

Preview

Squeezed to death: Mechanical control of MLKL beyond RIPK3

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Prolonged confinement is a consequence of amoeboid cancer-cell invasion. In this issue of *Developmental Cell*, Zhang et al.¹ show that sustained mechanical stress activates PIEZO1-STAT1-dependent MLKL expression, leading to MLKL-dependent membrane-lytic death in cells without detectable RIPK3 and canonical MLKL phosphorylation, broadening current models of necroptotic competence.

During tumor progression, malignant cells progressively acquire the capacity to invade surrounding tissues, disseminate from the primary lesion, and withstand therapeutic and immune pressures. A central component of this phenotypic plasticity is the mesenchymal-to-amoeboid transition (MAT), which enables cancer cells to adopt a highly deformable, weakly adhesive, and rapidly migrating state that facilitates movement through dense extracellular matrices and narrow tissue spaces. In this setting, mechanical confinement is not merely a physical consequence of tumor growth and invasion but an active determinant of cancer-cell behavior and fate.²

In this issue of *Developmental Cell*, Zhang et al.¹ ask what happens when this mechanically constrained amoeboid state is sustained over time. Rather than focusing on the acute acquisition of amoeboid migration, the authors examine prolonged confinement, which they define as long-term MAT (L-MAT). They show that persistent mechanical stress activates a PIEZO1-STAT1-MLKL axis that progressively restricts migration and ultimately triggers MLKL-dependent membrane-lytic cell death. Remarkably, this occurs in cell models lacking detectable RIPK3, challenging the canonical model of RIPK3-dependent MLKL activation.

Necroptosis is generally portrayed as a tightly ordered signaling pathway. Following appropriate upstream cues, RIPK3 phosphorylates MLKL, promoting the conformational rearrangements that

allow MLKL to oligomerize, accumulate at cellular membranes, and ultimately compromise membrane integrity.^{3–5} This architecture has made RIPK3 almost synonymous with the ability of a cell to execute necroptosis. Indeed, RIPK3-deficient cancer cells are commonly considered resistant to canonical necroptotic stimuli. The current study asks whether MLKL can, nevertheless, engage its membrane-disrupting function independently of RIPK3.⁶

Through pharmacological and genetic approaches, the authors identify PIEZO1 as the mechanosensor responsible for translating prolonged confinement into MLKL induction. PIEZO1-dependent calcium signaling promotes STAT1 activation, which in turn drives MLKL transcription. Genetic deletion of PIEZO1 or STAT1 reduces MLKL induction and cell death, whereas PIEZO1 activation is sufficient to increase MLKL expression. Thus, rather than activating a pre-existing pool of MLKL through RIPK3, sustained mechanical signaling progressively increases the abundance of the terminal executioner itself.

The most intriguing aspect of the study is that this increase in endogenous MLKL appears sufficient to cross an activation threshold. Zhang et al. describe a physiologically induced transcriptional program that raises MLKL to a level at which membrane localization and cell death occur without detectable RIPK3 and canonical MLKL phosphorylation. This suggests that MLKL abundance itself can become a determinant

of the threshold for membrane engagement and lytic activity.

This observation raises a fundamental question about what defines an “active” MLKL molecule. If increased endogenous MLKL abundance permits oligomerization and membrane engagement without the canonical phosphorylation event, then phosphorylation may represent a major route by which MLKL overcomes its auto-inhibited state rather than the only conceivable route to membrane engagement.⁴ Whether increased local concentration favors spontaneous conformational rearrangement, oligomerization, membrane partitioning, or engagement of additional regulatory partners remains to be determined.

MLKL is a lipid-binding membrane disruptor whose N-terminal executioner domain engages anionic phospholipids, including phosphoinositides, during canonical membrane permeabilization.⁷ Under prolonged confinement, increased MLKL abundance could cooperate with changes in membrane geometry, tension, lipid organization, or local protein crowding to favor membrane-proximal oligomerization. In this model, membrane engagement would not simply be the terminal consequence of MLKL activation but could help stabilize or amplify a membrane-active state once local MLKL concentration becomes sufficiently high. Membrane curvature can allosterically regulate protein structure and function, raising the possibility that confinement remodels the membrane environment to favor a membrane-competent MLKL



conformation and a threshold sufficient for spontaneous oligomerization and lytic activity.⁸ Whether confinement changes membrane accessibility or lipid organization to facilitate MLKL activity remains an important question.

The implications also extend to how MLKL activation is detected experimentally and in preclinical disease models. Phosphorylation of the MLKL (p-SER345) activation loop has become one of the principal biochemical and histological markers used to infer ongoing necroptosis.⁹ Zhang et al. now provide evidence that MLKL-dependent membrane-lytic death can occur without detectable canonical phosphorylation. If this principle extends to other physiological contexts, phospho-MLKL may identify canonical RIPK3-linked MLKL activation rather than serve as a universal indicator of MLKL involvement in cell death.

In inflammatory settings, STING-driven interferon signaling has been shown to induce ZBP1 and MLKL, thereby establishing a cellular state that permits ZBP1-RIPK3-MLKL-dependent necroptosis independently of TNFR1 and FADD, and without requiring pharmacological caspase inhibition.¹⁰ In that setting, transcriptional priming changes how the necroptotic pathway is accessed, but RIPK3 remains essential. Zhang et al. now reveal a complementary mechanism in which mechanical PIEZO1-Ca²⁺-STAT1 signaling increases MLKL itself to a level at which detectable canonical RIPK3-mediated MLKL phosphorylation is no longer required.

Together, these findings suggest that different initiating stimuli may establish distinct states of necroptotic competence. Mechanical PIEZO1 signaling appears to alter the MLKL abundance sufficiently to enable MLKL-dependent lytic death in the absence of RIPK3. Transcriptional priming may therefore determine not simply whether a cell can undergo necroptosis but which molecular architecture it chooses to use.

Sustained interferon responses can strongly alter the expression of necroptotic components. If MLKL abundance itself can contribute to activation, then biologically relevant MLKL activity may not be restricted to the fraction detectable as phospho-MLKL. This raises

a broader question for interferon biology: under what inflammatory conditions might sufficiently elevated MLKL acquire functions or contribute to cell death in ways that conventional phospho-MLKL measurements fail to capture?

PIEZO1 operates in numerous mechanically challenged cell types, including amoeboid immune cells, such as neutrophils, lymphocytes, dendritic cells, and macrophages. The authors raise the possibility that mechanically induced PIEZO1 signaling could engage MLKL in these additional settings. It remains unclear whether mechanical and cytokine-derived signals cooperate with, compete with, or substitute for one another in determining MLKL abundance and activation thresholds in inflammatory, fibrotic, vascular, or immune-mediated diseases.

In the cancer setting, MLKL activation also precedes terminal membrane rupture. Cells undergoing L-MAT progressively lose polarity and amoeboid motility, whereas MLKL-deficient cells remain migratory. Experimentally induced MLKL activation disrupts polarized cortical actin flow and preferentially restricts movement through strongly confined environments. These findings suggest that MLKL may influence cellular mechanics before its terminal membrane-disrupting activity becomes evident, although the molecular basis of this pre-lytic function remains unresolved.

Finally, spatial transcriptomic analyses suggest that this pathway may impose a selective constraint during tumor progression. MLKL expression is higher in less aggressive tumor regions and reduced in invasive and metastatic regions, while RIPK3 expression remains low and spatially uncoupled from MLKL. These observations are consistent with a model in which cells capable of mounting the confinement-induced MLKL response lose motility or die, whereas MLKL-low cells preferentially persist. However, the human spatial data are correlative and do not establish that PIEZO1-STAT1-MLKL signaling causally selects metastatic clones *in vivo*.

By connecting mechanical confinement to transcriptional control of a pseudokinase executioner, Zhang et al. expand the conceptual boundaries of

necroptosis. Their work suggests that MLKL should perhaps be viewed not simply as the terminal substrate of RIPK3 but as a context-sensitive molecular switch whose activation threshold can be reached through fundamentally different biochemical routes. In doing so, the study raises a broader possibility: the route to regulated necrotic death may be dictated as much by the cellular state established before death as by the acute signal that ultimately triggers it.

DECLARATION OF INTERESTS

The author declares no conflicts of interest.

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