Differential regulation of the Nedd4-2 ubiquitin ligase during neurite outgrowth

P.J. Donovan and P. Poronnik, School of Biomedical Sciences, The University of Queensland, St Lucia, Qld, Australia.

The ubiquitin protein ligase, Nedd4-2 has been shown in heterologous expression systems to regulate the cell surface levels of neuronal ion channels and receptors. A recent study in mammalian neurons showed that Nedd4-2 mediated the survival of nerve growth factor (NGF) dependent neurons by ubiquitinating the receptor TrkA. However, the role of Nedd4-2 in neurotrophin mediated neural differentiation remains unknown. The aim of this study was to investigate the role of Nedd4-2 in neurite outgrowth. PC12 cells were cultured with NGF (50ng/μl) for 48 hours, which resulted in significant differentiation of the cells with strong neurite outgrowth. This was paralleled by a 3-fold increase in Nedd4-2 protein (300±29%, n=3, $p<0.05$) as determined by Western blotting. Similarly, when PC12 cells were differentiated by transfection of the small GTPase, k-Ras, there was a strong differentiation response with 70±4% of the cells growing neurites. In contrast to NGF, in this case neurite outgrowth was accompanied by a reduction in Nedd4-2 protein expression to 39±3.7% (n=4, $p<0.05$) of control levels. These data suggest that endogenous Nedd4-2 negatively regulates k-Ras induced neurite outgrowth. If the suppression of Nedd4-2 was required for differentiation, we hypothesized that overexpression of Nedd4-2 should prevent neurite outgrowth. Indeed, when Nedd4-2 was co-transfected into PC12 cells with k-Ras, the number of cells sending out neurites was reduced by 50.5± 4.6% (n=4, $p<0.05$) compared with controls. Taken together these data suggest that Nedd4-2 has differential roles in the regulation of neurite outgrowth and that Nedd4-2 is an important factor in directing specificity of neurotrophin signaling.