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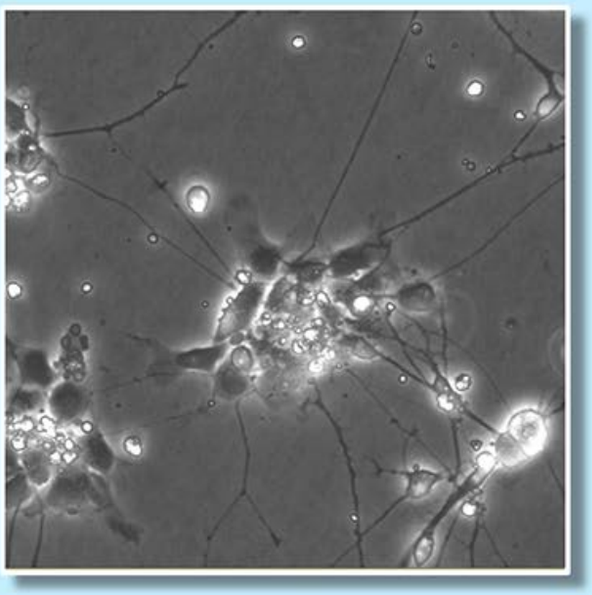

Neuronal Cells Differentiated from Human Embryonic Stem Cells

Dynamic Changes in the Function of Apoptotic Genes During Differentiation and Development

Apoptosis is the genetically encoded pathway that results in programmed cell death. Apoptosis occurs in response to stimuli including cell fate determinants, cellular stress, and DNA damage. Different cell types have different thresholds to undergo apoptosis. For example, undifferentiated human embryonic stem cells (hESCs) are extremely sensitive to apoptotic stimuli, while neurons are extremely resistant. In most cell types, apoptotic stimuli result in the activation of the pro-apoptotic protein BAX. Typically, inactive BAX is found in the cytosol and translocates to the mitochondria when the cell encounters an apoptotic stimulus. At the mitochondria, BAX activates the cascade of events that leads to cell death. We previously showed that in hESCs, BAX exists in a constitutively active conformation at the Golgi apparatus, which increases the vulnerability of hESCs to apoptotic stimuli. This finding encouraged us to investigate the changes in the other key apoptotic factors as cells differentiate from a sensitive hESC state to a resistant neuron state. To do so, hESCs were differentiated into neurons (nociceptors) using the Struder protocol. Immunofluorescence and quantitative real time PCR verified that the differentiation into nociceptor neurons was successful. Changes in gene expression levels of various genes involved in the cell death pathway provide understanding into the dynamic mechanisms by which distinct thresholds for apoptosis may be reached for different cell types upon differentiation. Additionally, specific investigation into the changes of BAX activation during development was of interest. We wished to use a mouse model with a functional GFP tagged mBAX transgene to study BAX activation during development. In order to do so, it was necessary to make a construct with GFP tagged mBAX and check its translocation and function in a mouse cell line. This was done by cloning the mBAX gene downstream of GFP and transfecting the resulting plasmid into wild-type and BAX/BAK double knockout mouse embryonic fibroblasts (MEFs). Expression and function of mBAX was verified using etoposide treatments, conducting Western blots for BAX and cleaved caspase 3, and immunofluorescence of active BAX. Our results convincingly suggested that GFP tagged mBAX was functional. Future work will include generating a mouse with GFP tagged BAX to continue investigating the dynamic changes in BAX activation during development.