RESEARCH HIGHLIGHT

MRE11 mobilizes CGAS and drives ZBP1-dependent necroptosis

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Recent data from Cho et al. demonstrate that the DNA repair protein MRE11 liberates CGAS from nucleosomes to license type I interferon secretion upon oncogenic stress and ionizing radiation. MRE11-mediated CGAS activation culminates in ZBP1-driven necroptosis, which is essential for limiting mammary oncogenesis.

Mammalian cells are endowed with numerous systems that detect infectious threats, including (but not limited to) a variety of receptors that react to exogenous and/or ectopic nucleic acids by promoting cellular and organismal defense responses, which often involve the secretion of antimicrobial and pro-inflammatory cytokines like type I interferon (IFN).[1] While these systems have emerged as part of the pathogen-host co-evolution, abundant preclinical and clinical findings demonstrate that the recognition of immunogenic endogenous RNA and DNA species that are formed (or change subcellular localization) in response to stress is paramount for the preservation of organismal homeostasis, especially in the context of malignant transformation.[2] A proficient response to aberrant or ectopic RNA and DNA species accumulating in cancer cells exposed to common cancer therapeutics (notably DNA-damaging agents such as radiotherapy) also appears to be critical for the elicitation of therapeutically-relevant anticancer immunity.[2] Intriguingly, multiple proteins involved in the DNA damage response (DDR) including the exonuclease MRE11 homolog, double strand break repair nuclease (MRE11) are intimately connected to the sensing of endogenous nucleic acids.[3] Specifically, cytosolic MRE11 promotes type I IFN secretion in response to DNA damage via a stimulator of interferon response cGAMP interactor 1 (STING1)- and interferon regulatory factor 3 (IRF3)-dependent mechanism.[4]

Recent data from Cho and colleagues extend our mechanistic understanding of this process by demonstrating that MRE11 promotes STING1 signaling by displacing the DNA sensor and STING1 activator cyclic GMP–AMP synthase (CGAS) from inhibitory interactions with chromatin. They also demonstrate that the ability of MRE11 to elicit proficient CGAS responses can culminate in the initiation of cell death via Z-DNA binding protein 1 (ZBP1)-driven necroptosis.[5]

Cho et al. established an in vivo CRISPR screen harnessing a transgenic mouse model with conditional alleles for *Mycn* and *cas9* expression and *Trp53* deficiency (*Rosa26*$^{LSL\text{-}Mync/LSL\text{-}cas9}$;*Trp53*$^{fl/fl}$) coupled with the orthotopic injection of Cre-expressing adenoviruses, with the ultimate goal of testing the impact of genetically imposed DDR alterations on CGAS activation. Amongst the top hits of this screen were well-established tumor suppressors such as breast cancer 2, early onset (*Brca2*) and partner and localizer of BRCA2 (*Palb2*), as well as *Mre11*. Following up on the latter hit, the authors found that delivery of an sgRNA specific for *Mre11* significantly shortened tumor latency and resulted in the development of more aggressive mammary neoplasms exhibiting abundant mitotic aberrations, compared to a control sgRNA.[5] These data suggested that MRE11 expression mediates oncosuppressive effects in mouse models of MYCN-amplified TP53-deficient breast cancer.

To gain mechanistic insight into these observations, Cho et al. targeted *Mre11* in primary mouse mammary epithelial cells (pMMECs) established from *Rosa26*$^{LSL\text{-}Mync/LSL\text{-}cas9}$;*Trp53*$^{fl/fl}$ mice, highlighting an expected defect in cell cycle arrest at the G_2/M transition as well as an unexpected impairment in post-mitotic arrest. Additional experiments showed that the inability of *Mre11*-defective pMMECs to exit the cell cycle as competently as their control counterparts was associated with a reduced accumulation of micronuclei and cytosolic CGAS foci, coupled with a reduced expression of several interferon-stimulated genes (ISGs) including interferon beta 1 (*Ifnb1*). Notably, pharmacological STING1 antagonism limited cell cycle exit in control pMMECs, while pharmacological STING1 agonism stimulated it (as well as ISG expression) in *Mre11*-defective pMMECs, suggesting that MRE11 and CGAS function upstream of STING1 activation in this setting. Moreover, *Cgas* or *Sting1* deletion phenocopied the effect of *Mre11* deletion on cell cycle progression, pointing to the existence of an MRE11-CGAS-STING1 signaling axis that regulates proliferation and type I IFN secretion in genomically unstable breast cancer cells.[5]

Cho et al. next demonstrated that MRE11 is required for CGAS activation, STING1 signaling and ISG induction in a variety of cell types transfected with interferogenic nucleic acids or nucleosomal core particles (NCPs), or exposed to radiotherapy. Extending previous findings,[4] both MRE11-binding partners RAD50 double strand break repair protein (RAD50) and nibrin (NBN) — which form the so-called MRE11–RAD50–NBN (MRN) complex — but not MRE11 exonuclease activity, were found to be required for the CGAS stimulation by MRE11, as were MRE11 DNA-binding domains. MRE11 frequently co-localized with CGAS upon transfection with NCPs, suggesting that the MRN complex could directly modulate CGAS signaling, especially as CGAS is inhibited by histone acidic patches (APs),[6,7] and as the MRN complex generally binds to the ends of double-stranded DNA (dsDNA) molecules.[5]

To test this possibility, Cho et al. harnessed time-resolved fluorescence resonance energy transfer (TR-FRET), ultimately

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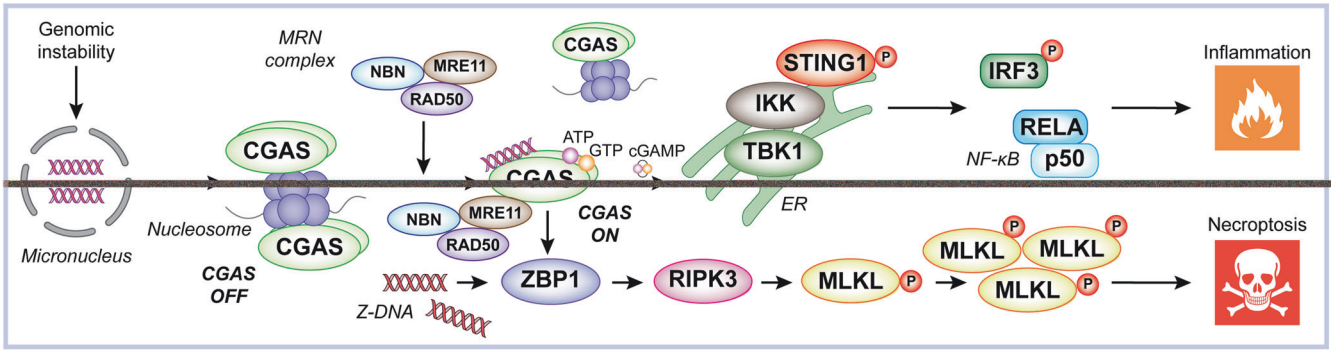

Fig. 1 MRE11 displaces CGAS from inhibitory interactions with nucleosomes. Genomically unstable cells display increased levels of spontaneous micronucleation. Micronuclei are poor CGAS activators due to inhibitory interactions between CGAS and nucleosomes. Recent data indicate that the heteromeric complex formed by MRE11, RAD50 and NBN, which is commonly known as MRN complex, effectively displaces CGAS from nucleosomes. This results in STING1 activation by 2′3′-cyclic GMP–AMP (cGAMP) and the consequent initiation of transcriptional pro-inflammatory programs. Moreover, MRE11 enables the accumulation of Z-DNA in response to genomic instability, ultimately driving ZBP1-dependent necroptosis via RIPK3 and MLKL. ER, endoplasmic reticulum; IKK, inhibitor of κB kinase; RELA, RELA proto-oncogene, NF-κB subunit; p50 (official name: NFKB1), nuclear factor kappa B subunit 1; TBK1, TANK-binding kinase 1.

demonstrating that CGAS binds AP-containing (but not AP-mutated) nucleosomes, and that the MRN complex can displace about half of NCP-bound CGAS molecules. Importantly, the MRN-mediated displacement of CGAS from NCPs restored CGAS activity, irrespective of MRE11 exonuclease activity. Photobleaching experiments in human triple-negative breast cancer MDA-MB-231 cells exposed to a DNA-damaging agent confirmed these observations, pointing to a key role for MRE11 in the displacement of CGAS from inhibitory chromatin patches during the DDR.[5]

To obtain additional insights into the impact of MRE11-driven CGAS signaling on mammary tumorigenesis, Cho et al. compared MRE11-deficient pMMECs to their MRE11-competent counterparts by single-cell RNA sequencing. They found that MRE11-expressing pMMECs, but not *Mre11*$^{-/-}$ pMMECs, manifested the upregulation of multiple ISGs, including *Zbp1*. ZBP1 senses Z-DNA and Z-RNA, the left-handed conformations of dsDNA and dsRNA, respectively, resulting in the activation of receptor interacting serine/threonine kinase 3 (RIPK3) and consequent initiation of mixed lineage kinase domain like pseudokinase (MLKL)-dependent necroptosis.[8]

By contrast to their wild-type counterparts, *Mre11*$^{-/-}$ pMMECs failed to spontaneously accumulate Z-DNA and hence to initiate the ZBP1-dependent cascade leading to MLKL phosphorylation. In line with these findings, genetic disruption of CGAS, ZBP1 or RIPK3 decreased MLKL phosphorylation in MRE11-competent pMMECs, while pharmacological activation of STING1 was able to bypass MRE11 loss and restore MLKL activation in MRE11-defective pMMECs. Moreover, *Zbp1* deletion phenocopied the effects of *Mre11* deletion on cell cycle, overall pointing to the existence of a MRE11-driven ZBP1-mediated signal transduction pathway that is elicited in the context of mammary oncogenesis driven by *MYCN* amplification and TP53 defects. Indeed, high *ZBP1* levels were found to correlate with signatures of immune cell infiltration and improved overall survival in breast cancer patients from The Cancer Genome Atlas (TCGA) and other public transcriptional datasets.[5]

In conclusion, Cho and collaborators have delineated a new molecular cascade connecting components of the DDR to the activation of CGAS and the induction of ZBP1-dependent necroptosis[2] (Fig. 1). Whether this signal transduction mechanism exhibits any degree of crosstalk with mitochondrial DNA sensing by CGAS as elicited by DNA-damaging agents including radiotherapy[9] remains to be elucidated. Similarly, whether the expression levels of adenosine deaminase RNA specific (ADAR), which destabilizes Z-RNA species,[10] limits oncosuppression driven by MRE11 remains to be formally investigated. Despite these and other unresolved issues, preventing CGAS inhibition by nucleosomes (as MRE11 does) stands out as a promising strategy to limit tumor progression and increase tumor sensitivity to a variety of therapies. Additional work will clarify the translational potential of this approach.

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ADDITIONAL INFORMATION

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