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DEVELOPMENT AND DISEASE

Alzheimer's protein in embryonic pruning

> "…APP is cleaved on trophic deprivation and N-APP binding activates DR6, triggering axonal degeneration through caspase 6."

During development excess neurons and axons are eliminated to refine neuronal connections. The underlying molecular mechanism for this selection process has remained largely unknown. Now, Tessier-Lavigne and colleagues have revealed that signalling of the amyloid precursor protein (APP) through the death receptor DR6 (also known as TNFRSF21) is central in this selection process, a finding with potential relevance to neurodegenerative disease.

Screening the tumour necrosis factor receptor family — members of which are well known for their roles in apoptotic signalling — for potential mediators of the selection process, the authors identified DR6 as a candidate as it was expressed at low levels in neuronal progenitors in the spinal cord but at high levels in differentiating neurons.

Compromising the function of DR6 by using antibodies against it, small interfering RNA knock down or genetic deletion delayed neuronal cell death after trophic deprivation. This signalling event was caspase 3 dependent. To investigate whether axonal pruning is also regulated through DR6, the authors used a compartmented cell culture chamber in which axons can grow under a partition into a nerve growth factor (NGF)-containing side chamber. Depletion of NGF from the side chamber led to axonal degeneration, but this degeneration was delayed in neurons cultured from *Dr6*-knockout mice or when control neurons were treated with antibodies against DR6. *In vivo* studies likewise showed a delay in the pruning of retino-collicular projections in *Dr6*-knockout mice.

Investigating the downstream signalling of DR6 after trophic deprivation, the authors found that inhibition of BAX, an effector of the pro-apoptotic pathway, blocked axonal degeneration. Downstream of BAX, inhibition of caspase 6 but not caspase 3 also blocked this degeneration.

The authors next investigated whether DR6 is activated by ligand binding. Recombinant DR6 ecto-domain blocked degeneration and bound to axons before but not after trophic deprivation. The authors therefore proposed that trophic deprivation triggers shedding of a pro-apoptotic ligand that is inactive when bound to the membrane, and they investigated APP as a potential DR6 ligand: APP is expressed during development, is cleaved by secretases and is tied to degeneration through its links to Alzheimer's disease (AD). Indeed, the authors showed that the amino terminus of APP (N-APP) binds to DR6. Moreover, when this binding was inhibited in neuronal cultures, pro-degenerative signalling was impaired. Further results showed that APP is cleaved on trophic deprivation and that N-APP binding activates DR6, triggering axonal degeneration through caspase 6. Interestingly, degeneration induced by synthetic amyloid-β (Aβ), the main constituent of amyloid plaques in AD, was not blocked by genetic deletion of *Dr6*, suggesting that Aβ is not involved in developmental pruning.

The authors propose that this embryonic pruning mechanism, involving N-APP binding to DR6, may be abnormally activated in AD and contribute to its initiation or progression, either alone or together with other mechanisms such as Aβ toxicity. In support, they cite evidence that *DR6* is expressed in adult neurons and enriched in regions like the hippocampus that are vulnerable in AD, that N-APP immunoreactivity is associated with plaques, and that activated caspase 6 is associated with plaques and tangles in AD.

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ORIGINAL RESEARCH PAPER Nikolaev, A. *et al.* APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature* **457**, 981–989 (2009)