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CrossTalk opposing view: the triggering and progression of the cell death machinery can occur without cell volume perturbations

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Cell death is accompanied by the dissipation of ion gradients across the plasma membrane which, in turn, may cause cell volume perturbations by altering the content of intracellular osmolytes and osmotically obliged water. It is well documented that cells maintain their volume with an accuracy of 2–3% and volume perturbations beyond this range affect diverse cellular functions including membrane electrical potential, oxygen burst, metabolism, proliferation and gene expression (Lang *et al.* 1993; Hoffmann *et al.* 2009). Considering this, the contrasting volume behaviour in cells undergoing two morphologically distinct modes of death, apoptosis and necrosis, was classified by Okada and co-workers as necrotic volume increase (NVI) and apoptotic volume decrease (AVD; Okada *et al.* 2001). It is true that in many studies of apoptosis where cell volume was accurately measured, an early shrinkage was detected. This decrease is different from, and precedes the mechanical reduction of the cell size caused by separation of apoptotic bodies. In several reports, however, the absence of AVD during the early morphological stages of apoptosis was noted. In analysing this controversy, one should consider the fact that the most popular cell volume measurement methods, such as light scattering and Coulter electronic sizing are only applicable to suspended cells, which restricts the choice of experimental models (Orlov *et al.* 2013). At the same time, in many studies of adherent cells, shrinkage was equated to the loss of spreading, which is clearly a separate phenomenon. To address these difficulties, we have developed two techniques that allow the measurement of volumes of unperturbed, substrate-attached cells: dual-image surface reconstruction (DISUR; Boudreault & Grygorczyk, 2004; Groulx *et al.* 2006; Fels *et al.* 2009) and transmission-through-dye microscopy (Model, 2012). We employed DISUR to assess the volume of single vascular smooth muscle cells transfected with E1A-adenoviral protein (E1A-VSMC; Platonova *et al.* 2012). In the absence of growth factors, these cells undergo rapid death, presenting biochemical markers of 'classic' apoptosis. Remarkably, after a 30–60 min lag-phase, the volume of serum-deprived E1A-VSMC did not decrease, but increased by ∼40%, preceding caspase-3 activation and chromatin cleavage. Swollen cells then underwent abrupt apoptotic collapse, manifested by plasma membrane budding, with formation of numerous apoptotic bodies. By contrast to this case, the collapse of E1A-VSMC in the presence of staurosporine followed a ∼30% decrease of their volume. A similar behaviour was observed in HeLa cells treated with actinomycin D, where the swelling phase could last up to several hours; however, shrinkage did occur eventually, following mitochondrial depolarization and condensation of chromatin (Kasim *et al.* 2013). A different, but also a complex pattern of volume dynamics was observed in Ehrlich ascites tumour cells exposed to cisplatin (Poulsen *et al.* 2010). In the initial phase, the cell volume decreased by 6–8%, then recovered and finally dropped by ∼30% during the executive phase of apoptosis. These results demonstrate that shrinkage may be absent during certain stages of irreversible apoptotic damage and, therefore, caution is called for when making generalizations about the universal nature of apoptotic shrinkage. Keeping this uncertainty in mind, we propose to use the acronyms DVD (dying cell volume decrease) and DVI (dying cell volume increase) to indicate the shrinking and swelling stages in dying cells.

The other important question is whether primary shrinkage serves as a signalling mechanism controlling downstream apoptotic reactions or merely a passive consequence (presumably, physiologically

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important) of ion loss. Because monovalent ions may have other roles besides controlling intracellular water (Orlov & Hamet, 2006), a rigorous proof that shrinkage is necessary for apoptosis would require that it be specifically inhibited without affecting ion concentrations. However (as discussed below), the typical experiment designed to demonstrate the role of shrinkage involves inhibition of ion channels or changing the composition of cellular media. Both approaches result in drastic alteration of intracellular ion concentrations, making it difficult to separate these two factors. But even the experiments with inhibition of ion channels have produced divergent results. Thus, Maeno and co-workers reported that in HeLa, U937, PC12 and NG108-15 cell lines, non-selective inhibitors of anion channels (DIDS, NPPB and phloretin) and of K^+ channels (5 mM Ba^{2+}) completely blocked DVD and increased cell survival (Maeno *et al.* 2000). By contrast, both DIDS and NPPB inhibited DVD but did not affect caspase-3 activation and DNA fragmentation in staurosporine-treated cortical neurons (Wei *et al.* 2012). The IK_{Ca} inhibitor clotrimazole blocked both DVD and the death of staurosporine-treated glioblastima cells, but not of cells exposed to TRAIL (tumour necrosis factor-related apoptosis-inducing ligand), a trigger of the extrinsic apoptosis pathway (McFerrin *et al.* 2012). A very modest inhibition or the absence of any protective action of K^+ channel blockers were documented in some other studies (for recent reviews, see Lang *et al.* 2007; Hoffmann *et al.* 2009; Orlov *et al.* 2013).

The second type of experiment designed to address the role of cell volume in apoptosis employs non-isotonic solutions. Since the initial observation on immune system cells (Bortner & Cidlowski, 1996), numerous research teams have reported that hyperosmotic shrinkage, triggered by an increase of osmolality up to 600–700 mosmol kg^{-1}, results in the death of mammalian cells (for reviews, see Bortner & Cidlowski, 2007; Hoffmann *et al.* 2009). It should be noted, however, that a similar elevation of osmolality only slightly augmented apoptosis in SH-SY5Y neuroblastoma cells (Matthew & Feldman, 1996) and had no effect on MDCK (Orlov *et al.* 1996), Cos-7, GH_3 and HeLa cells (Bortner & Cidlowski, 1996). Moreover, Lang and co-workers observed that a 20–30% increase in osmolality inhibited, rather than triggered, apoptosis in Fas-L-treated Jurkat cells (Gulbins *et al.* 1997). Thus, it appears that experiments where cell volume shrinkage was artificially induced by hyperosmotic shock, do not unequivocally indicate that shrinkage favours apoptosis, but rather suggest that the effect is cell specific.

It is known that the majority of cells exposed to hypotonic solutions undergo rapid regulatory volume decrease, while subsequent normalization of the medium osmolality leads to their shrinkage. We have observed that this protocol reduced the volume of E1A-VSMC by ∼2-fold but did not affect their survival (Orlov *et al.* 2004). By contrast, a ∼20–30% attenuation of cell volume, triggered by isosmotic substitution of Cl^- by gluconate or aspartate or of Na^+ by *N*-methyl-D-glucamine, resulted in the death of HeLa and U937 cells with all the canonical markers of apoptosis present (Maeno *et al.* 2006; Nukui *et al.* 2006).

In conclusion, recent data show cell type-specific rather than ubiquitous cell volume behaviour in cells treated with death stimuli and suggest that cell volume perturbations are not always sufficient for the triggering and progression of the cell death machinery.

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