TRANSCRIPTION FACTOR Max IN PROGRAMMED CELL DEATH AND NEUROPROTECTION

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We investigated the expression and subcellular distribution, as well as a protective role of the transcription factor Max upon programmed cell death (PCD) in the central nervous system (CNS), using retinal ganglion cells (RGC) as a model neuron. In healthy RGC, Max is found essentially within the nucleus, and immunoreactivity increases during postnatal development. Explants from retinas of 2 week-old rats were maintained in vitro for 6-72h, during which axon damage at explantation resulted in retrograde RGC degeneration and apoptosis. Max content and distribution were studied by immunohistochemistry and western blots following subcellular fractionation. Upon axon damage, Max rapidly disappeared from the nucleus and was found in the cytoplasm of RGC, preceding nuclear condensation and cell degeneration. Nuclear exclusion of Max still ocurred under treatments with the pan-caspase inhibitor BAF (100µM), the calpain inhibitor calpeptin (0.001-100µM), or the Crm1 exportin inhibitor leptomycin B (3-30nM), among which only BAF prevented PCD. Low temperature at short incubation times (4°C, 12h) prevented both nuclear exclusion and PCD, whereas longer periods at 4°C resulted in RGC death without nuclear exclustion. Whereas both the transcription inhibitor actinomycin D (30µg/ml), and the translation inhibitor anisomycin (1µg/ml) prevented RGC death, only anisomycin prevented nuclear exclusion of Max. The proteasome inhibitor lactacystin (5µM) prevented the loss of nuclear Max, whereas newly-synthesized Max still accumulated in the cytoplasm of the axon-damaged RGC. Overexpression of Max using a recombinant adeno-associated viral vector resulted in increased levels of nuclear Max and prevented RGC degeneration both in vitro and in vivo. The results show that the nuclear exclusion of Max is an integral event of PCD in the CNS. Mechanisms of nuclear exclusion are upstream of caspase activation, and involve nuclear proteasomal degradation coupled with blockade of nucleo-cytoplasmic transport. Moreover, overexpressed Max is neuroprotective and its overexpression may lead to novel gene therapeutic approaches to neurodegenerative conditions. (Supported by CNPq, CAPES, PRONEX-MCT, FAPERJ)