mRNA was ex-pressed and translated to functional protein in normal DRG neurons. Soon after sciatic nerve injury (2 hours), DAPK1 was significantly ($p<0.05$, 2.2 fold) up-regulated in the injured L4 and L5 DRG compared with contralateral uninjured side. However, 7 days after axotomy a profound decrease was observed in DAPK1 level, with further reduction that reached its minimum level at 14 days postoperatively. In addition, at 7 days after injury, most of DAPK1 positive injured neurons were ATF3 positive, while after 14 days this correlation was not observed as DAPK1 immunoreactivity decreased in injured ATF3 positive neurons. Interestingly, DAPK1, p53 and Bax exhibited almost same expression pattern in axotomized lumbar DRG. The results also revealed that sciatic nerve injury had no effects on the gene expression of ERK5, P38 and AKT at all studied time points. Moreover, NMDA NR2B mRNA expression increased at 7 days and contin-ued to up-regulate significantly until 14 days postoperatively ($p<0.05$, 3.6 fold). In a contrast, our immu-nofluorescence results showed a decrease in its protein level in DRG neurons during this time period; however, a strong positive NMDA NR2B immunoreactivety appeared in the satellite cells that surround injured large-sized neurons in L4 and L5 DRG neurons. In addition, immunofluorescence double labeling revealed that DAPK1 and NMDA NR2B are co-localized in normal and injured DRG neurons. In conclusion, the down-regulations of DAPK1 following sciatic nerve injury along with other vital pro-apoptotic players promoting neuronal survival might shed light on the mechanisms of peripheral nerve regeneration. We also suggest that NMDA might modulate neuropathic pain through satellite cell but not neurons 7 and 14 days after PNS injury.