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SAMHD1 and the innate immune response to cytosolic DNA during DNA replication

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Cytosolic DNA of endogenous or exogenous origin is sensed by the cGAS-STING pathway to activate innate immune responses. Besides microbial DNA, this pathway detects self-DNA in the cytoplasm of damaged or abnormal cells and plays a central role in antitumor immunity. The mechanism by which cytosolic DNA accumulates under genotoxic stress conditions is currently unclear, but recent studies on factors mutated in the Aicardi-Goutières syndrome cells, such as SAMHD1, RNase H2 and TREX1, are shedding new light on this key process. In particular, these studies indicate that the rupture of micronuclei and the release of ssDNA fragments during the processing of stalled replication forks and chromosome breaks represent potent inducers of the cGAS-STING pathway.

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Introduction

The innate immune system is the first line of host defense against a broad range of pathogens, such as viruses, bacteria, fungi and parasites. It acts by directly sensing pathogens-associated molecular patterns through a set of pattern recognition receptors that stimulate downstream signaling cascades leading to the production of type I interferons (IFNs) and other pro-inflammatory cytokines. This system operates on the premise that no free DNA should be present in the cytosol and therefore recognizes cytosolic DNA as non-self [1]. This DNA is recognized by a variety of nucleic acids sensors that converge on STING (STimulation of Interferon Genes) to transactivate innate immune response genes [2]. A key player in this process is the cyclic GMP-AMP synthase (cGAS), which binds double-stranded DNA (dsDNA) in a sequence-independent manner and produces cyclic-GMP-AMP. This second messenger binds STING and induces TBK1 activation, IRF3 phosphorylation and induction of type I IFNs and other cytokine genes [1,2].

Besides pathogens, the innate immune system also responds to tissue damage by sensing damage-associated molecular patterns, including cytosolic DNA of endogenous origin. The cGAS-STING pathway is activated by cytosolic DNA accumulating after ionizing radiation [3] or exposure to a variety of chemotherapeutic agents targeting DNA replication forks [4–6]. DNA sensing pathways act as a double-edged sword in cancer development in a context-dependent manner. On the one hand, the cGAS-STING pathway contributes to the elimination of abnormal cells by different means, including induction of senescence and senescence-associated secretory phenotype (SASP) [7••,8•,9•], the upregulation of immunoreceptor ligands of natural killer cells [5,6,10] and the stimulation of adaptive immune responses [11,12]. On the other hand, the chronic induction of DNA sensing pathways by genomic instability promotes metastasis in cancer cells in a STING-dependent manner [13••].

The cGAS-STING pathway is not only triggered by genotoxic agents, but also by endogenous sources of DNA damage, such as telomere shortening and oncogene-induced DNA damage [14,15,16••]. This pathway is frequently suppressed in human cancers, enabling malignant cells to escape immune surveillance [15,17]. This is reminiscent of the inactivation of p53 and other DNA damage response (DDR) factors in response to oncogene-induced DNA damage [18]. Since innate immunity and DDR pathways share multiple components [19], it is likely that these processes cooperate to prevent tumorigenesis [20•]. However, the interplay between DDR factors and the cGAS-STING pathway remains poorly characterized at the molecular level. Here, we discuss recent findings on three genes frequently mutated in human interferonopathies that shed new light on the mechanism by which DNA damage activates the interferon response.

Insights from the Aicardi-Goutières syndrome

The cGAS-STING pathway is constitutively activated in a rare Mendelian disorder called the Aicardi-Goutières

syndrome (AGS) and in other related interferonopathies, which are characterized by a chronic upregulation of type I IFNs [21,22]. AGS is caused by germline mutations in genes encoding factors involved in nucleic acids metabolism, such as TREX1, RNase H2 and SAMHD1.

TREX1 (Three Prime Repair Exonuclease 1) is a major 3′-5′ exonuclease that is anchored to the outer face of the nuclear membrane and degrades both single-stranded and double-stranded DNA (ssDNA and dsDNA), although it preferentially binds ssDNA [4,23,24]. *Trex1*−/− mouse embryonic fibroblasts (MEFs) accumulate cytosolic DNA of endogenous origin, including endogenous retroelements, and show a chronic activation of type I IFNs [4,25]. Importantly, TREX1 regulates radiotherapy-induced tumor immunogenicity by controlling the amount of IFN-stimulatory DNA present in cancer cells [26•,27•] and prevents SASP [9•].

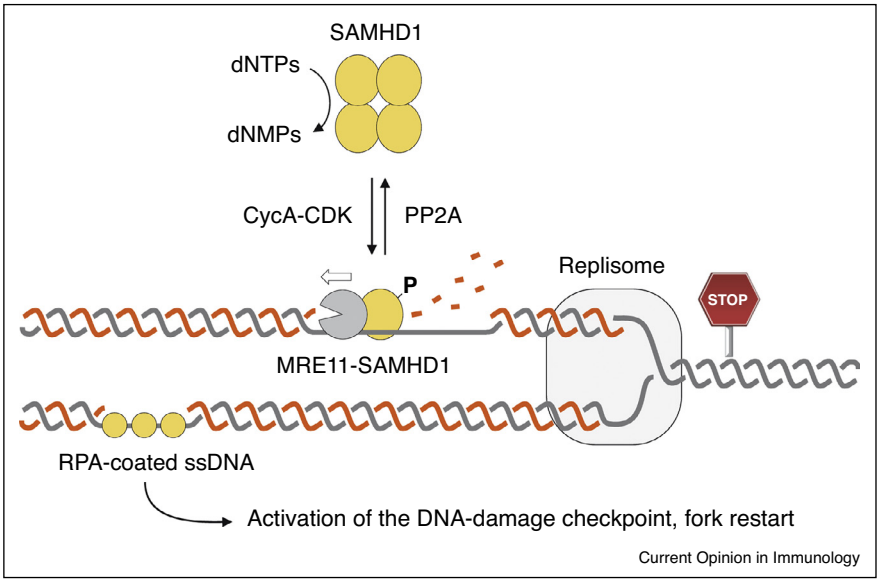
RNase H2 plays a central role in the removal of ribonucleotides misincorporated into DNA during DNA replication and in the resolution of co-transcriptional RNA:DNA hybrids [28]. RNase H2-deficient cells display spontaneous replication stress and accumulate DSBs in a Top1-dependent manner [29,30]. Moreover, recent evidence indicate that RNase H2-deficient cells accumulate micronuclei, which eventually become permeable to cGAS and trigger IFN production [31•,32••]. These micronuclei could result from the persistence of unrepaired DSBs during mitosis, as recently reported in gamma-irradiated cells [33••].

SAMHD1 (sterile alpha motif and HD-domain containing protein 1) is a restriction factor for HIV-1 infection and a deoxynucleoside triphosphate (dNTP) hydrolase that depletes intracellular dNTP pools in non-cycling cells [34,35]. SAMHD1 is also recruited to DNA repair foci in response to DNA damage [36,37•] and plays a key role in DSB repair by promoting the resection of DNA ends [37•,38••]. SAMHD1 is mutated in 17% of AGS patients [39] and *Samhd1* −/− mice show a chronic induction of type I IFNs in a cGAS-dependent and STING-dependent manner [40]. Together, these data suggest that SAMHD1 plays a unique function at the interface between DNA repair and inflammation through the regulation of dNTP pools and/or the processing of chromosome breaks.

SAMHD1 and the replication stress response

DNA replication is a complex process depending on the coordinated activation of thousands of replication origins and the unconstrained progression of replication forks, through the action of DNA polymerases, helicases and accessory proteins forming the replisome [41]. This process is frequently challenged by events of endogenous or exogenous origin, collectively referred to as DNA replication stress, leading to replication fork slowing or stalling (Figure 1). Stalled forks are detected by the ATR checkpoint kinase, which binds RPA-coated ssDNA and

Figure 1



Dual role of SAMHD1 during DNA replication. In G_1, SAMHD1 forms a tetramer and acts as a dNTP hydrolase. This function is important to maintain balanced dNTP pools. Upon entry into S-phase, the phosphorylation of SAMHD1 by CycA-CDK on T592 activates a novel role of SAMHD1 in the stimulation of the exonuclease activity of MRE11. This function is important for the resection of nascent DNA strands (orange) on both strands of stalled replication forks. This resection activity generates ssDNA gaps that are covered by the ssDNA-binding complex RPA and that are important for the activation of the DNA damage checkpoint and for replication resumption.

activates the effector kinase CHK1 to prevent premature entry into mitosis and promote replication resumption [30,42].

Depleted or imbalanced dNTP pools represent a common source of replication stress, caused by genotoxic drugs or by mutations affecting dNTP metabolism [30]. Since SAMHD1 plays a major role in this process, efforts have been made to monitor the impact of SAMHD1 mutations on DNA replication. SAMHD1-depleted cells show deregulated dNTP pools, impaired replication fork progression and increased mutagenesis [38••,43,44]. However, the regulation of dNTP pools is not the only function of SAMHD1 during the S-phase of the cell cycle. Indeed, it has been recently reported that SAMHD1 interacts physically with the MRE11 nuclease and stimulates its exonuclease activity [38••]. MRE11 is recruited to stalled forks to promote the degradation of nascent DNA strands through its 3′-5′ exonuclease activity [45]. This activity is impaired in SAMHD1-depleted cells, which limits the formation of RPA-coated ssDNA, activation of the ATR−CHK1 pathway and fork restart [38••]. This function of SAMHD1 at stalled forks is reminiscent of its role in DNA end resection [37•].

SAMHD1 is phosphorylated on T592 by the cyclin-dependent kinases CDK1 and CDK2 associated with cyclin A2 [46] and is dephosphorylated at the M/G1 transition by the PP2A-B55α phosphatase [47]. Phosphorylation of T592 leads to the dissociation of SAMHD1 tetramers upon entry into S phase and was proposed to downregulate its dNTPase and/or HIV-1 restriction activities [48–51]. Since the phosphorylation of T592 is also required to activate the MRE11-dependent resection activity of SAMHD1, it may act as a switch between the nuclease and dNTPase functions of SAMHD1 (Figure 1). In the absence of SAMHD1, cells are therefore exposed to a significant level of endogenous replication stress resulting from both a deregulated pool of dNTPs and a reduced ability to signal and repair arrested forks. This may explain why SAMHD1 is mutated in chronic lymphoid leukemia (CLL) and in other cancers [36].

SAMHD1 prevents the accumulation of cytosolic ssDNA

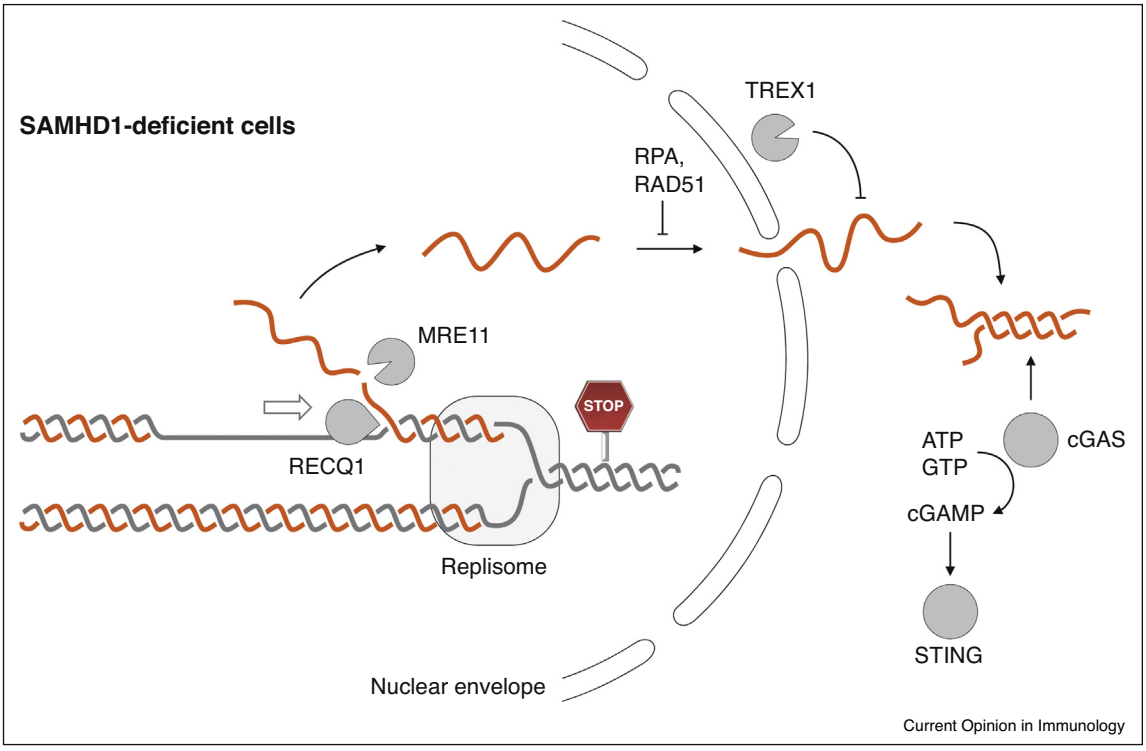
Different sources of replication stress induce the accumulation of cytosolic DNA and the activation of the cGAS-STING pathway [6,12] but the mechanisms involved have remained unclear. In Xenopus egg extracts, the processing of DSBs by the MRE11 nuclease generates ssDNA oligos that are also detected in human cells exposed to ionizing radiation and could represent byproducts of DNA end resection [26•,52]. Moreover, depletion of the ssDNA binding protein RAD51 and of factors regulating DNA end resection results in the accumulation of cytosolic ssDNA and the activation of innate immune signaling [24,26•,53]. Since SAMHD1 promotes resection at both DSBs and stalled forks, an attractive possibility could be that SAMHD1-deficient cells activate the cGAS-STING pathway because of the aberrant processing of stalled forks. This view is supported by the fact that, in the absence of SAMHD1, nascent DNA is displaced by the RECQ1 DNA helicase, leading to the formation of a flap that can be cleaved by the endonuclease activity of MRE11 [38••]. The resulting ssDNA fragments would eventually accumulate in the cytosol and activate the cGAS-STING pathway (Figure 2). This model is consistent with the fact that the depletion of RECQ1 with siRNAs or the inhibition of MRE11 with Mirin prevents the accumulation of cytosolic ssDNA in SAMHD1-deficient cells [38••]. However, it is worth noting that this study was mostly performed using transformed cells lines, which often display a downregulation of STING expression [15,17,54]. An important question that remains to be addressed is whether replication stress also contributes to chronic inflammation in primary cells and in AGS patients. A *Samhd1* morphant zebrafish model recapitulating the brain features of AGS [55] could be instrumental to address this question, unlike *Samhd1* knockout mouse models that lack brain phenotypes [56].

Another unanswered question is how cGAS senses cytosolic ssDNA in SAMHD1-depented cells, considering that it preferentially binds to dsDNA [1,2]. Since fork resection operates on both DNA strands and since repetitive DNA sequences are abundant in the human genome, it could be that ssDNA fragments reanneal in the cytosol to form dsDNA duplexes that are long enough to activate cGAS [57,58]. Alternatively, other sensors than cGAS could be involved in the detection of ssDNA fragments resulting from the processing of DSBs and stalled forks. This is consistent with the fact that the induction of type I IFNs in SAMHD1-depleted cells was only partially reduced in the absence of cGAS [38••]. A non-canonical IFI6-STING pathway inducing type I IFN production in response to DNA damage has been recently reported [59••] and could act in parallel with the cGAS-STING pathway to signal genotoxic stress.

Conclusion and perspectives

The recent characterization of the molecular function of several factors mutated in the Aicardi-Goutières syndrome has shed new light on the connections between the DNA damage response and innate immunity in a pathological context. The next challenge is to understand how these processes operate in primary cells. The picture that emerges from the different studies mentioned above is that self-DNA of different origins (retrotransposons, damaged mitochondria, DNA repair byproducts . . .) is kept under check in the cytosol by the TREX1 nuclease.

Figure 2



Aberrant fork processing in SAMHD1-deficient cells. In the absence of SAMHD1, nascent DNA is displaced by the RECQ1 helicase and is cleaved by the endonuclease activity of MRE11, leading to the accumulation of cytosolic ssDNA fragments. Despite the presence of the TREX1 nuclease, these fragments reanneal and activate the cGAS-STING pathway. Intriguingly, the RECQ1-dependent displacement of nascent DNA strands does not activate the DNA damage checkpoint to the same extent as MRE11-dependent fork resection.

Under replication stress conditions, altered fork processing and/or chromosome missegregation could increase the accumulation of cytosolic DNA, overwhelming the buffering capacity of TREX1. Intriguingly, the presence of cGAS in the nucleus of cycling cells has recently been reported [8•,60••]. How cells prevent cGAS from sensing nuclear DNA is currently unknown, but it may involve its titration by circular RNA species, as recently found in long-term hematopoietic stem cells [60••]. Another challenge is to determine whether nuclear ssDNA fragments passively diffuse into the cytosol or whether this process is regulated. Interestingly, it has been recently reported that RPA and RAD51 sequester ssDNA in the nucleus [24]. Since RPA is present in limiting amount and is rapidly mobilized by stalled forks under severe replication stress conditions [61], it is tempting to speculate that RPA exhaustion helps the immune system detect cells with a heavily damaged genome by increasing cytosolic DNA and activating the STING pathway. Along the same line, many genotoxic agents classically used as chemotherapeutic agents increase tumor immunogenicity by increasing T cell influx and sensitizing tumors to immune checkpoint inhibition [62,63]. A better understanding of the mechanisms by which genotoxic agents induce type I IFNs and trigger immunogenic cell death should therefore promote the development of more efficient anticancer treatments combining the effects of chemotherapy and immunotherapy.

Conflict of interest statement

Nothing declared.

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