Apoptotic Processes in Megakaryocytes and Platelets

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It is becoming increasingly clear that most mammalian cells are capable of undergoing apoptosis and that, within particular lineages, specific apoptotic pathways have evolved to regulate survival and turnover. The role of apoptosis in the megakaryocyte lineage is an intriguing one. Various insults, such as chemotherapeutics, autoantibodies, and human immunodeficiency virus (HIV), have been suggested to induce the apoptotic death of megakaryocytes and/or their progenitors. Conversely, apoptotic processes have been implicated in megakaryocyte development and platelet production. Platelets also contain functional apoptotic pathways, which circumscribe their survival. It has even been suggested that platelet activation responses involve components of the apoptotic machinery, highlighting a potential role for apoptotic processes in hemostasis and thrombosis. This review discusses the current state of knowledge about how apoptosis and apoptotic proteins contribute to the generation and function of megakaryocytes and platelets.
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Apoptosis is a programmed form of cell death. Morphologically, it is defined by cell membrane blebbing, cell shrinkage, chromatin condensation, and DNA fragmentation. Apoptotic cell corpses are swiftly consumed by neighboring phagocytes, a process that is immunogenically silent and non-inflammatory. Throughout gestation, the removal of apoptotic cells ensures efficient tissue remodeling of the developing embryo. In the adult, apoptosis is necessary for the turnover of functionally expended, damaged, or infected cells. Evasion of apoptosis contributes to the survival of malignant cells,[1] and promotes the development of autoimmune conditions, such as systemic lupus erythematosus (SLE).[2] In contrast, excessive apoptotic cell death is a component of many pathological conditions, such as stroke (neurons[3]), heart attack (cardiomyocytes[4]), and diabetes (pancreatic beta cells[5]).

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APOPTOSIS: TWO BIOCHEMICAL PATHWAYS TO CELL DEATH

Metazoan cells possess two highly conserved pathways that regulate their apoptotic death (Figure 1).[6] Both pathways share a common endpoint: the activation of proteolytic enzymes called caspases, which mediate the rapid dismantling of cells. In viable cells, caspases normally reside in the cytosol as inactive precursors. The initiator/apical caspases, such as caspase-9 and caspase-8, are autocatalytically activated and proteolytically activate the effector/executioner caspases-3 and -7, which rapidly cleave essential intracellular substrates resulting in cell death.

One apoptosis pathway is the intrinsic, or mitochondrial pathway. The intrinsic pathway is governed by the interaction between members of the Bcl-2 family of proteins, which comprises three subgroups: the multi-domain pro-survival proteins Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, and A1/Bfl-1; the multi-domain pro-death proteins Bak, Bax, and Bok; and the BH3-only domain pro-death proteins Bad, Bim, Bid, Bik, Noxa, Puma, Bmf, and Hrk/DP5.[6] Cell survival requires that Bak and Bax (and possibly Bok) are kept in check.[7,8] Once they become activated, Bak and Bax oligomerize in the mitochondrial outer membrane, thereby inducing mitochondrial outer membrane permeabilization (MOMP).[8–10] MOMP is considered to be the point of commitment to cell death as it permits the efficient efflux of apoptogenic factors, such as cytochrome *c,* from the mitochondria into the cytosol. Once released, cytochrome *c* binds apoptotic protease-activating factor 1 (Apaf-1), which re-

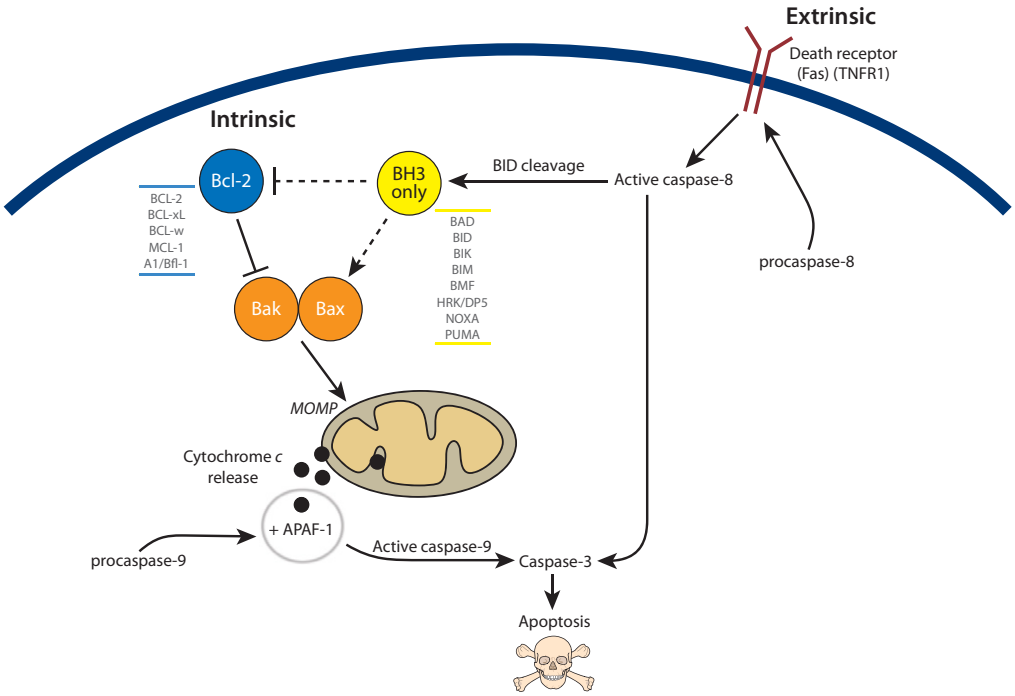

Figure 1. The intrinsic and extrinsic apoptosis pathways. The intrinsic apoptosis pathway is regulated by the Bcl-2 family of pro-survival and pro-death proteins. Stress signals activate the BH3-only proteins, which leads to the activation of Bak and Bax. Consequently, Bak/Bax oligomerize and permeabilize the mitochondria. Diffusion of cytochrome *c* through the mitochondria drives caspase activation. The extrinsic pathway is induced upon ligand binding to death receptors. This initiates recruitment of adaptor proteins to the death receptor intracellular domain (death domain), which results in cleavage and activation of caspase-8. Active caspase-8 can directly activate caspase-3. Alternatively, it can trigger the intrinsic pathway by cleaving Bid to produce tBid.

cruits pro-caspase-9 and initiates downstream caspase activation.[11,12]

Exactly how Bcl-2 family pro-survival proteins restrain Bak and Bax, and how the latter become activated is the subject of considerable debate. It is well established that internal or external stress signals, such as DNA damage-induced activation of p53, trigger the activation of the pro-death BH3-only proteins. Two models propose that BH3-only proteins then either (1) interact directly with and activate Bak/Bax,[13] or (2) disrupt the physical interaction between Bak/Bax and the pro-survival Bcl-2 proteins, allowing Bak and Bax to be unleashed.[14]

An alternate apoptosis pathway, known as the extrinsic pathway, is triggered by ligands, such as Fas-L or tumor necrosis factor (TNF)-α, binding to cell surface receptors of the TNF family.[15] This leads to recruitment of adaptor proteins to the receptor's cytosolic death domain and subsequent activation of caspase-8.[16,17] The pathway can then bypass the Bcl-2 family and mitochondria via the direct activation of caspase-3 by caspase-8.[18] In some cells, caspase-8 is capable of amplifying the apoptotic cascade by triggering the intrinsic pathway. It does so by cleaving the pro-death BH3-only protein Bid to its truncated form, tBid, which initiates Bak- and Bax-dependent MOMP.[19–22]

APOPTOTIC DEATH OF MEGAKARYOCYTES

Megakaryocytes presumably need to prevent the inadvertent activation of the apoptotic machinery in order to survive and produce platelets. This may be relatively straightforward at steady state, but in a range of pathophysiological settings, megakaryocytes and their progenitors are targets of signals that appear to induce apoptotic death. A prime example is cancer chemotherapy. Since the 1960s, when the link was first established, a wide range of chemotherapeutic agents have been demonstrated to cause thrombocytopenia,[23] but the cell types targeted by specific drugs and the mechanisms by which they kill them are not well understood. Given the ability of cytotoxic agents to trigger apoptotic pathways in tumor cells,[24] it is likely that similar processes underlie, at least in part, chemotherapy-induced thrombocytopenia. Furthermore, some of the decrease in platelet production observed in idiopathic or immune thrombocytopenia purpura (ITP) patients may be explained by antibody-induced megakaryocyte damage and apoptotic death.[25–28] It has even been suggested that human immunodeficiency virus (HIV) infection may trigger megakaryocyte apoptosis.[29,30]

Compelling evidence that specific molecular pathways control apoptosis in megakaryocytes came from a