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RESEARCH HIGHLIGHT

Heartbreakers: innate sensors ZBP1 and cGAS linked to cardiotoxicity

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As an innate immune sensor, ZBP1 senses infections and cellular damage, triggering the formation of a PANoptosome complex containing caspase-8 and RIPKs that activates the inflammasome, drives inflammatory cell death, and produces proinflammatory cytokines; however, ZBP1's role in mitochondrial damage was not previously known. A recent publication in *Cell* by Lei et al. shows that ZBP1 senses mitochondrial genome instability and forms a complex with cGAS and RIPKs to sustain type I interferon signaling and drive cardiotoxicity.

The innate immune system senses infections and disruptions in cellular homeostasis through pattern recognition receptors (PRRs). PRRs sense pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) and activate intracellular signaling cascades to elicit proinflammatory immune responses and cell death. Inflammation and cell death are distinct yet highly interconnected and mutually regulated processes critical for innate immune responses and organismal homeostasis, but they can also drive pathogenesis.

Cytosolic nucleic acid (NA)-sensing PRRs, such as RIG-I, AIM2, and cGAS, are vital for recognizing endogenous and pathogen-derived RNA and DNA to activate inflammatory immune responses and regulate interferon (IFN) production. The DNA sensor cGAS is critical for inflammation in infection, cellular stress, nuclear or mitochondrial DNA damage, and tissue damage.[1] Recent studies have identified ZBP1 as another critical NA sensor in inflammation. ZBP1 was grouped into a conserved family of Z-DNA binding proteins based on its Z-DNA binding domain, which is expected to bind the unique left-handed helix Z-form of DNA[2] in a structure-specific manner.[3] While ZBP1 was studied biochemically for many years, its potential as an innate immune sensor remained unclear until it was unexpectedly discovered to be the essential sensor for influenza A virus (IAV) to induce NLRP3 inflammasome activation and inflammatory cell death, PANoptosis.[4] It is now known that infection with RNA viruses, including IAV and SARS-CoV-2, as well as homeostatic perturbations[5] can activate ZBP1-dependent innate immune and inflammatory cell death responses (Fig. 1a). Additionally, ZBP1 forms a PANoptosome complex with another NA sensor, AIM2, to drive inflammatory signaling and PANoptosis in response to herpes simplex virus 1 and *Francisella novicida* infections,[6] suggesting that ZBP1 can cooperatively sense aberrant NAs. However, whether ZBP1 can synergize and form complexes with NA sensors beyond AIM2 remained unknown.

A recent study has now identified that mitochondrial genome instability leads to the formation of a ZBP1–cGAS–RIPK1–RIPK3 complex in response to mtDNA leakage, and this complex drives sustained activation of the IFN-I response and IFN-stimulated gene (ISG) production (Fig. 1b).[7] Previously, persistent mitochondrial genome instability caused by a heterozygous mutation of transcription factor A, mitochondrial (*Tfam*$^{+/-}$) was found to induce cytosolic mtDNA release, cGAS-STING activation, and the production of ISGs.[8] Building on these findings, NA sensors beyond cGAS were also found to respond to mitochondrial genome instability. Specifically, ZBP1-dependent production of ISGs was observed in both genetic and pharmacological models of mitochondrial genome instability, including genetic disruption of *TFAM*, loss of DNA topoisomerase III alpha, or loss of the type IB topoisomerase TOP1MT, as well as treatment with the DNA-damaging agent Doxorubicin (DOXO).[7]

ZBP1 contains a Z-DNA binding domain; therefore, it is expected to induce inflammatory responses upon sensing of endogenous or pathogen-derived Z-NAs.[9] When mitochondrial genome instability occurs, B-DNA to Z-DNA transitions can be driven by negative supercoiling, which could enable ZBP1 sensing of Z-DNA to drive the ISG response. While levels of Z-DNA in the mitochondria and cytosol increased during occurrences of mitochondrial genome instability, this was ZBP1-dependent,[7] suggesting that in addition to sensing Z-DNA, ZBP1 also stabilizes Z-DNA generated in response to mtDNA instability. However, experimentally measuring Z-DNA remains challenging, with limited reagents and validation available, and future work will be needed to provide multiple lines of evidence to fully understand the role of Z-DNA as a specific ligand that binds to ZBP1 and its function in mitochondrial genome instability.

Other NA sensors, including cGAS,[8] can also respond to cytosolic DNA, and cGAS activity was increased in response to mitochondrial genome instability.[7] Together, cGAS and ZBP1 potentiated the ISG production in response to mtDNA instability,[7] suggesting a molecular link between their functions in this context. Indeed, ZBP1 and cGAS interacted in the cytosol both in the presence and absence of DNA.[7] Furthermore, the ZBP1 RHIM domains were more important for this interaction than the Zα domains were, and the RHIM-containing proteins RIPK1 and RIPK3 were also present in the cytosolic ZBP1–cGAS complex.[7] The kinase activities of RIPK1 and RIPK3 promoted STAT1 phosphorylation to sustain IFN-I signaling,[7] suggesting that the ZBP1–cGAS–RIPK1–RIPK3 complex was critical for the IFN

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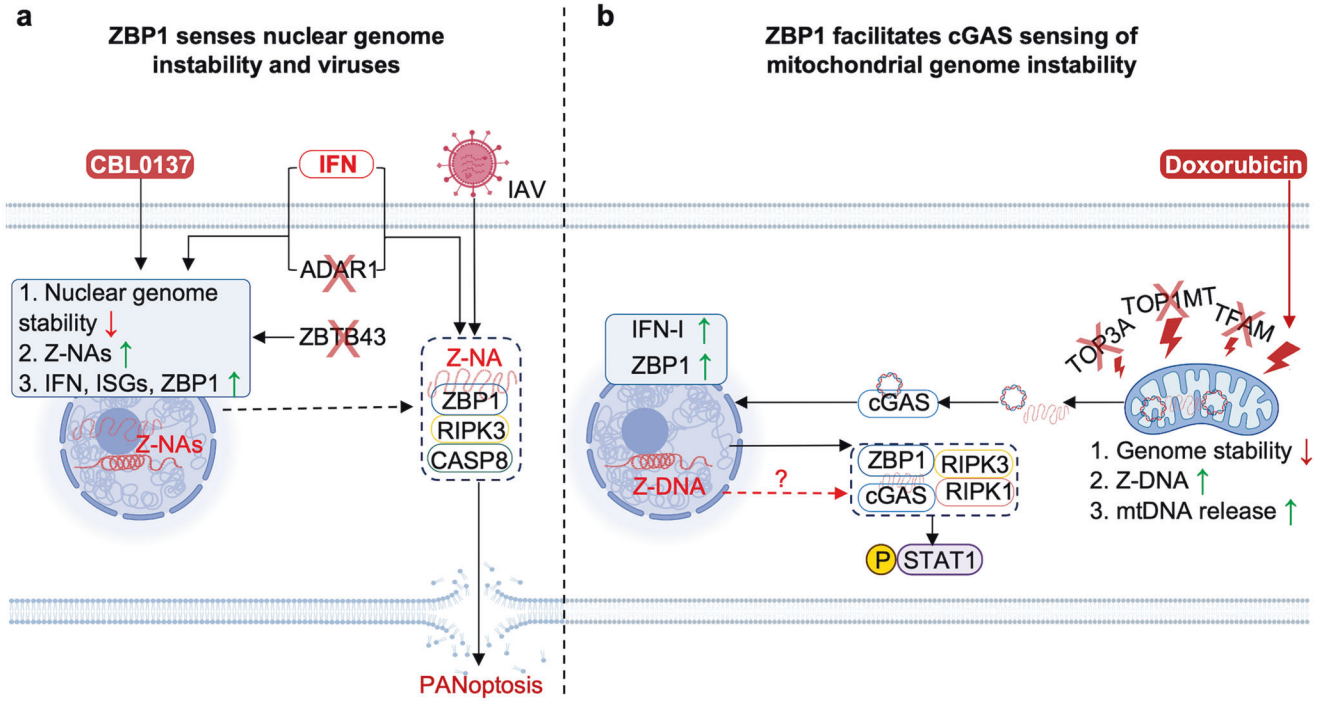

Fig. 1 ZBP1 sensing of infection and nuclear or mitochondrial genome instability. a ZBP1 responds to NAs in the cytosol. Viral infection, such as IAV, induces Z-NA accumulation in the nucleus and cytosol, driving ZBP1-dependent cell death and inflammation. Genetic deficiency of the ZBP1 negative regulator ADAR1 also leads to Z-NA accumulation in the nucleus and cytosol and contributes to the activation of IFN signaling and ZBP1-dependent cell death. Genetic deficiency of *ZBTB43* or exogenous treatment with CBL0137 similarly induce Z-NA accumulation, and CBL0137 triggers ZBP1-dependent cell death. **b** Mitochondrial genome instability causes mtDNA leakage into the cytosol to activate the ZBP1-cGAS signaling pathway and induce the formation of a ZBP1–cGAS–RIPK1–RIPK3 complex to promote STAT1 phosphorylation and sustain IFN-I signaling.

response to mitochondrial genome instability. Given the known connection between ZBP1 and RIPK3 in inducing PANoptosis,[4,5] it is possible that cell death also has a role in this context. While knockdown of *Mlkl* did not reduce the IFN-I response to mitochondrial genome instability,[7] PANoptosis may still be occurring through other cell death executioners, and this requires further evaluation.

The cytosolic formation of the ZBP1–cGAS complex also has physiological implications. In vivo, treatment with DOXO, which mimics clinical DOXO-induced cardiotoxicity (DIC), can induce mitochondrial genome instability.[10] The expression of *Zbp1* and other ISGs was elevated in the heart for weeks after DOXO exposure.[7] Additionally, DOXO triggered a robust ZBP1-, STING-, and IFNAR-dependent cardiac IFN-I response and cardiac dysfunction.[7] Together, these findings suggest that ZBP1 is a critical innate immune regulator of DIC and cardiomyopathy through the formation of the ZBP1–cGAS complex to regulate IFN-I signaling.

Overall, the discovery of a ZBP1–cGAS–RIPK1–RIPK3 complex that responds to stressed mitochondria links mitochondrial damage to IFN production and inflammatory responses. This concept is similar to the formation of other ZBP1 complexes, such as the ZBP1–NLRP3–RIPK3–caspase-8[4] and ZBP1–AIM2–Pyrin–RIPK3–caspase-8[6] complexes, that drive cell death in response to infection and cellular damage. These findings shed new light on how organellar genome instability drives ZBP1-dependent immune responses. In the future, it will be important to understand whether ZBP1 traffics from the cytosol to the mitochondria for early sensing and the extent to which ZBP1-cGAS signaling activation contributes to cell death. An improved understanding of how genome instability drives inflammation and the molecular mechanisms of ZBP1-dependent innate immunity will guide potential treatment options for cardiomyopathy, as well as other mtDNA-mediated inflammatory responses.

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ADDITIONAL INFORMATION

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