Dissecting the role of lysosomal proteases in programmed cell death

Cell death is a fundamental biological process required for tissue homeostasis, immune defense, and development, and its dysregulation contributes to malignancy, chronic inflammation, and neurodegenerative disorders. Although apoptosis proceeds through non-lyticand immunologically silent cellular disassembly whereas pyroptosis, pro inflammatory cell death, culminates in membrane rupture, the proteolytic checkpoints that determine these divergent outcomes remain incompletely defined. Previous studies have suggested cooperative roles for lysosomal cathepsins during NLRP3 inflammasome activation; however, the specific contribution of individual lysosomal proteases, and how these depend on the nature of the stimulus or cellular context, has not been resolved. This gap reflects the widespread use of non-selective inhibitors and total-protein measurements, which do not directly reflect enzyme activity. To address these limitations, selective chemical tools were applied to delineate the functional roles of individual cysteine cathepsins in regulated cell death (RCD), with emphasis on their intersection with pyroptotic execution.

Gasdermin D (GSDMD) cleavage represents the central event driving pyroptosis, and accumulating evidence indicates that several cysteine proteases can influence this step in addition to caspase-1. To investigate these interactions and overall role of these enzymes in pyroptosis, in-vitro proteolysis assays, immunoblotting, live-cell imaging, and high-dimensional single-cell analysis (mass cytometry, CyTOF) were combined with tailor-made inhibitors and activity-based probes (ABPs) generated using Hybrid Combinatorial Substrate Library (HyCoSuL) chemistry. Experimental perturbations included the K^+ ionophore nigericin to activate canonical inflammasome and the lysosomotropic dipeptide methyl ester L-leucyl-L-leucine methyl ester (LLOMe) to induce lysosomal membrane permeabilization (LMP). ABPs enabled direct visualization of catalytic activity in live cells, circumventing the inherent limitations of antibody-based detection of zymogens versus active enzymes, while a custom CyTOF panel incorporating TOF-ABPs and canonical markers provided a high-resolution temporal map of proteolytic events at the single-cell level.

The results indicate that cysteine cathepsins are capable of interfacing with pyroptotic signaling; however, under physiological conditions, the canonical caspase-1, GSDMD pathway remains the primary driver of membrane rupture. Kinetic analyses using selective inhibitors identified cathepsin B as the principal lysosomal protease mediating necrotic-like lysis downstream of LMP, highlighting its non-redundant role in lysosome dependent cell death (LDCD). Although cathepsin S and other family members can cleave pyroptotic substrates in vitro, their engagement appears highly context-dependent, becoming prominent under conditions of profound lysosomal stress. Collectively, these findings support a model in which inflammasome signaling operates as a proteolytic network rather than a strictly linear cascade, with auxiliary cysteine proteases capable of amplifying or modifying the final outcome. Cathepsins may play a supportive role in IL-1β and IL-18 processing and, in certain settings, provide alternative routes to lytic cell death when caspase-1-mediated mechanisms are compromised. This functional redundancy likely represents an evolutionary safeguard that preserves the ability of infected or damaged cells to undergo lytic demise and release immunostimulatory signals even when canonical pyroptosis is inhibited.