Hypertonic stress and cell death. Focus on “Multiple cell death pathways are independently activated by lethal hypertonicity in renal epithelial cells”

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Programmed cell death has been synonymous with caspase-mediated apoptosis for many years, until the recent discovery of caspase-independent mechanisms of cell death. Programmed necrosis is a form of caspase-independent cell death and is currently intensively studied as mechanism of kidney cell injury. The inner core of the kidney, known as the renal medulla, is hypertonic due to a specialized counter current arrangement of tubules, vascular elements and transporters. This regional hypertonicity is critical for body fluid osmoregulation as it provides the osmotic driving force for urinary concentration. The hypertonic environment of the kidney medulla can also promote cell death, if the physiologic cell protective mechanisms are impaired (4). Hypertonic cell stress can induce several pathways that lead to cell
death and the distinction between hypertonicity-induced apoptosis and necrosis is becoming increasingly complex.

Cells in the inner medulla are constantly exposed to high NaCl and urea concentrations, which induce osmotic stress that impairs function of intracellular proteins and disrupts the structural integrity of cell organelles. This may lead to increased reactive oxygen species formation, cytoskeletal rearrangements, decreased DNA replication, increased mitochondrial depolarization and damaged DNA proteins (6). To counter the hyperosmotic stress, renal medullary cells accumulate chaperones such as heat shock proteins and compatible organic osmolytes. Sudden increases in fluid tonicity or impaired protective responses can activate or induce cell death pathways. Hypertonicity-dependent mitochondrial and death-receptor pathways of apoptosis have been demonstrated in different cell lines (1). Cytochrome c release is an important initial step following hypertonicity-dependent activation of the mitochondrial pro-apoptotic pathway in kidney cells (8). The study by Choi et al. (...) in this issue of American Journal of Physiology-Cell Physiology offers new insights into the caspase-dependent and independent death pathways that are activated by hypertonic stress in renal epithelial cells.

Using a collecting duct cell line from mouse kidney inner medulla (IMCD3), Choi et al show that acute, lethal hypertonicity independently activates the mitochondrial, lysosomal and death receptor pathways of apoptosis. They show that increased permeability of mitochondrial or lysosomal membranes is an early activation step,
leading to cytochrome c and cathepsin B release, respectively. Further, they
demonstrate that neither of these early activation steps requires caspase activation.
Moreover, these hypertonicity-induced cell death pathways are variably activated in
different renal epithelial-derived cell lines. The findings in this study show that
there is significant overlap between hypertonicity-induced activation of pathways
leading to apoptosis versus programmed necrosis.

Inner medullary cells \textit{in vivo} can survive and function despite interstitial tonicities
of over 1200 mosmol/kgH$_2$O. In contrast, in culture, IMCD3 cells do not survive an
acute increase in NaCl concentration above 600 mosmol/kgH$_2$O (7). This greater
osmotic tolerance \textit{in vivo} is partially due to both slower proliferation rates and
gradual, slower rate of tonicity changes, as dictated by the need to generate
concentrated urine to adjust osmolality of body fluids. These allow cells to adjust
more slowly and to initiate compatible osmotic stress responses (9). A possible
explanation for the small amount of cell death \textit{in vivo} is that there is iso-volumetric
regulation during the gradual increase in tissue tonicity (due to the accumulation of
osmolytes), which prevents cell shrinkage-induced initiation of apoptotic and
necrotic pathways (2, 5). However, the mechanisms that lead to the initiation of
programmed cell death or programmed necrosis are still poorly understood. The
cell's sensing of a hypertonic environment may involve membrane-spanning
receptors, such as integrins (3). There are still many questions to be answered: How
does cell shrinkage activate apoptotic versus necrotic pathways? Which step defines
whether caspase-dependent or independent pathways will be activated? Is
hypertonicity-mediated cell death important in certain clinical settings, such as NSAID-induced acute kidney injury?

With emerging new tools to study cell death pathways (i.e. triple-knockout mice, conditional tubular knockout systems) the relative contribution of classic apoptotic pathways versus programmed necrosis in hypertonicity-induced cell death will be unraveled. Interconnections in signaling pathways, cell cycle regulation and mechanisms of organelle membrane alterations between these different forms of organized cell death will likely be found. Enhanced understanding of the regulatory steps and these interconnections open up therapeutic opportunities that might lead to prevention of drug-induced cell injury or age-related impaired kidney function.


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Legend

Fig. 1: Hypertonicity-induced programmed cell death in kidney medullary cells: Hypertonic stress can activate either receptor-mediated, lysosomal-dependent or mitochondrial death pathways, which may lead to either programmed necrosis or apoptosis and can be induced either by caspases or by caspase-independent fashion.
Hypertonicity

Death Receptor

C8 homodimer

Cathepsin B

Bax/Bak

Cytochrome c

Mitochondrion

Nucleus

Lysosome

apoptosis

C3

C6

C7

APAF1