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RESEARCH HIGHLIGHT

DDX3X: stressing the NLRP3 inflammasome

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Stress granules and inflammasomes are intracellular complexes that assemble in response to cellular stress signals, enabling cells to persist and perish, respectively. In a recent study published in *Nature*, Samir et al. identify the helicase DDX3X as a central decision maker in the formation of pro-survival stress granules or pro-death NLRP3 inflammasomes.

Stress granules, formed in response to cell stress stimuli, consist of accumulated messenger ribonucleoproteins (mRNPs), RNA-binding proteins and other non-RNA-binding proteins.[1] These cytoplasmic structures are pro-survival, enabling cells to respond to and withstand changes in homeostatic flux. On the other hand, cytosolic multimeric innate immune signaling complexes known as inflammasomes are also formed in response to certain cellular stress signals. However, inflammasome assembly and activation leads to execution of the programmed cell death pathway of pyroptosis,[2] accompanied by secretion of the inflammatory cytokines, interleukin (IL)-1β and IL-18. The molecular mechanisms governing the crosstalk between two contrasting cell fate pathways had not, until recently, been well understood.

In a recent study published in *Nature*, Samir et al. identified the DEAD-box family member and helicase, DDX3X, as a major contributor to both the formation of stress granules and inflammasome complexes.[3] In mouse bone marrow-derived macrophages (BMDMs) primed with LPS, stress granules are formed following stimulation with the osmotic stress-inducing agent arsenite, whereas inflammasome complexes are formed following stimulation with the ionophore and NLRP3 inflammasome activator, nigericin. A potential crosstalk between the two signaling pathways emerged when the authors found that BMDMs pre-treated with arsenite followed by nigericin preferentially accumulated stress granules and prevented formation of inflammasome complexes (Fig. 1).[3] Arsenite-induced inhibition of inflammasome assembly is reflected by the reduction in the activation of the inflammasome executors caspase-1 and pro-pyroptotic factor gasdermin D, and in the release of IL-1β or IL-18. Arsenite appears to be specific to the inhibition of the inflammasome triggered by NLRP3 activators, but not that by AIM2, NAIP-NLRC4 and Pyrin inflammasome activators.[3]

To elucidate the identity of a potential factor responsible for the crosstalk between stress granule formation and NLRP3 inflammasome activation, the authors employed an affinity purification mass spectrometry-based approach. Using this technique, the helicase DDX3X emerged as a binding partner of NLRP3.[3] The interaction between DDX3X and NLRP3 appears to occur in LPS-primed BMDMs that were left untreated or treated with nigericin (Fig. 1).[3] A strong interaction between DDX3X and the NACHT domain of NLRP3 was found. This finding raises the question of whether DDX3X might interact with the other NLRP3 partner, NEK7, which also binds directly to the NACHT domain of NLRP3.[4–7] The role of DDX3X was further investigated via a genetic approach using a conditional knockout mouse strain lacking DDX3X in the myeloid compartment (called $Ddx3x^{fl/fl}LysM^{cre}$). BMDMs from $Ddx3x^{fl/fl}LysM^{cre}$ mice displayed a reduction in the number of arsenite-induced stress granules as well as abrogated nigericin-induced NLRP3 activation compared to $Ddx3x^{fl/f}$ BMDMs.[3] These data suggest that DDX3X might be a shared commodity between stress granules and the NLRP3 inflammasome (Fig. 1). In addition, stochastic optical reconstruction microscopy (STORM) revealed that DDX3X progressively colocalized with the inflammasome adapter protein ASC in a time-dependent manner, suggesting recruitment of DDX3X to the NLRP3-ASC complex (Fig. 1).[3]

The central requirement of DDX3X in the formation of stress granules and the NLRP3 inflammasome indicates that this helicase provides a molecular switch between survival and death in response to certain stress signals. Indeed, a time course experiment revealed that BMDMs pre-treated with arsenite resisted activation of the NLRP3 inflammasome and pyroptosis, whereas cells stimulated with an NLRP3 activator prior to treatment of arsenite proceeded to pyroptosis unhindered. These data suggest that the timing of the stress signal dictates the cell fate outcome. It is possible that formation of the stress granule might deplete the available pool of DDX3X such that the NLRP3-DDX3X complex cannot be formed even in the presence of a potent inflammasome activator (Fig. 1). What evolutionary advantages would be conferred by stress granule-mediated blockade of the NLRP3 inflammasome? Perhaps stress granules might allow the cell to persist and recover until the pro-death signal disappears. Alternatively, cells with stress granules might delay formation of the NLRP3 inflammasome transiently but will inevitably succumb to pyroptosis over time.

Given the insight into DDX3X as a regulator of cell fate decisions, several additional questions remain to be explored. DDX3X is encoded on the X-chromosome.[8] The related DDX3Y encoded on the Y-chromosome shares 90% homology with DDX3X.[8] The high degree of similarity between these two DDX3 isoforms suggests potential functional redundancy.[8] It would be worthwhile to understand whether sex-specific differences exist in the crosstalk between stress granule formation and NLRP3 inflammasome assembly, considering differences in immune functions between male and female mice in loss-of-function models of DDX3X have been reported.[8] In addition, the crosstalk between cellular stress and NLRP3 inflammasome assembly could be elucidated further. It would be worthwhile to determine whether DDX3X is the sole regulator or whether DDX3X is one of many accessory proteins utilized in this crosstalk. Furthermore,

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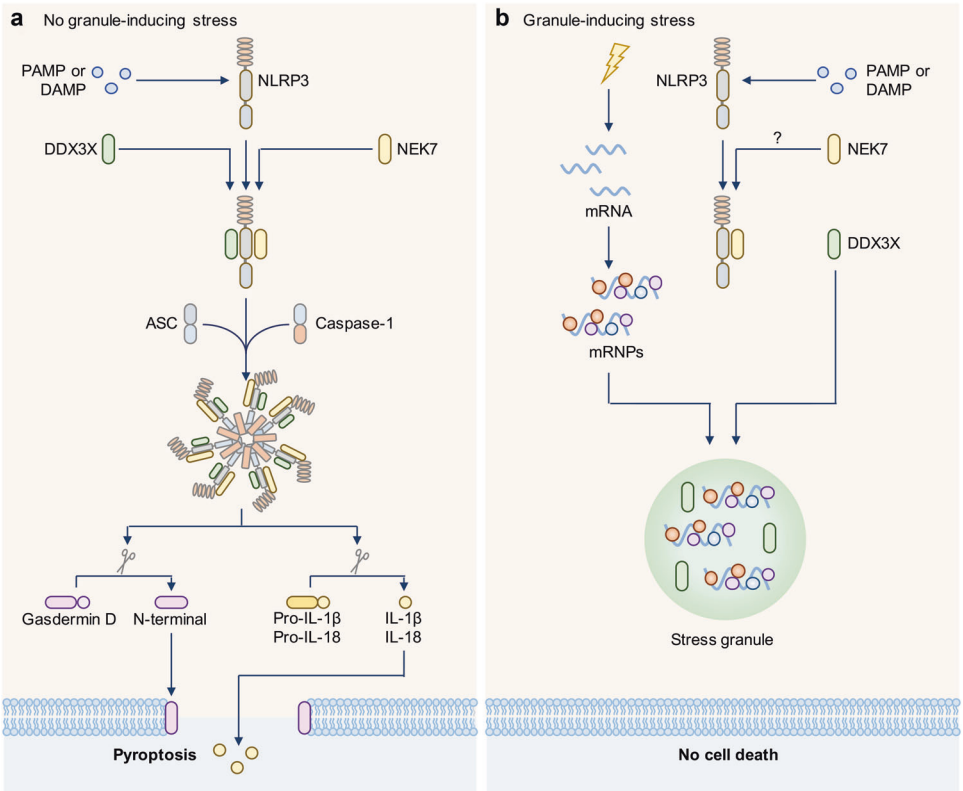

Fig. 1 DDX3X is a building block for the NLRP3 inflammasome and stress granules

DDX3X interacts with both TBK and IKKε and enhances signaling via the IRF3, IRF7 and NF-κB pathways.[8] How exactly DDX3X interprets cellular stress cues to initiate specific signaling outcomes remains to be determined.

The findings by Samir et al. establish DDX3X as a building block for the formation of stress granules and NLRP3 inflammasome complexes in response to cellular stresses. This crosstalk might inform mechanisms of disease manifestation. Aberrations in both the assembly or disassembly of stress granules and NLRP3 inflammasome activation have been linked to neurodegenerative diseases, such as Alzheimer's Disease.[9,10] Investigations into the DDX3X tug of war between stress granules and inflammasomes in neurodegenerative disease might enable discovery of targeted therapeutics.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Protter, D. S. W. & Parker, R. *Trends Cell Biol.* **26**, 668–679 (2016).
2. Shi, J., Gao, W. & Shao, F. *Trends Biochem Sci.* **42**, 245–254 (2017).
3. Samir, P. et al. *Nature* **573**, 590–594 (2019).
4. He, Y., Zeng, M. Y., Yang, D., Motro, B. & Nunez, G. *Nature* **530**, 354–357 (2016).
5. Shi, H. et al. *Nat. Immunol.* **17**, 250–258 (2016).
6. Schmid-Burgk, J. L. et al. *J. Biol. Chem.* **291**, 103–109 (2016).
7. Sharif, H. et al. *Nature* **570**, 338–343 (2019).
8. Szappanos, D. et al. *PLoS Pathog.* **14**, e1007397 (2018).
9. Wolozin, B. *Mol. Neurodegener.* **7**, 56 (2012).
10. Heneka, M. T., McManus, R. M. & Latz, E. *Nat. Rev. Neurosci.* **19**, 610–621 (2018).