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Nuclear waste ignites cGAS

DNA damage can lead to activation of the cytosolic sensor cyclic GMP–AMP synthase (cGAS) and inflammatory gene expression, but it has been unclear how self DNA from the nucleus accesses the cytoplasm. Three independent studies now show that cytosolic chromatin can activate cGAS and trigger an innate immune response following DNA damage.

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Activation of cGAS by DNA leads to the generation of a second messenger, cGAMP, which subsequently induces a type I interferon (IFN) response via the adaptor stimulator of IFN genes (STING). As mutations in nucleases have been associated with cGAS- or STING-dependent inflammatory disease, Mackenzie *et al.* explored whether endogenous DNA damage generates ligands for cGAS. They found that micronuclei (which are small nuclei comprising chromosomal DNA not incorporated into daughter nuclei during mitosis) were increased in frequency in mouse embryonic fibroblasts (MEFs) double-deficient for RNase H2 and the tumour suppressor p53. Micronuclei were also more frequent in *Rnaseh2b*$^{A174T/A174T}$ mice, which are a model for the autoinflammatory disease Aicardi–Goutières syndrome. This suggested that micronuclei may be a source of immunostimulatory DNA. Indeed, experiments using labelled cGAS showed that it associated with micronuclei in *Rnaseh2b*$^{-/-}$ *Trp53*$^{-/-}$ MEFs, irradiated MEFs and cells from a human osteosarcoma cell line. Furthermore, localization of cGAS in micronuclei was associated with the induction of IFN-stimulated genes (ISGs).

As a nuclear envelope normally encloses micronuclei, it was unclear how cGAS accesses DNA in these structures. Mackenzie *et al.* found that most cGAS-positive micronuclei have ruptured nuclear envelopes, and used live imaging to show that cGAS rapidly enters micronuclei following the loss of membrane integrity. It had been suggested that DNA fragments may leak through the nuclear envelope during cell interphase and trigger cGAS. However, as micronuclei are generated during mitosis, the authors hypothesized that such activation of cGAS would be cell-cycle dependent. Confirming this, when DNA-damaged MEFs were arrested in the G0 phase of the cell cycle they did not form micronuclei or activate an innate immune response.

Harding *et al.* set out to address why radiotherapy and chemotherapies take days to activate inflammatory signalling despite inducing double-stranded breaks (DSBs) in DNA within minutes. They noted that DSB-induced activation of the transcription factor STAT1 in irradiated cells from a mammary epithelial cell line (MCF10A) correlated with the presence of misshaped nuclei and micronuclei. However, MCF10A cells that had excessive DNA damage did not show STAT1 activation owing to the activity of cell cycle checkpoints. Similarly to Mackenzie *et al.*, Harding *et al.* found that micronuclei formation correlated with inflammatory gene expression and was dependent on cell-cycle progression. STAT1 can be phosphorylated downstream of cGAS activation and the authors showed that knockout of cGAS or STING reduced the induction of ISGs following cell irradiation. Again in agreement with Mackenzie *et al.*, Harding *et al.* found that cGAS localizes to micronuclei following mitosis and showed that loss of micronuclear envelope integrity is necessary for cGAS to access damaged DNA.

Glück *et al.* were interested in how senescent cells secrete various inflammatory mediators, collectively referred to as the senescence-associated secretory phenotype (SASP). Preliminary experiments suggested that cGAS-deficient cells were protected against cellular senescence. In agreement with this, under conditions of oxidative stress, wild-type MEFs rapidly entered senescence, whereas cGAS- or STING-deficient MEFs showed a compromised senescence phenotype. Further experiments indicated that cGAS is required for the SASP and this seemed to be due, at least in part, to cGAS-dependent induction of type I IFNs.

A common feature of senescent cells is the presence of cytosolic chromatin fragments (CCFs) as a result of nuclear envelope degradation. The authors found that cGAS colocalized with these CCFs in senescent cells and showed that knockdown of the nuclear envelope protein lamin B1 in MEFs induced chromatin leakage into the cytosol that correlated with an elevated ISG response. Finally, the induction of cellular senescence by irradiation or oncogene activation triggered a loss of nuclear envelope integrity and led to cGAS activation.

In each of these studies, the authors point to the importance of cGAS detection of cytosolic chromatin for immune surveillance of cancer cells, in which micronuclei and chromosomal instability are common features. Indeed, using a mouse model of melanoma, Harding *et al.* found that STING signalling was necessary to activate an antitumour immune response to distal tumours following combination therapy with immune checkpoint blockade and radiation.

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ORIGINAL ARTICLES Mackenzie, K. J. *et al.* cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature* http://dx.doi.org/10.1038/nature23449 (2017) | Harding, S. M. *et al.* Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* http://dx.doi.org/10.1038/nature23470 (2017) | Glück, S. *et al.* Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nat. Cell Biol.* http://dx.doi.org/10.1038/ncb3586 (2017)