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Correspondence

Vital dyes and virtual deaths

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Dear Editor,

In 2009 and 2012, *Cell Death & Differentiation* has published recommendations of the Nomenclature Committee on Cell Death (NCCD) for the *Classification of cell death* and *Molecular definitions of cell death subroutines*, respectively. Under the subtitle *When is a cell 'dead'*?, and in an accompanying table entitled *Molecular or morphological criteria to define dead cells*, the 2009 paper featured a set of three criteria; the fulfillment of any single one is considered to indicate cell death.[1] The first criterion in this list is *Loss of plasma membrane integrity*, and parameters commonly used to assess integrity of the plasma membrane were mentioned, namely exclusion from cells of vital dyes like trypan blue or propidium iodide. It should be noted that injuries of the plasma membrane may be transient. This is now widely accepted, and here some key publications related to this issue are reviewed. For instance, mechanical stress can cause lesions of the plasma membrane spanning several micrometers, but they can be rapidly repaired by calcium influx-dependent mechanisms.[2] Similarly, plasma membrane perforation by various pore forming proteins may be reversible.[3] Some pore-forming proteins, for example, streptolysin O, render cells permeable for vital dyes and even for macromolecules, whereas small pore-forming proteins like α-toxin of *S. aureus* form lesions too small to permit flux of these molecules. However, all kinds of membrane lesions cause a drop of potassium and cellular ATP concentrations, which however, may be reversible if reconstitution of membrane integrity is achieved in time. Transient plasma membrane damage may not only trigger transcriptional responses, and release of mediators,[4] but also cell cycle progression.[5] Therefore, loss of membrane integrity is not even a reliable predictor of imminent death. Cellular tools for mending perforated membranes are diverse and comprise calcium influx-dependent or calcium influx-independent mechanisms. However, vesicle traffic emerges as a common denominator of resealing processes,[6–8] which appear to be evolutionarily conserved and operate *in vivo*.[8] As all definitions of cell death are necessarily 'package deals' with the tacit assumption that the observed changes are irreversible, investigators are facing the question for how long they have to observe an indicator of a presumed, irreversible process before they may actually regard it as such. As membrane repair depends on the energy consuming task of vesicle traffic, irreversibility of loss of membrane integrity may be ascertained when cellular ATP is depleted. Although more work is required to fully understand and appreciate the mechanisms and relevance of membrane repair, the available evidence warrants a reassessment of *Loss of plasma membrane integrity* in itself as a *bona fide* criterion to define cell death.

Conflict of Interest

The author declares no conflict of interest.

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