A cell death deep cut

Isha N. Srivastava[1,2], Scott J. Dixon[*1]

https://doi.org/10.15302/vita.2026.04.0027

A paper recently published at *Vita* by Li *et al.*[1] reports that necrotic cell death results in proteolytic cleavage of proteins in a unique pattern compared to apoptosis, generating new epitopes that could be used to track non-apoptotic cell death in normal processes and diseases.

Cell death can be executed by apoptotic or necrotic (non-apoptotic) mechanisms[2]. These cell death mechanisms are biochemically distinct. For example, unlike apoptosis, non-apoptotic forms of cell death are typically characterized by early disruption of plasma membrane integrity. Distinct intracellular proteases, which cleave substrate proteins, are activated in apoptosis and some forms of non-apoptotic cell death to drive cell death and release of immune modulators[3,4]. Cysteine aspartyl (caspase) proteases have important roles in apoptosis and some forms of non-apoptotic cell death, like necroptosis and pyroptosis. Whether other proteases are involved in cell death regulation, and the full spectrum of protease substrates cleaved during cell death is unclear. Now, Li *et al.*[1] report a large-scale comparative study of apoptosis and three forms of non-apoptotic cell death: necroptosis, ferroptosis, and pyroptosis, finding a novel role for extracellular proteases in non-apoptotic cell death activated in cultured cells and also *in vivo*.

Using high-resolution, label-free imaging of cultured cells, Li *et al.*[1] first find that nuclear envelope disruption is shared amongst non-apoptotic cell death mechanisms, including necroptosis and ferroptosis. Notably, the nuclear envelope marker lamin-B1 is lost during the induction of necroptosis and ferroptosis but not apoptosis. Lamins B1 and A/C are classic apoptotic caspase substrates and yield proteolytic cleavage products of defined sizes in response to apoptotic stimuli. Induction of necroptosis or ferroptosis in the same cells also resulted in lamin cleavage, but yielded products with molecular masses that were distinct from the canonical apoptotic cleavage products. A ~50 kDa lamin-B1 cleavage product uniquely observed in necroptotic and ferroptotic cells in culture was also detected *in vivo* in a mouse kidney injury model, suggesting that this proteolytic processing event was physiologically relevant.

How these unusual lamin-B1 cleavage products were created was initially unclear. The protease inhibitor leupeptin prevented the formation of the ~50 kDa fragment in necroptotic or ferroptotic cells, without altering the cleavage of lamin-B1 in apoptotic cells. Interestingly, the non-apoptotic cleavage of lamin-B1 occurred only following membrane rupture. This suggested that extracellular, leupeptin-sensitive proteases may “invade” into permeabilized necrotic cells to access intracellular substrates late in the cell death execution process (Fig. 1). By contrast, invasion of extracellular proteases into apoptotic cells is not possible as plasma membrane integrity is generally maintained for longer periods of time, and instead proteins are cleaved by intracellular proteases, explaining why unique lamin cleavage products were detected in non-apoptotic vs apoptotic cells. While leupeptin treatment blocked lamin-B1 processing, this inhibitor did not prevent cell death, indicating that these protein cleavage events are markers of non-apoptotic cell death, but not a functional requirement for the execution of cell death.

Hundreds of protein substrates for apoptotic caspases have been defined using proteomic approaches[5]. A major question concerned whether protein cleavage by invading extracellular proteases was limited to the lamins or extended to other proteins. Using a proteomics approach, the authors found that necroptosis and apoptosis yielded distinct global patterns of protein cleavage involving hundreds of different proteins in multiple cellular compartments, both *in vitro* and *in vivo*. In necroptotic cells, protein cleavage after Arg and Lys residues was consistent with the activities of trypsin-like proteases that are present in the extracellular environment, including trypsin-like serine proteases of the PRSS and TRY families of enzymes. The invasion of these enzymes appears to be a feature conserved between necroptosis, ferroptosis, and likely other forms of non-apoptotic cell death. Practically, this process of extracellular protease invasion will yield many protein epitopes that can serve as potential new markers for non-apoptotic cell death, as demonstrated here with the development of antibodies against two proteins, cleaved ABHD16A and GLUD1. Antibodies against these cleaved products were validated using models of mouse acute kidney injury and hepatic ischemia-reperfusion injury and ileitis, as well as human diabetic nephropathy, establishing this cleavage signature as a feature of non-apoptotic cell death in disease across organs and species. Whereas the entry of extracellular proteases into necrotic cells does not alter the course of cell death in the physiological context, the resultant cleavage events alter the proteome in a way that promotes efficient phagocytic clearance of necrotic nuclei. The authors demonstrate that blocking proteolytic cleavage in necrotic cells provokes autoimmune responses in mice, suggesting that proteolysis occurring inside necrotic cells may help prevent intracellular self-antigens from triggering immune responses.

The work of Li *et al.*[1] opens the door to multiple future lines of investigation. Apoptotic protein cleavage has a complex interplay with other post-translational modifications like phosphorylation[6]. It would be interesting to examine how the

1. Department of Biology, Stanford University, Stanford, CA, USA. 2. Department of Neurology, Stanford University, Stanford, CA, USA. *Correspondence: Scott J. Dixon (sjdixon@stanford.edu)

Published: April 28, 2026

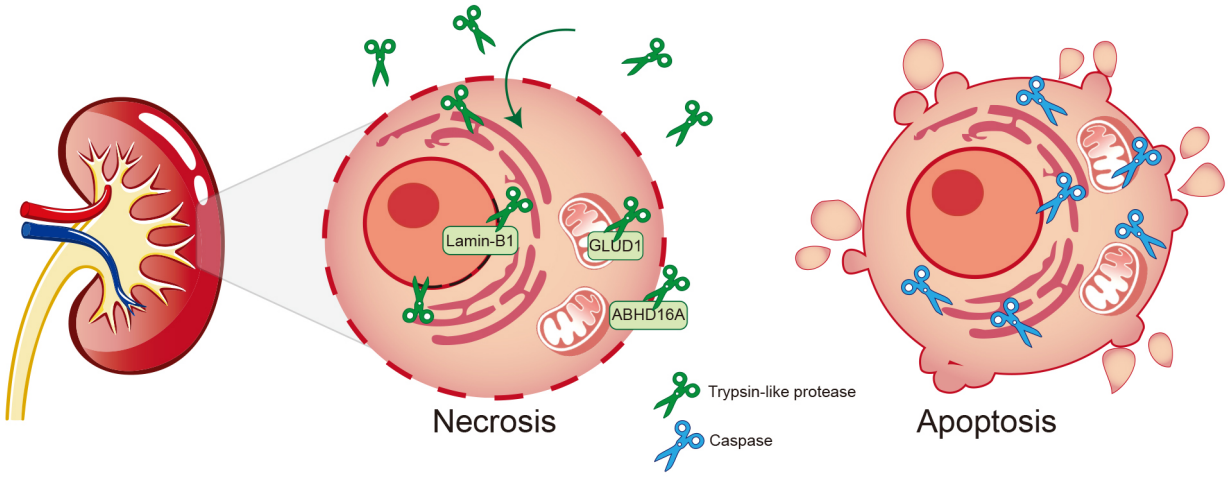

Fig. 1 Extracellular proteases invade necrotic cells to cleave substrates. Unlike apoptosis (right), where intracellular caspases cleave substrates within an intact plasma membrane, necrotic membrane rupture (left) allows extracellular trypsin-like proteases (PRSS family, plasmin) to enter the dying cell and cleave diverse substrates including lamin-B1, GLUD1, and ABHD16A. Li *et al*. detect these cleavage products in mouse kidney ischemic injury and other disease contexts.

unique processing events catalyzed by invading extracellular proteases interact with phosphorylation and other modifications to existing substrates. While this study focused mainly on cleaved proteins shared across non-apoptotic vs apoptotic conditions, cleavage events unique to individual non-apoptotic pathways may also exist. Non-apoptotic forms of cell death have distinct mechanisms of membrane disruption that may alter which extracellular proteases can gain access to intracellular substrates, potentially resulting in unique cleavage events. For example, ferroptotic lipid peroxidation at the plasma membrane[7] or certain intracellular organelles[8] could uniquely change the susceptibility of membrane-associated proteins to attack from invading proteases, yielding unique epitopes that can distinguish further between different modes of non-apoptotic cell death. This would be especially valuable for ferroptosis where few specific post-translational markers of this form of cell death are known[9]. Applying the markers identified in this study to a greater number of diseases could help map the spectrum of non-apoptotic mechanisms occurring *in vivo*, though biomarkers activated by multiple pathways will require careful interpretation. The findings of Li *et al*.[1] suggest that despite differences in the initiation of non-apoptotic cell death, diverse mechanisms likely converge at the very end, resulting in a largely unified protein proteolysis phenotype. Sometimes, the deepest cuts are ultimately the most satisfying.

COMPETING INTERESTS

I.N.S. declares no competing interests. S.J.D. holds patents related to ferroptosis.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Scott J. Dixon.