Pro-apoptotic Bcl2 family proteins Bax and Bak and their role in mammalian embryonic development

Elizabeth Allen, student no. 213170048

Dr. John C. McDermott

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Since its discovery, apoptosis, or the act of programmed cell death, has been established as crucial to the normal development and homeostasis of many animals.[10] There are two main apoptotic pathways: the extrinsic and intrinsic pathways, which converge on the same final execution pathway leading to the death of the cell. The extrinsic signaling pathway is triggered by transmembrane receptors, which are themselves activated by factors external to the cell. In contrast, the intrinsic pathway is so named because it is induced by signals from within the cell. Intrinsic apoptosis can be triggered by the absence of necessary compounds, such as growth factors or hormones, or injury to the cell caused by stimuli such as radiation or toxins.[6]

The cascade of events in the intrinsic apoptotic pathway begins in the mitochondria. Changes in the inner mitochondrial membrane cause an opening of pores in the membrane, causing the release of pro-apoptotic proteins into the cytosol and loss of mitochondrial transmembrane potential.[21] These pro-apoptotic proteins which are released belong to the Bcl2 family, which consists of a number of proteins that are crucial to the control and regulation of the apoptotic pathways.[2] One model hypothesizes that the balance of pro- and anti-apoptotic Bcl2 members at a critical checkpoint determines whether the cell will commit to apoptosis in response to a death stimuli.[17] Two of the proteins involved in the intrinsic pathway are Bax and Bak, closely related pro-apoptotic Bcl2 family members that show widespread tissue distribution. Activation of Bax and Bak help open pores in the mitochondrial outer membrane, and this step represents the point of no return for the apoptotic cascade.[6]

Under normal conditions, Bax and Bak help trigger intrinsic apoptosis in certain parts of the developing embryo. This helps shape the bodily structures of the embryo, such as through the detachment or fusion of epithelial sheets.[19] Apoptosis also effectively prunes away cells which did not develop successfully

or are otherwise not needed in the adult organism. However, defects in apoptotic regulation can lead to insufficient cell death, which has been implicated in a number of abnormalities in the developing embryo. The majority of research done on Bax and Bak deficiency in vertebrates has been done in mice, by genetically engineering mouse models containing double knockouts of these genes, abbreviated as Bax (-/-) Bak (-/-) mice or DKO (double knockout) mice. Studies of these mice provide insight into the role of Bax and Bak during embryogenesis.

Mice deficient only in Bak were found to develop normally, did not appear to have any observable defects, and were capable of reproduction. Cells isolated from the Bak-deficient mice showed a normal death response when apoptotis was induced through the cell intrinsic pathway.[14] Mice that are deficient in Bax alone were also shown to have limited abnormalities in development.[11] These findings indicate that Bak and Bax not an essential regulator of apoptosis on their own, and that their function may be redundant with each other, or with other pro-apoptotic members of the Bcl2 family.

However, in mice with a double knockout of both Bax and Bak genes, numerous developmental defects as well as a high rate of lethality were observed. The most common causes of death were omphalocele, a birth defect of the abdominal wall causing organs to protrude outside the body, and excephaly, a condition where the brain exists outside the skull.[9] These phenotypes indicate that Bax and Bak deficiency is lethal primarily due to failure of dorsal and ventral midline fusion. Only around 10 percent of double knockout mice survived, with most dying before or at birth.[1] However, mature Bax and Bak-deficient mice displayed a number of abnormal phenotypic traits, such as cleft palate, retainment of the interdigital webs and failure of the vaginal opening to form in females.[13,9] Many of the developmental defects caused by Bax and Bak have also been found to affect the nervous system

and the immune system in particular, two areas where cells are initially overproduced during development and later selectively removed through apoptosis.

During the normal development of the immune system, T and B cells with antigen receptors that are aggressive to self-antigens are eliminated by apoptosis, to prevent the immune system from attacking the cells of their own body. When the intrinsic apoptotic pathway is impaired by Bak and Bax deficiency, these defective cells may escape destruction and contribute to the development of autoimmune diseases.[22] In mice, Bax and Bak have been shown to be essential regulators of B cell death, both during development and at maturity. Double knockout of the Bax and Bak genes results in B cells that are highly resistant to stimuli that would normally induce cell death, such as radiation and the chemical etoposide.[22] The spleen and lymph nodes were also massively enlarged in these mice, due to accumulation of B and T cells that would normally die during development or in the maintenance of tissue homeostasis. The massively enlarged spleen was also found to contain a large increase in the number of plasma cells and histiocytes. Spleen cells and lymphocytes from the mice were found to display enhanced survival and resistance to apoptosis induced by cytotoxic stimuli.[13] These results indicate that Bax and Bak are regulators of the intrinsic apoptotic pathway in lymphoid and myeloid cells.

Studies using mice whose bone marrow had been reconstituted with cells from Bak and Bax deficient mice showed defects in T-cell development within the thymus.[15] The loss of Bak and Bax in mice has also been shown to cause pathology of the lymph nodes and elevated levels of gamma immunoglobin, leading the immune system to attack the kidneys and lead to inflammation of the glomeruli.[22] These mice also possessed autoantibodies against a number of other organs, and in many cases led to autoimmune-induced damage to the blood vessels of the spleen and pancreas.[15] Elevated white blood

cell counts were also observed in the Bax and Bak deficiency mice, with significant increases in myeloid and lymphoid cells, both within circulation as well as in the lymphoid organs.[13]

The hematopoietic system was also found to have many abnormalities in Bax and Bak deficiency mice. Such defects include elevated numbers of granulocytes, lymphocytes, and hematopoietic progenitor cells.[8] In a study of human cell lines displaying hematopotietic maglinancies, approximately 21 percent were found to have mutations of Bax.[16] Discoveries of Bax mutations have also been found in human colonic carcinomas.[20] These observations suggest that loss of function mutations in pro-apoptotic Bcl2 family members could contribute to the development of cancer in the blood and colon.

In addition to the immune system, the nervous system of the developing vertebrate also produces a large number of excess cells. Neuronal cell death via apoptosis plays a prominent role in the targeted removal of these excess cells during specific developmental periods.[18] Within the context of the nervous system, this means neurons which failed to successfully make synaptic connections with other neurons. Neuronal death is largely controlled by trophic factor deprivation, with nerve growth factor (NGF) being an example of such a factor. In wild-type neonatal mice, neurons of the superior cervical ganglion are dependent on nerve growth factor for survival.[12] In its absence, virtually all neurons die by apoptosis within 2 days.[5] In this way, growth factor deprivation can be used to test whether neurons begin apoptosis in the absence of appropriate stimuli. To examine the effect of nerve growth factor deprivation in Bax deficient mice, sympathetic neurons from the superior cervical ganglion of neonatal Bax -/- mice were placed in a medium containing anti-NGF antibodies. These neurons were found to survive nerve growth factor deprivation for 6 days, indicating that Bax plays a role in the initiation of cell death by trophic factor deprivation.[3] Observation of the brains of Bax

deficient mice also show that there were increased numbers of neurons present in the superior cervical ganglia.[3]

The brains of adult bak -/- bax -/- mice were found to contain increased numbers of neural progenitor cells, as well as an increased number of cells in other areas of brain where neural progenitors were thought not to exist. For example, the amount of neurons observed in the mid-corpus callosum were present in twice the density observed in wild-type mice.[14]

Neuronal progenitor cell cultures from these mice were exposed to a variety of pro-apoptotic stimuli in vitro, to assess whether a resistance to apoptosis was responsible for these cells' ability to persist in the adult brain. Neural progenitor cells from wid-type and bak -/- bax -/- mice were treated with two chemicals known to induce apoptosis, etoposide and staurosporine. After 24 hours, the majority of wild-type cells died, while the bak -/- bax -/- neural progenitor cells showed resistance to apoptosis.[14] It is interesting to note that neural progenitor cells from single knockout bak -/- or bax -/- deficient mice did not have a significant difference in cell death compared with those of wild-type mice. This is another indiciation that the functions of these genes may be overlapping or redundant.

In addition to the pruning of neurons at later stages of development, described above, the formation of the neural tube early in development is also dependent upon normal patterns of apoptosis. In mice, Bax and Bak double knockout has been shown to lead to failure of neural tube closure, leading to conditions such as spina bifida and exencephaly and a high rate of lethality.[9]

To examine the effect of Bak and Bax deficiency in a human model, cerebral organoids have been created from Bax/Bak double knockout human pluripotent stem cells, which are able to recapitulate

many developmental processes of the fetal human brain.[7] Loss of Bax and Bak produced defects in corticogenesis, in particular the loss of correct organization and proper cell development in the cortex.[7] Mitochondria from the double knockout organoids also showed abnormal morphology, appearing more fragmented than in controls.[7] Neural rosettes developed from human stem cells also failed to close[7], which is consistent with previous studies which assert that midline fusion during development is dependent on apoptosis.[24] Human embryonic stem cells have also been found to keep Bax in its preactivated state at the Golgi.[4] When the cell experiences DNA damage, the active Bax protein is translocated to the mitochondria from the Golgi. By maintaining Bax in its active form, this may allow for rapid initiation of apoptosis in the early stages of development, by eliminating cells with mutations before they can multiply.

The body of research that has been conducted thus far on the genes Bak and Bax has shown that both genes play a role in encouraging a cell to commit to intrinsic apoptosis, particularly in embryogenesis, where excess cells are created and must be pruned away during the course of development. The importance of Bax and Bak in the midline fusion of the embryo is especially critical, as failure of neural tube closure is likely to be lethal. Interestingly, many of the developmental abnormalities observed in Bax (−/−) Bak (−/−) double knockout mice were not observed in mice deficient in only one of the genes. This suggests that the two proteins may have overlapping functions in the cell, or perhaps their redundancy is to ensure normal cell death processes in the event that one of the genes is nonfunctional. Further study is needed to elucidate the ways in which Bak and Bax fulfill similar functions in the apoptotic process, and the ways in which they differ.

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