Akt-Mediated Survival of Oligodendrocytes Induced by Neuregulins

Flores, A.I. et al. (2000) *J. Neurosci.* **20**, 7622.

Myelination of axons is an essential role of oligodendrocytes in the CNS. During development, oligodendrocyte cell number is regulated by proliferation of progenitors and apoptotic cell death. Significant data indicate that the neuregulin family of growth factors is important for oligodendrocyte development. Indeed, neuregulins on the surface of axons play a role in the maintenance of oligodendrocytes and their progenitors. Neuregulins have been implicated in survival of cells in the oligodendrocyte lineage, but the precise signaling pathway remains to be established, since they signal through both PI 3-kinase and MAP kinases. We focused this study on whether neuregulins induce cell survival in the oligodendrocyte lineage through the PI 3-kinase/Akt pathway.

Our studies clearly indicate that neuregulin acts as a survival factor in the oligodendrocyte lineage and activates the Akt signaling pathway. To establish whether the Akt signaling pathway is the primary pathway involved in neuregulin-induced survival, we overexpressed a dominant negative form of Akt (AA-Akt) in primary cultures of oligodendrocytes and their progenitors by adenovirus-mediated gene transfer. (AA-Akt contains a replacement alanine at both of the phosphorylation sites.) Our results indicate that dominant-negative Akt effectively induced apoptosis in neuregulin-protected cells as compared with wildtype (wt) or control virus infection.

To assess the mechanism by which AA-Akt induces apoptosis in the oligodendrocyte lineage, we studied caspase activation in infected cultures of oligodendrocyte progenitors using CaspACE™ FITC-VAD-FMK In Situ Marker (Cat.# G7461, G7462). Oligodendrocyte progenitors were infected for 24 or 48 hours and then transferred to medium with 0.5% FBS for an additional 20–24 hours before incubation with the fluoro-isothiocyanate conjugate of the cell permeable irreversible caspase inhibitor VAD-FMK. Our results indicate that the number of caspase-positive oligodendrocyte progenitors was dramatically increased in the dominant-negative Akt-expressing cells as compared with mock- or wt-infected cells. Essentially, all caspase-positive oligodendrocyte progenitors, NG2-positive (Figure 1) or A2B5-positive (data not shown here), showed condensed DAPI-stained nuclei, suggesting that AA-Akt overexpression induces caspase-mediated apoptosis in oligodendrocyte progenitors.

Summary by Ana I. Flores and Wendy B. Macklin, Ph.D.

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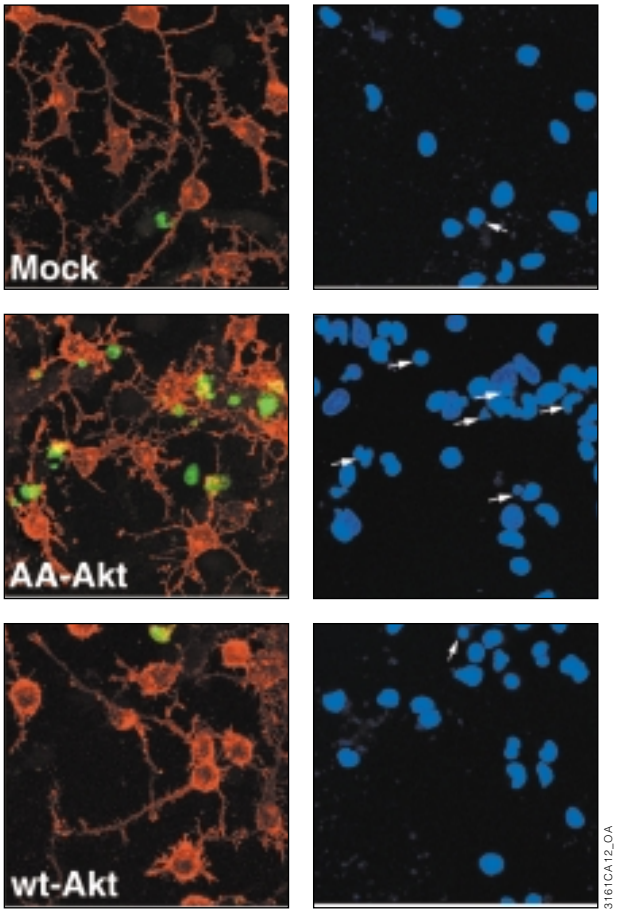

▲ Figure 1. Dominant-negative Akt induced caspase activation in primary cultures of NG2-positive oligodendrocyte progenitors. Oligodendrocyte progenitors were mock-infected or infected with adenovirus expressing wt-Akt (50MOI) or AA-Akt (10MOI) for 24 hours. After infection, cells were subjected to caspase activation analysis using Promega's CaspACE™ FITC-VAD-FMK In Situ Marker (green) and NG2 staining (red). Condensed nuclei were detected by DAPI staining (indicated by arrows). Representative data from one of three independent experiments is shown. Details on infection protocol and staining methods may be found in Flores A.I. *et al.* (2000) *J. Neurosci.* **20**, 7622. (Images reprinted with the kind permission of Dr. Wendy Macklin, The Lerner Research Institute, Cleveland Clinic Foundation and The Society for Neuroscience. Figure Copyright 2000 by the Society for Neuroscience.)

Ordering Information

Product	Size	Cat. #
CaspACE™ FITC-VAD-FMK In Situ Marker	50µl	G7461
	125µl	G7462

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