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News and Commentary

Apoptotic volume decrease and the incredible shrinking cell

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Apoptosis is a physiological process inherent in all cells, intended to eliminate a specific group of cells at a given time or in response to a particular signal to preserve the overall well being of the organism. This mode of cell death has been defined by a distinct set of both morphological and biochemical characteristics, which collectively identify this process. The predominant signatures of apoptosis include the loss of cell volume or cell shrinkage, nuclear condensation, internucleosomal DNA fragmentation, and apoptotic body formation. These phenotypic features distinguish apoptosis from other types of cell death including necrosis.[1,2] As our understanding of apoptosis has progressed, other cellular characteristics such as externalization of the phosphatidylserine, changes associated with the mitochondria, and the activation of caspases have been added to the definition for this mode of cell death. However, many of these characteristics are both cell type and death signal dependent, calling into question their universality. In contrast, the loss of cell volume or cell shrinkage, termed apoptotic volume decrease (AVD) has been a ubiquitous aspect of apoptosis that ironically has been largely ignored, particularly from the viewpoint of regulation.

The loss of cell volume during apoptosis has been viewed as a passive process occurring to facilitate the breakdown of the cell into smaller, apoptotic bodies, aiding their eventual engulfment by neighboring cells or macrophages. However, recent studies on the role of AVD in the death process have led to a new understanding of how and why cells shrink, as well as the molecular processes regulating this event during apoptosis. In contrast to earlier dogma, several studies have now suggested that AVD plays an active and critical role during cell death regulating in particular the activity of apoptotic nucleases and the activation of caspases.[3–5] What has also become evident is that although the morphological phenomenon of cell shrinkage may be important, the underlying movement of ions plays a functional role in setting a threshold for the cell to commit suicide. Thus, an appreciation of ion movement, particularly changes in intracellular sodium and potassium in the dying cell, holds the key to understanding the role AVD during apoptosis.

Maintenance of a normal cell size is an energetically demanding endeavor, particularly in response to adverse environmental conditions. The cell uses a tremendous amount of ATP to preserve cellular homeostasis, and a majority of this energy goes towards maintaining an ionic balance required for life through the constitutive activity of the Na^+/K^+-ATPase. Thus, it is vital for a cell to maintain a normal cell size and ionic balance to remain healthy and viable. Under anisotonic conditions, either hyperosmotic or hypoosmotic, a cell can shrink or swell respectively, mainly through loss or gain of intracellular water. However under these conditions, most cells have the ability to compensate for changes in cell volume through a set of volume regulatory mechanisms, which permits the cells to achieve a near normal cell size and thus maintain cellular homeostasis.[6] These inherent regulatory mechanisms depend in part on changes in the intracellular ionic environment and serves as a line of defense to protect the cells from unfavorable conditions. Unfortunately, the mechanisms responsible for the activation of these volume regulatory responses are not completely understood. Additionally, the question as to what signals may be generated upon alterations in cell volume also remains to be determined, specifically in relation to apoptosis.[7]

Interestingly during apoptosis, AVD does not appear to be compensated to any relative degree by a regulatory volume increase response (RVI; Figure 1), such that when the cell shrinks, it remains shrunken. In fact, a regulatory volume decrease response (RVD) observed in many cells under hypotonic conditions is thought to facilitate AVD during apoptosis.[8] This difference between anisotonic and apoptotic changes in cell volume may depend on how the cell initially perceives alterations in its cell size. Under anisotonic conditions, the cells initially change size due to a movement of intracellular water either into or out of the cell. However during apoptosis, a movement of ions has been shown to underlie AVD. Therefore the question is does this initial ionic movement, as opposed to water, render the cells unable to activate inherent volume regulatory mechanisms in the cell to compensate for the change in cell size during the apoptotic process? Additionally during anisotonic conditions, the cells rapidly change volume (in seconds) permitting an observable change in cell size that can then be used as a base line for measuring inherent compensatory volume regulatory mechanisms in the cell. In contrast during apoptosis, AVD appears to occur much slower, over a period of minutes. Thus if cells are attempting to compensate for a loss of cell volume during apoptosis via RVD response, in the continual presence of signals which are directing the loss of cell volume, the aforementioned RVD response may not be observed based on simple morphological techniques. Essentially, the signals to shrink and swell the cells are balancing themselves out. A dilemma then arises if the signal(s) for a cell to shrink is stronger than the signal(s) for a

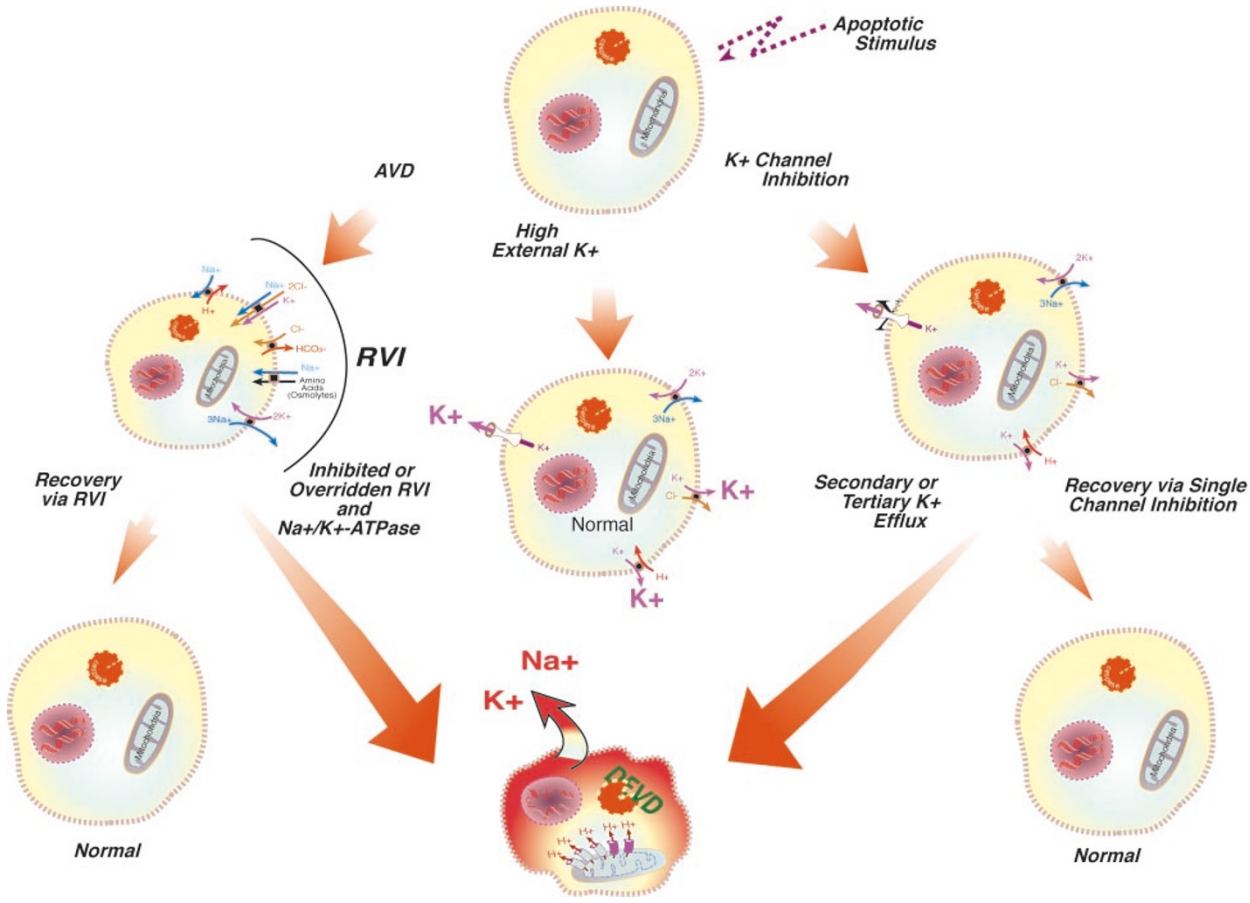

Figure 1 A schematic view for the role of intracellular potassium in relation to AVD during apoptosis. Upon apoptotic stimulation, the cell must decide how to accomplish the cell death program, including orchestrating a movement of ions, specifically potassium. Inherent volume regulatory mechanisms, intervention by high external potassium, or inhibition of a specific potassium pathway can promote cell recovery. However, the overall importance of the apoptotic process to eliminate these cells once activated imply that inhibition or overriding the protective volume regulatory response, or the activation of multiple potassium efflux pathways may occur to ensure the completion of the cell death process

cell to compensate for the loss of cell volume, thus favoring AVD and eventual cell death. This leads to the question if volume regulatory mechanisms are inhibited during apoptosis, or are they active but overridden by the intensity of the cell death signal?

During apoptosis, it has now been well documented that the loss of intracellular ions, specifically potassium, underlies AVD (reviewed in[9,10]). Potassium is well suited for this role due to its high concentration in the cytosol compared to other intracellular ions. However, the overall decrease in intracellular potassium may result not only from the efflux of intracellular potassium, but also from the inhibition of potassium uptake resulting from inhibition of the Na^+/K^+-ATPase. Recently, Na^+/K^+-ATPase inhibition was shown to occur during both anti-Fas and glucocorticoid induced apoptosis of lymphoid cells.[11,12] Additionally, to maintain electroneutrality, the loss of anions, particularly chloride should accompany the loss of potassium. Several recent studies have suggested that the anionic pathway triggered during AVD may be similar to that observed during an RVD response, supporting the notion of an active RVD response during apoptosis.[8,13] Stimulation of the CD95 receptor in Jurkat cells results in the activation of an outwardly rectifying chloride channel.[13] Additionally, the regulation of this channel has been shown to occur through a Src-like tyrosine kinase, a mechanism similar to the one observed in the regulation of the swelling-induced chloride channels in lymphocytes.[14] Thus a link between AVD and cell volume regulation may exist, but remains to be completely defined under various apoptotic stimuli.

What has eluded detection during the AVD process is the exact pathway in which potassium is lost. Attempts to inhibit the loss of intracellular potassium during apoptosis in numerous model systems through the use of potassium channel blockers have resulted in varied success, ranging from no effect to attenuating the amount of AVD.[15–17] To date, no universal potassium channel inhibitor has been identified that completely prevents apoptosis under all cell death conditions, in spite of the common occurrence of AVD as a distinguishing characteristic of cell death. Interestingly in some model systems, inhibition of potassium channels has been shown to induce cell death. For example it has been reported that the potassium channel blocker 4-AP induced apoptosis in HepG2 cells,[18] while

clofilium, a different potassium channel blocker, induced apoptosis in HL-60 cells,[19] suggesting diverse roles for potassium channels during the cell death process.

The question remains, why has the mechanism of potassium efflux during apoptosis eluded our detection, in spite of its common occurrence during the programmed cell death process? The answer to this question appears to have many forms. First, potassium channels make up one of the most diverse groups of ion channels; classified into distinct sub-families based on their transmembrane and pore-forming domains.[20] To date, more than 60 pore-forming subunits for potassium channels have been cloned in mammals alone. Thus, the diversity of these protein complexes on its own does not lend itself for a straightforward identification of potassium channels associated with apoptosis. Furthermore, the modulation of potassium channels by cytoplasmic factors such as kinases, phosphatases, and other ions adds an additional level of complexity to these proteins. While many regulatory proteins such as Bcl-2 family members, caspases, and IAP's have been identified and are known to play a critical role in the apoptotic process, the regulation of these proteins by cytoplasmic kinases and phosphotases is not completely understood, and the regulation of potassium channels are no exception.

A second consideration in why the mechanism of potassium efflux has eluded our detection is the idea that potassium channel characteristics may change during the cell death process. These channel characteristics may include changes in ion selectivity, changes in the gating properties of the channel, either activation or inactivation, changes in drug sensitivity, and changes in channel trafficking. These changes may be brought about by modifications in the channels themselves, such as alterations in their phosphorylation status, through alterations in regulatory proteins associated with the channels as mentioned earlier, or through modification of specific plasma membrane proteins neighboring the channel. For example, it has been shown that activation of Fas leads to the inhibition of voltage-dependent n-type potassium channels,[21] and reactive oxygen species (ROS) can also modulate potassium channel activity.[22] As the study of ion channels continues apart from its participation in the death process, it is probably safe to assume that many of the signaling pathways that regulate channel activity will probably also play a role during apoptosis.

Finally, an appreciation of the overall importance of this cell death process needs to be considered. This cellular suicide process is critical for cellular homeostasis. Thus insufficient or excessive apoptosis can lead to and contribute to various disease states. When apoptosis is triggered, it is imperative that the cell death process be completed. Aborted apoptosis may lead to necrosis, with the ensuing inflammatory response disrupting cellular homeostasis, therefore countering the original purpose of this naturally occurring physiological death process. While these ideas appear rather obvious and simplistic, insights into the nature of AVD can be drawn. As the importance of ion movements as they relate specifically to the regulation of the common apoptotic machinery continues to be established, an apoptotic cell must ensure that the process is completed, including the efflux of potassium during AVD. If a primary pathway for ion loss is inhibited due to the addition of a specific channel blocker, a previously dormant secondary or tertiary pathway may be activated. Because of the importance of completing the cell death process once a cell is triggered to die, it is imperative that the cell utilizes alternative means for potassium efflux in the event of inhibition of the primary ionic pathway. Therefore, multiple pathways for this loss of intracellular potassium probably exist (Figure 1).

In support of this latter hypothesis, moderate success has been achieved when high potassium, at a level similar to the intracellular concentration of potassium, is used in the external environment to prevent AVD (Figure 1). The presence of high potassium on the outside of the cell simply impedes the efflux or loss of potassium regardless of the ionic pathway invoked during apoptosis, and has been shown to prevent the activation of other apoptotic components during the cell death process. The inhibition of cell death due to high extracellular potassium has been shown in a number of diverse apoptotic model systems including oxygen-induced cell death in PC12 cells,[23] serum deprivation or staurosporin treatment of mouse neocortical neurons,[24] and anti-Fas treated Jurkat cells.[3] Recently, the protective effect of high extracellular potassium were shown to inhibit numerous characteristics of the apoptotic process, including not only caspase activation and apoptotic nuclease activity, but also the externalization of phosphatidylserine, mitochondrial depolarization, and release of cytochrome *c*.[25] Additionally, high extracellular potassium prevented the formation of the active apoptosome complex,[25,26] again suggesting the importance of AVD and potassium loss during the cell death process.

In summary, apoptosis is a unique and well-orchestrated series of events leading to cellular suicide of which AVD plays an important role. Clearly an ionic component exists in the apoptotic program that has emerged and participates not only in the facilitation of the death program, but also in the repression of the cell death machinery. It is this repressive aspect of intracellular ions that appears to act as a checkpoint during apoptosis in which the cell not only decides whether to live or die, but how to accomplish this goal. Once the decision to die has been made, a specific movement of ions must occur, whether it be inhibiting or overriding the inherent volume regulatory responses used to protect the cell, or activating multiple pathways to permit the efflux of potassium from the cell. What is also apparent is that once the decision has been made to die, success is only measured with the unobstructed completion of the programmed cell death process. As with any worthwhile scientific endeavor, the study of AVD has increased our understanding of the role this process plays during apoptosis, however it has naturally led to more questions then answers. Questions that will continue to define apoptosis.

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