p53 and Innate Immune Signaling in Development and Cancer: Insights from a Hematologic Model of Genome Instability

Christian Zierhut

Genome instability due to the loss of DNA repair factors can drive developmental defects, autoinflammatory disease, and cancer. Two major signaling pathways are activated by genome instability—DNA damage checkpoint signaling, leading to p53 activation, and innate immunity, largely driven by the DNA sensor cGAS. Like p53, cGAS is thought to drive cell death and senescence during genotoxic stress in addition to its canonical inflammatory functions, but its role during cellular differentiation and carcinogenesis is poorly understood. Furthermore, it is heavily debated whether the cGAS pathway primarily has tumor-suppressive or oncogenic functions. A study in this issue of *Cancer Research* used a hematopoietic lineage-specific knockout of the ribonucleotide repair gene *Rnaseh2b* to introduce genotoxic stress, resulting in severe hematopoiesis defects and increased incidence of hematologic cancers. These two effects were driven by and antagonized by p53, respectively. Surprisingly, despite increased innate immune signaling, the cGAS pathway did not seem to play a role in either process. These findings suggest that innate immune responses to genotoxic stress may be more subtle and context-specific than appreciated, indicating that a more detailed understanding of pathway activation and signaling consequences is needed.

See related article by Dressel et al., p. 2858

The genetic material of every cell is constantly exposed to mutagenic forces, many of which are a consequence of cellular metabolism. To antagonize these forces, elaborate mechanisms exist that reverse these lesions or deal with their consequences in a way that maintains genetic information and genome structure. A major potential source of genome instability is the erroneous incorporation of ribonucleotides during DNA replication (1). If left unrepaired, this can lead to a variety of mutagenic consequences, most dramatically large-scale chromosomal instability. To counteract these effects, ribonucleotide excision repair initiated by the RNaseH2 enzyme complex can remove and replace DNA-incorporated ribonucleotides. Congenital mutations in components of RNaseH2 manifest as Aicardi–Goutières syndrome (AGS), characterized by increased systemic interferon levels and hyperinflammation. The major driver of this inflammation seems to be the innate immune DNA sensor cGAS (2–4), which under normal conditions responds to the DNA of pathogens but under conditions of genotoxic stress may also be activated by self-DNA (4). Several experiments have suggested that chromosomal abnormalities such as micronuclei, single chromosomes, or chromosome fragments that can form their own separate nucleus-like structures may permit cGAS activation by self-DNA during genotoxic stress such as that caused by RNaseH2 deficiency (3, 4).

Given that cancer is a disease promoted by DNA damage and mutations, it is not surprising that mutations in genes encoding DNA damage response factors can drive cancer. Most famously, the p53 tumor suppressor is extensively lost and mutated in cancer. Selective pressure to lose p53 function exists at least in part because its activation by DNA damage signaling can lead to apoptosis as well as a terminal proliferation arrest known as senescence. These fates can also be driven by cGAS (4), leading to suggestions of its possible function as a tumor suppressor. However, cGAS has also been postulated to have oncogenic functions, potentially driving tumor development and metastasis (4, 5). To add to the complexity, cGAS can function both cell intrinsically and cell extrinsically (through cytokine production and signal transfer between cells) within tumor cells as well as nontumor cells within the tumor microenvironment.

To investigate the pathology associated with AGS and to shed light on the consequences of genome instability, Dressel and colleagues generated mice carrying conditional alleles of the RNaseH2 subunit B, allowing its removal within the hematopoietic system (6). This led to an overall drop in blood cells, likely due to the reduced proliferative competence of hematopoietic stem cells (HSC), which the authors showed both when RNaseH2-deficient cells were cultured *in vitro* and when they were used for transplantation into irradiated mice. In searching for a cause for the proliferation defect, the authors noted higher levels of genome instability of *Rnaseh2b*$^{-/-}$ cells, as evidenced by increased γH2AX phosphorylation (a marker for DNA damage) and an increased fraction of micronucleation within erythrocytes and bone marrow cells. Genomically unstable cells are often removed by apoptotic pathways driven by p53, and indeed deletion of a single copy of *Trp53* rescued blood cell counts and allowed *Rnaseh2b*$^{-/-}$ HSCs to repopulate irradiated mice (6).

Genome instability is a double-edged sword; on the one hand, it can drive cell death and senescence, but on the other hand it may drive genetic variability and tumorigenesis. Patients carrying AGS mutations of *RNASEH2B* do not show obvious cancer predisposition, although this may be a consequence of their extremely short lifespan. However, there is some evidence that somatic loss of *RNASEH2B* or non-AGS mutations of *RNASEH2B* may correlate with carcinogenesis, suggesting its potential function as a tumor suppressor (7, 8). The situation is often complicated by the fact that the *RNASEH2B* locus is close to the gene encoding the tumor suppressor RB1, and tumor-associated large deletions in this region frequently cover both genes,

Division of Cancer Biology, Institute of Cancer Research, London, United Kingdom.

Corresponding Author: Christian Zierhut, Division of Cancer Biology, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, United Kingdom. E-mail: christian.zierhut@icr.ac.uk

Cancer Res 2023;83:2807–8

doi: 10.1158/0008-5472.CAN-23-1855

making it difficult to assess whether *RNASEH2B* is a tumor suppressor gene. The clean genetic model generated by the authors allowed them to address this question (6). Indeed, they found that *Rnaseh2b*$^{-/-}$ mice had a dramatically reduced lifespan, which was mostly due to the development of hematologic cancers, although some animals died from other hematologic defects or infection. As expected, p53 loss, while rescuing blood cell counts, accelerated tumorigenesis in *Rnaseh2b*$^{-/-}$ animals. Altogether these data showed that, similar to other tissue-specific models (7), RNaseH2 can act as a tumor suppressor within the hematopoietic system.

The authors next turned to investigate how RNaseH2B deficiency might affect cGAS signaling, and whether cGAS contributed to the observed hematologic defects and carcinogenesis. Surprisingly, loss of neither cGAS nor STING showed any rescue of blood cell counts, and there was no impact on the development of hematologic cancers. How can these findings be reconciled with previous literature suggesting cell death/survival and oncogenic/tumor-suppressive functions of cGAS during genotoxic stress? It is currently unclear how the levels or type of genome instability generated by RNaseH2B deficiency compare with other types of genome instability, such as that caused by ionizing radiation or chromosome missegregation (4, 5). The authors note that not all the observed inflammatory gene induction caused by RNaseH2B loss depended on cGAS (6), suggesting a possible involvement of other innate immune pathways such as RIG-I or Toll-like receptor signaling. However, inflammatory gene expression was largely abrogated by deletion of the type I IFN receptor IFNAR1, and like cGAS or STING knockouts, this did not impact the hematologic defects and carcinogenesis in *Rnaseh2b*$^{-/-}$ animals (6). At present, we do not know whether this finding represents tumor specificity or cell type specificity; the authors note that there is evidence for downregulation of cGAS in HSCs, but at this point, we also do not know whether there are any contributions by signaling within nontumor cells. It is also unclear whether the levels of genome instability caused by RNaseH2B deficiency are not high enough to elicit a robust and functional cGAS response. In line with this hypothesis, hematologic RNaseH2B defects did not cause systemic inflammation. Furthermore, although loss of RNaseH2B in mouse embryonic fibroblasts can lead to inflammatory gene expression via cGAS, single-cell mRNA sequencing and comparison of responses in bulk populations with the maximal pathway activation (achieved by DNA transfection) indicated lower levels of signaling (3). This may not be unique to the type or level of genome instability caused by RNaseH2B loss; other types of genome instability may also result in lower signaling when compared with the maximum achieved by DNA transfection (4, 9). To potentially address this, the authors irradiated HSCs (albeit at a fairly low dose for a single fraction), but they again found no obvious impact from the cGAS pathway to the changes in cellular proliferation *in vitro* or the efficiency of transplantation into irradiated mice. It is possible that, due to the dominance of p53, cells are killed before they achieve a level of genotoxic stress sufficient to activate cGAS, and future work with double mutants may shed light on this. However, this option appears to be unlikely as at least some of the spontaneously occurring cancer cells in *Rnaseh2b*$^{-/-}$ *cGAS*$^{-/-}$ mice were likely to carry *Trp53* mutations.

The potentially low levels of inflammation and low impact of cGAS in the authors' model of genome instability is compatible with the high level of chromosome missegregation that may occur during early mouse development (10), which does not appear to correlate with increased inflammatory signaling. Overall, a major takeaway from this new study is that the contribution of the cGAS pathway to organismal consequences of genome stability may be more complex than we had previously anticipated.

Authors' Disclosures

No disclosures were reported.

Acknowledgments

Work in the laboratory of C. Zierhut is supported by a Cancer Research UK Career Development Fellowship (RCCFEL\100092) and by the Cancer Research UK Radiation Research Centre of Excellence at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust (grant ref: A28724). The author thanks Lauren Davies for critical reading of this article.

Received June 21, 2023; accepted June 23, 2023; published first September 1, 2023.

References

1. Williams JS, Kunkel TA. Ribonucleotide incorporation by eukaryotic B-family replicases and its implications for genome stability. Annu Rev Biochem 2022;91:133–55.
2. Pokatayev V, Hasin N, Chon H, Cerritelli SM, Sakhuja K, Ward JM, et al. RNase H2 catalytic core Aicardi-Goutières syndrome-related mutant invokes cGAS-STING innate immune-sensing pathway in mice. J Exp Med 2016;213:329–36.
3. Mackenzie KJ, Carroll P, Martin C-A, Murina O, Fluteau A, Simpson DJ, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature 2017;548:461–5.
4. Zierhut C, Funabiki H. Regulation and consequences of cGAS activation by self-DNA. Trends Cell Biol 2020;30:594–605.
5. Kwon J, Bakhoum SF. The cytosolic DNA-sensing cGAS-STING pathway in cancer. Cancer Discov 2020;10:26–39.
6. Dressel N, Natusch L, Munz CM, Ramon SC, Morcos MNF, Loff A, et al. Activation of the cGAS/STING axis in genome-damaged hematopoietic cells does not impact blood cell formation or leukemogenesis. Cancer Res 2023;83:2858–72.
7. Kellner V, Luke B. Molecular and physiological consequences of faulty eukaryotic ribonucleotide excision repair. EMBO J 2020;39:e102309.
8. Polaczek R, Schürmann P, Speith L-M, Geffers R, Dürst M, Hillemanns P, et al. Germline variation of ribonuclease H2 genes in ovarian cancer patients. J Ovarian Res 2020;13:146.
9. Mohr L, Toufektchan E, von Morgen P, Chu K, Kapoor A, Maciejowski J. ER-directed TREX1 limits cGAS activation at micronuclei. Mol Cell 2021;81:724–38.
10. Vázquez-Diez C, Yamagata K, Trivedi S, Haverfield J, FitzHarris G. Micronucleus formation causes perpetual unilateral chromosome inheritance in mouse embryos. Proc Natl Acad Sci U S A 2016;113:626–31.