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Innate Immune Sensing and Signaling: Co-opted for Genome Surveillance? *Implications for Tumorigenesis*

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Abstract

Innate immune signaling is traditionally associated with the response to pathogenic infection. However, emerging evidence suggests that nuclear innate immune sensors and their downstream pathways may also serve as a critical mechanism for genome surveillance. This review explores a model in which DNA sensors such as mouse IFI204 and IFI205 (IFI16 in humans) localize to replication forks, where they detect endogenous aberrant DNA structures and initiate an interferon-stimulated gene (ISG) transcriptional program. A key output of this transcriptional program is ISG15, which we find conjugated to fork-associated proteins and facilitates recruitment of the replication fork protection complex, thereby stabilizing replication forks under physiological conditions. We discuss how nuclear innate immune sensors mediate replication stress sensing and examine the broad consequences of downstream ISG transcription across diverse contexts—including its impact on genome stability and its dual roles in modulating tumor cell behavior and the tumor microenvironment. These findings suggest that the innate immune system, through its nuclear DNA sensing arm, may be evolutionarily co-opted for genome surveillance and may influence tumor initiation and therapy resistance. Understanding how innate immune signaling intersects with replication stress could offer mechanistic insights into tumor development and reveal novel therapeutic targets.

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Hexiao Wang: Writing – review & editing, Writing – original draft, Funding acquisition. **John HJ Petrini:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

In this review article, we will present evidence that innate immune sensors and effectors promote the fidelity of DNA replication by effecting replication fork surveillance. Our model posits that nuclear innate immune DNA sensors are constitutively associated with the DNA replication fork. Thus situated, they tonically—meaning steadily, at a low level, and without an acute trigger—activate a low-level interferon-stimulated gene (ISG) transcriptional program upon detection of aberrant DNA structures that arise sporadically (and rarely) during normal DNA replication. A key output of the ISG program is the ubiquitin like modifier interferon-stimulated gene 15 (ISG15). We found that ISG15 is conjugated to replication fork constituents with known roles in DNA replication, and we have shown that recruitment of the replication fork protection complex to stressed forks, comprising Timeless, Tipin, Claspin, and TopBP1, is compromised in the absence of ISG15. Accordingly, ISG15 deficiency results in tonic DNA replication stress, typified by checkpoint kinase 1 (CHK1) activation, a high frequency of replication fork collapse, DNA bridges between daughter cells, and sensitivity to Aphidicolin [1].

The ISG transcriptional program is chronically induced in breast epithelial cells undergoing persistent genomic instability associated with a hypomorphic mutation in the meiotic recombination 11 homolog A (MRE11) complex, $Mre11^{ATLD1}$ [2, 3]—or potentially in other genome maintenance factors such as breast cancer type 1/2 susceptibility proteins (BRCA1/2). The ISG transcriptional program is associated with changes in the chromatin landscape of the affected cells and renders them susceptible to oncogene (HER2) induced breast cancer [4].

The underlying mechanism(s) of that susceptibility remain an open question. Available evidence indicates that heterochromatic changes triggered by oncogene activation depend on both the MRE11 complex and p53 and are essential for tumor suppression. Disruption of the MRE11 complex-p53 axis suppresses the deposition of heterochromatic marks and renders mammary epithelium highly susceptible to oncogene induced breast cancer [5]. We will discuss the relevant innate immune sensors and effectors with a focus on their implications in genome integrity. Their roles in innate immunity have been extensively reviewed and we have directed the reader to relevant reviews where appropriate.

2. Innate immune signaling and sensors

Innate immunity is an evolutionarily conserved defense mechanism that responds to both external and internal signals. The response is initiated when pattern-recognition receptors (PRRs) detect conserved molecular signatures—pathogen-associated molecular patterns (PAMPs) from microbes or damage-associated molecular patterns (DAMPs) released by injured cells. Ligand binding triggers PRRs to recruit or activate adaptor proteins, such as myeloid differentiation primary response 88 (MyD88), TIR-domain–containing adaptor-inducing interferon-β (TRIF), mitochondrial antiviral signaling protein (MAVS), and stimulator of interferon genes (STING), initiating downstream signaling cascades that activate the transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interferon regulatory factor 3/7 (IRF3/7), and activator protein 1 (AP-1).

These factors drive expression of pro-inflammatory cytokines and ISGs, which together establish an antiviral and antipathogen state and coordinate the early immune response [6, 7].

PRRs are classified into six major families based on structure and domain functions, with each family characterized by distinct ligand specificity, localization, and signaling pathways. These include Toll-like receptors (TLRs) [7–10], NOD-like receptors (NLRs) [11–15], RIG-I-like receptors (RLRs) [16], C-type lectin receptors (CLRs) [17], AIM2-like receptors (ALRs) [18–20], and OAS-like receptors (OLRs) [21, 22]. A summary of their key features is provided in Table 1.

The outcomes of PRR activation depend on both the identity of the ligand and its subcellular localization, which together determine the specific receptors engaged and the downstream signaling cascade activated. Nucleic acids are particularly important ligands. They may be exogenous (pathogen-derived) or endogenous (arising from cellular injury or dysregulation). Regardless of origin, nucleic acids in inappropriate compartments serve as danger signals. Endosomal TLRs detect nucleic acids within endosomes [8], whereas cytoplasmic RNA and DNA are sensed by RLRs (e.g., retinoic acid-inducible gene I (RIG-I)) and OLRs (e.g., cyclic GMP-AMP synthase (cGAS)), respectively. Both RLR and cGAS engagement trigger a type I interferon (IFN)–mediated antiviral response [16, 23]. Additionally, cytoplasmic DNA sensed by absent in melanoma 2 (AIM2) activates the inflammasome, leading to maturation of interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) [20].

In addition to the six canonical PRR families, several other nucleic acid sensors have been described in innate immune signaling. One such example is Z-DNA binding protein 1 (ZBP1; also known as DAI or DLM-1). ZBP1 has recently been shown to induce necroptosis through receptor-interacting protein kinase-3 (RIPK3), particularly during viral infection and replicative stress [24–27]. In the context of dysfunctional telomeres, ZBP1 is upregulated downstream of cGAS–STING and becomes activated upon binding telomeric-repeat-containing RNA (TERRA), leading to MAVS-dependent signaling and amplification of the immune response [27].

3. Nuclear innate immune sensing

For the most part, research on nucleic acid sensing has been focused on cytosolic pathways, since cytoplasmic nucleic acids are the clearest indicators of infection or damage. However, several innate immune receptors are operative in the nucleus. For example, the heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1) is a recently characterized nuclear DNA sensor that recognizes viral DNA and initiates type I IFN production. Discovered in a screen for nuclear sensors of HSV-1 infection, hnRNPA2B1 binds viral DNA in the nucleus, undergoes homodimerization and demethylation, then translocates to the cytosol to activate the TBK1–IRF3 pathway and drive IFN-α/β expression [28].

Interferon-inducible protein 16 (IFI16) is a nuclear DNA sensor belonging to the ALR family [29]. It possesses an N-terminal pyrin domain and two C-terminal hematopoietic expression, interferon-inducible nature, and nuclear localization (HIN) domains with

oligonucleotide-binding (OB) folds that recognize single- and double-stranded DNA (ssDNA and dsDNA) in a sequence-independent manner [30]. Unlike AIM2, which functions primarily in the cytosol, IFI16 contains an N-terminal nuclear localization signal (NLS) and localizes to the nucleus to detect pathogenic DNA [31, 32]. Upon sensing HSV-1 DNA, IFI16 translocates to the cytoplasm, recruits STING and activates TBK1–IRF3 and NF-κB signaling to induce IFN responses [33–35].

In addition to DNA sensors, nuclear RNA receptors have been described. Scaffold attachment factor A (SAFA) binds viral RNA in the nucleus to promote antiviral gene expression [36]. RIG-I, traditionally viewed as a cytosolic RNA sensor, can shuttle into the nucleus to detect influenza A virus RNA [37]. Moreover, ZBP1 recognizes Z-form RNA structures during viral infection and triggers necroptosis, contributing to innate immune defense [24].

How do nuclear receptors distinguish self-DNA/RNA from non-self pathogenic nucleic acids? The nuclear DNA sensor IFI16, for example, recognizes DNA in a sequence-independent manner, and current evidence does not support a preference for specific DNA structures [18, 38]. This suggests that detection of aberrant DNA necessarily in its natural cellular compartment is likely to be more complex than detecting cytosolic nucleic acids. It is conceivable that the length or accessibility of DNA serves as a discrimination cue [39, 40]. Whatever the basis for recognition may be, nuclear DNA sensors effectively initiate the response to viral DNA in the nucleus. In this regard, it must be considered that nuclear DNA innate immune sensors are able to engage aberrant structures that ensue from DNA replication stress and genetic contexts associated with genomic instability. For example, structures such as extended single-stranded DNA at stalled replication forks, R-loops, or extrachromosomal fragments generated by damage may all expose DNA in ways that can be recognized by nuclear innate immune receptors (Figure 1). Such engagement can initiate a DNA damage response (DDR) that contributes to maintenance of genome stability. Supporting this idea, hnRNPA2B1 modulates RPA dynamics during normal DNA replication and in cells experiencing genotoxic stress [41]. Similarly, unlike its cooperation with cGAS in recognizing cytosolic DNA [34, 42], IFI16 can also activate STING in a cGAS independent manner following nuclear DNA damage and promote downstream NF-κB signaling [19].

Although the mechanisms underlying the relationship between innate immune receptors and the DDR remain to be fully defined, their localization to replication forks and specificity for DNA position them ideally for replication fork surveillance. It remains an open “chicken vs. egg” question whether genome surveillance or the response to pathogenic infection came first during evolution.

4. Nuclear DNA Sensing for Replication Stress

To fulfill the role of replication fork surveillance, nuclear DNA sensors must localize to the replication fork. Supporting this, we recently found that hnRNPA2B1, along with mouse IFI204 (interferon activated gene 204) and IFI205 (interferon activated gene 205) —ALR family members related to human IFI16—are present at the DNA replication

fork in mouse embryonic fibroblasts (MEFs), as shown using Isolation of Proteins on Nascent DNA (iPOND) experiments. Moreover, IFI205 and IFI204 are further enriched at stressed replication forks [1]. These genes are themselves interferon-stimulated and can be transcriptionally upregulated following their activation as sensors at the replication fork. This establishes a positive feedback loop that facilitates the continued recruitment of these sensors, together with other downstream ISGs—such as ISG15—to the replication fork, thereby amplifying signaling and modulating fork stability. The signaling cascade initiated by these sensors is analogous to canonical innate immune pathways. Within this framework, the localization and functional attributes of these sensors strongly support their role as receptors operating at the replication fork.

Consistent with previous reports showing IFI205 mediates interferon signaling [43], our recent study found that IFI205 drives a chronic ISG transcriptional program in mouse mammary organoids with genomic instability due to deficiency in the MRE11 complex (*Mre11*$^{ATLD1/ATLD1}$). This phenotype depends on the DNA-binding capacity of IFI205, supporting its role in direct DNA sensing. These primary mammary epithelial cells are cultured without immortalization, preserving the intact functions of p53, p19^{ARF}, and p16^{INK4A}. Micronuclei and cytosolic double-stranded DNA are not significantly elevated in *Mre11*$^{ATLD1/ATLD1}$ organoids [4]. Consequently, cGAS is not a major contributor to ISG induction in this context. This is consistent with a recent study demonstrating that the MRE11 complex is required to displace cGAS from acidic-patch-mediated sequestration, thereby enabling its mobilization and activation by cytosolic dsDNA [44]. Hypomorphic mutations in *Mre11* thus impair cGAS activation in response to cytosolic dsDNA, further supporting the conclusion that DNA damage signaling in *Mre11*$^{ATLD1/ATLD1}$ organoids originates primarily from the nucleus. Despite involving distinct upstream sensors, *Sting* knockout in *Mre11*$^{ATLD1/ATLD1}$ organoids closely phenocopies *Ifi205* knockout in *Mre11*$^{ATLD1/ATLD1}$ organoids, suggesting that IFI205 may signal through STING after translocation to cytoplasm, consistent with previous reports on IFI16 [19].

In addition to its well-established role in sensing cytosolic DNA, cGAS has also been demonstrated to regulate replication dynamics within the nucleus. A fraction of cGAS associates with chromatin and interacts with replication fork proteins in a DNA binding–dependent manner. This chromatin-associated pool of cGAS acts independently of cGAMP and STING to decelerate replication forks, thereby maintaining fork stability and preventing replication-associated DNA damage. Loss of cGAS results in uncontrolled DNA replication, fork acceleration, genomic instability, and heightened sensitivity to genotoxic stress, revealing a noncanonical function of cGAS at the replication forks [45].

Although molecular, biochemical, and genetic evidence support the involvement of some innate immune sensors in sensing replication stress, the precise DNA structures they recognize and bind during damage remains unknown. Future studies are needed to reveal a more comprehensive understanding of its mechanism and to discover other previously under characterized nuclear DNA sensors involved in genome surveillance.

5. ISG transcription in non-infectious setting

A wide array of innate immune sensors detect diverse pathogenic and endogenous ligands, yet their downstream signaling converges on a limited set of core pathways, including mitogen-activated protein kinase (MAPK)/p38/Jun N-terminal kinase (JNK), transforming growth factor beta-activated kinase 1 (TAK1)/NF-κB, and IRF3/7 [6, 7]. These pathways ultimately trigger the transcription of ISGs, forming a central axis of the innate immune response.

ISGs are traditionally defined as genes that are upregulated in response to IFN stimulation [46]. To date, hundreds of ISGs have been identified across various cell types [47–49], with some studies suggesting that approximately 10% of the human genome is subject to IFN regulation [50]. While thresholds for classification differ among studies, ISGs represent a large and diverse group of genes with broad biological functions.

Despite their name, ISGs are not solely downstream of IFNs. As described above, they are commonly induced by a wide array of innate immune pathways, including those independent of IFN-α/β, IFN-γ and IFN-λ *per se* [51]. This is especially relevant in proliferating epithelial cells, which do not secrete high levels of IFNs. In such contexts, DNA damage becomes an important physiological trigger for ISG expression in the absence of infection [52, 53]. These ISGs can exert significant effects, beyond classical anti-viral defense, on both non-malignant and malignant cells [54].

5.1 Impact of ISG transcription on tumorigenesis and cancer therapy

ISG transcription has an important role in regulating cellular responses to DNA damage, as increasingly demonstrated by research in cancer. This function underscores the proposed dual nature of IFN signaling: acute, robust IFN responses activate cytotoxic pathways and promote tumor clearance, whereas chronic, low-level IFN signaling—marked by sustained ISG transcription—has been suggested to drive immune suppression, stromal remodeling, and therapeutic resistance. The duration, magnitude, and cellular origins collectively determine the functional consequences of ISG transcriptional programs in cancer [55].

During the acute phase of IFN signaling, cytotoxic ISGs are rapidly induced and contribute to cell death [55–57]. The underlying mechanism involves the activation of apoptotic pathways, and several ISGs have been directly implicated in apoptosis regulation. Examples include family with sequence similarity 14 (FAM14) [58], interferon-stimulated gene 54 (ISG54) [59], inositol hexakisphosphate kinase 2 (IP6K2) [60, 61], AIM2 [62], IFI16 [63], NEDD8 ultimate buster 1 (NUB1) [64], signal transducer and activator of transcription 1 (STAT1) [65].

Beyond their cell-intrinsic effects, ISGs also influence the microenvironment, which is particularly relevant in the context of cancer therapies such as irradiation and chemotherapy. ISGs are not only central to the innate immune response but also play a pivotal role in initiating immunogenic cell death, thereby triggering adaptive immune response activation. It is generally considered important in promoting T-cell mediated immunity against cancer cells [66]. For example, radiation therapy enhances C-X-C motif chemokine ligand 10

(CXCL10) production in myeloid cells, leading to increased infiltration of CD8+ T lymphocytes into the tumor microenvironment [67]. Driven by robust acute IFN signaling, ISG expression induces cell death through both intrinsic and extrinsic mechanisms. These coordinated responses facilitate the clearance of damaged or malignant cells and help preserve tissue homeostasis.

In contrast, chronic ISG transcription contributes to pro-survival effects. Constitutive overexpression of ISGs downstream of the STAT1/IFN-I pathway suppresses cytotoxic responses and induces pro-survival genes, rendering squamous cell carcinoma cells resistant to ionizing radiation [56, 68]. This phenomenon was later validated, leading to the identification of a subset of ISGs as the interferon-related DNA damage resistance signature (IRDS), which mediates resistance to both chemotherapy and radiation in cancer cells. The expression of these ISGs depends on high levels of unphosphorylated (U-) STAT1, U-STAT2, and IRF9 [69, 70]. While the mechanisms by which these ISGs promote resistance to DNA damage remain unclear, they may enhance genome stability (discussed further in the ISG15 section) and/or protect against DNA damage-induced cell death.

Recent studies provide some mechanistic insights. For example, 2’,5’-oligoadenylate synthetase 1 (OAS1), an IRDS gene, promotes cancer cell survival under chemotherapy and oxidative stress by adding AMP residues to the ends of poly (ADP-ribose) (PAR) chains [71]. This modification limits excessive PARylation, which would otherwise trigger a caspase-independent form of programmed cell death known as parthanatos [72]. Thus, OAS1 acts as a suppressor of parthanatos in response to DNA damage by restraining PAR chain elongation [71]. Other IRDS genes, such as LGP2, help suppress ISGs associated with cytotoxic stress, thereby protecting cancer cells from IR-induced damage [73].

In addition to resistance against DNA-damaging agents, other ISGs can also provide a pro-survival advantage in tumors with chromosomal instability (CIN). For example, in triple-negative breast cancer, cGAS–STING activation triggered by micronuclear rupture induces IL-6–STAT3 and non-canonical NF-κB signaling, which promote tumor growth and survival [74].

Chronic ISG transcription also exerts profound effects on the tumor microenvironment, contributing to its pro-survival role [55]. While ISG expression occurs in both epithelial and stromal compartments, the upstream triggers and downstream effectors may differ markedly depending on cellular context. Therefore, it is critical to determine the cellular origin of ISG expression within tumor tissues.

A recent study using fluorescence-activated cell sorting (FACS)-purified epithelial cells from pancreatic ductal adenocarcinoma (PDAC) demonstrated that cancer cells with high ISG expression can reprogram the surrounding stroma toward a pro-tumorigenic state, leading to more aggressive disease and poorer patient outcomes [75]. Consistent with this finding, persistent interferon signaling in melanoma cells induces STAT1-associated chromatin remodeling and sustained ISG expression, which drives both programmed death-ligand 1 (PDL1)-dependent and PDL1-independent mechanisms of immune evasion. It has been shown that elevated ISG levels in tumor cells—rather than immune cells—

underlie resistance to immune checkpoint blockade [76, 77]. Supporting this interpretation, preclinical studies revealed that administration of the JAK1 inhibitor, Itacitinib, following anti–programmed cell death protein 1 (PD-1) therapy enhances immune function and antitumor responses in mice. This strategy translated into a 67% response rate in a phase 2 clinical trial for metastatic non-small cell lung cancer [78].

As discussed above, most studies investigating the effects of chronic ISG transcription have focused on established cancer cells, where ISG expression is primarily driven by the cytosolic dsDNA/cGAS-STING pathway. This is largely due to the severe chromosomal instability that characterizes many cancer cells, and the cGAS–STING pathway, rather than being selected against during tumor evolution, may instead have conferred pro-tumorigenic functions [74, 79].

Our recent study extended this understanding to pre-malignant cells. Using a mouse mammary organoid system, we found that chronic ISG transcription is mediated by the nuclear immune sensor IFI205—a member of the mouse ALR family—presumably in response to genome instability caused by the *Mre11*$^{ATLD1/ATLD1}$ mutation. In this context, chronic ISG activation is associated with sustained changes in chromatin accessibility and primes the mammary epithelium for oncogene-induced tumorigenesis in implanted *Mre11*$^{ATLD1/ATLD1}$ organoids. *Mre11*$^{ATLD1/ATLD1}$ mammary organoids have cytosolic dsDNA levels comparable to wild-type mammary organoids and can develop into normal mammary tissue post-implantation. And, *Mre11*$^{ATLD1/ATLD1}$ mice did not spontaneously develop tumors in the absence of oncogene activation [2]. These features provide a valuable model for studying the earliest stages of cancer initiation and the influence of chronic ISG signaling. Genetic ablation of *Ifi205* in *Mre11*$^{ATLD1/ATLD1}$ mammary organoids suppresses ISG transcription, reverses the associated chromatin remodeling, and significantly delays oncogene-driven breast tumor formation after implantation. These findings suggest that chronic ISG transcription plays a pro-tumorigenic role not only in advanced malignancies but also at the very earliest stages of cancer development [4].

The mechanistic details underlying the ‘priming effect’ we propose—where chronic ISG transcription promotes oncogene-induced tumorigenesis—remain unclear. We hypothesize that this effect may result from widespread chromatin remodeling. IFN signaling has been shown to induce extensive chromatin changes, including the formation of new enhancers, disassembly of existing enhancers, modulation of chromatin accessibility, and alteration of histone marks. These chromatin changes are mediated by ISGs such as STATs and IRFs, which bind to gene regulatory elements and recruit chromatin-modifying enzymes [80–85]. When this remodeling occurs at ISG loci, it is generally associated with active transcription, thus accounting for their designation as ISGs. However, when such remodeling occurs at other loci, it does not alter gene expression acutely. Instead, the binding of transcription factors can lead to the deposition of epigenetic marks and changes in chromatin accessibility. The ensuing chromatin states may render these loci more responsive to future environmental cues, including oncogenic stimuli [86].

A similar principle—that epigenetic alterations can initiate tumorigenesis—was recently demonstrated. In a Drosophila model, transient perturbation of Polycomb-mediated

transcriptional silencing induced irreversible derepression of oncogenic drivers, including JAK–STAT pathway genes and the ZEB1 homologue zfh1, ultimately leading to malignant transformation [87].

These observations are consistent with the concept of epigenetic plasticity. Analogous to Waddington's epigenetic landscape of developmental specification—where differentiating cells roll downhill through branching canals—permissive chromatin states described above can be visualized as lowered canal walls [88, 89]. This lowers the threshold that would otherwise block pre-malignant cells from exploring alternative chromatin landscapes and transcriptional programs, some of which may confer tumorpromoting properties. If a particular chromatin configuration proves advantageous and heritable, it may give rise to the emergence of a new clone. This iterative trial process can be further amplified in the presence of genomic instability, which greatly enhances the likelihood of malignant transformation through clonal evolution. Such a model helps explain why somatic mutations alone do not fully account for the susceptibility of tumorigenesis. Future studies aimed at defining, monitoring, and quantifying epigenetic plasticity will be essential for elucidating the causal relationship between permissive chromatin states and malignant transformation.

5.2 Implication of ISGs in maintenance of genome integrity

Among the ISGs, ISG15 is unique due to its emerging role in maintaining replication fork stability. ISG15 is a ubiquitin-like (UBL) protein initially characterized for its antiviral roles in innate immunity [90, 91]. ISG15 has two ubiquitin-like β-grasp domains separated by a short linker [92]. The β-grasp fold of the C-terminal ubiquitin-like domain in ISG15 partially wraps around a short, flexible tail that terminates in diglycine residues, which are essential for conjugation to target proteins [93]. ISG15 exists in two major functional forms: a free (unconjugated) form, which can be secreted from cells, and a conjugated form, in which ISG15 is covalently attached to target proteins via a process known as ISGylation.

The secreted form of ISG15 acts as a cytokine-like molecule, promoting immune activation. It has been shown to stimulate IFN-γ production by natural killer (NK) cells and T cells, thereby enhancing adaptive immune responses [94].

In contrast, ISGylation—the covalent attachment of ISG15 to lysine residues on target proteins—is a process precisely analogous to ubiquitylation. ISGylation is carried out by a three-enzyme cascade: 1) E1 enzyme: Ubiquitin-activating enzyme E1-like protein (UBE1L) activates ISG15 in an ATP-dependent manner [95]. 2) E2 enzyme: ubiquitin-conjugating enzyme H8 (UBCH8) transfers activated ISG15 [96]. 3) E3 ligases: such as HECT and RLD domain containing E3 ubiquitin protein ligase 5 (HERC5, in humans), tripartite motif containing 25 (TRIM25), or ariadne RBR E3 ubiquitin protein ligase 1 (ARIH1), catalyze the final conjugation of ISG15 to specific target proteins [97–99]. While ubiquitylation commonly targets proteins for proteasomal degradation, ISGylation typically acts to stabilize proteins and suppress turnover. This occurs primarily through two mechanisms: first, by competing for the same lysine residues, ISGylation can sterically hinder ubiquitylation; and second, by modifying ubiquitin chains themselves, ISGylation can interfere with proteasomal recognition and degradation [100, 101].

Beyond modulating degradation, ISGylation serves broader regulatory roles by altering protein activity, subcellular localization, stability, and protein–protein interactions in a context-dependent manner. For the most part, studies of ISG15 have focused on its role in blocking viral entry, replication, and release. More recently, it has become clear that ISGylation also regulates essential cellular functions such as autophagy, protein synthesis, exosome secretion, and the DNA repair [54, 102, 103].

Our recent work has further elucidated ISG15's role in mitigating DNA replication stress. In MEFs, inducible knockout of Nijmegen breakage syndrome 1 (*Nbs1)* triggers replication stress and activates the innate immune pathway, leading to robust induction of ISG15 and ISGylation. ISG15 was found at replication forks even in wild-type cells and was further enriched following *Nbs1* deletion. Pulldown experiments of endogenously tagged ISG15 under denaturing conditions, followed by mass spectrometry, identified ISGylation of 43 chromatin-bound DNA replication and repair proteins, including DNA topoisomerase 2-alpha (TOP2A), structural maintenance of chromosomes 3 (SMC3), flap structure-specific endonuclease 1 (FEN1), and valosin containing protein (VCP) [1, 104].

Genetic ablation of *Isg15* resulted in spontaneous replication stress under otherwise unperturbed conditions, marked by activation of the S-phase checkpoint, increased replication fork stalling, micronuclei formation, chromosome aberrations, and hypersensitivity to aphidicolin [1]. These findings indicate that ISG15 is important for stabilizing replication forks during normal DNA replication.

Upon replication stress, ISG15-deficient cells also exhibited broad changes in the proteome associated with nascent DNA, with more proteins being depleted than enriched, suggesting ISG15 may stabilize protein occupancy at stalled forks. Although ISG15 was not required for the direct recruitment of individual ISGylated proteins like FEN1 or VCP to replication forks, its absence appeared to disrupt recruitment of certain factors, among them the fork protection complex comprising Timeless, Tipin, Claspin, and TopBP1 [1]. Analogous to how ubiquitylation and SUMOylation facilitate protein clustering at DNA lesions, ISGylation may function as a 'molecular glue' that stabilizes the fork protection complex during replication stress [105–107].

Consistent with the direct involvement of ISG15 in genome stability maintenance, a recent study demonstrated that ISG15 and ISGylation are required to preserve nascent DNA in BRCA1/2-deficient cells, where IFN-β treatment restores fork stability and viability in an ISG15-dependent manner [108]. This work further underscores the integral role of ISG15 in protecting stalled replication forks and extends its functional significance to BRCA-associated cancer vulnerabilities.

While important for the maintenance of replication fork stability, excess ISG15 may also be detrimental to cells, albeit through a non-covalent mechanism. It has been shown that ISG15 binds to the DNA helicase RECQ1 and enhances its fork restart activity, accelerating fork progression but also promoting replication stress and DNA damage when overexpressed [109]. The authors argue that the seemingly contradictory roles of ISG15 in replication fork stability are context dependent [108]. In a BRCA-proficient setting, ISG15 upregulation

accelerates DNA replication fork progression through RECQ1-mediated fork restart, leading to aberrant replication structures [109]. By contrast, in a BRCA-deficient setting, ISG15-mediated fork restart rescues fork integrity by counteracting reversed fork accumulation, which otherwise serves as entry points for deregulated nucleolytic activity [108].

However, it is also possible that these findings reflect the distinct contributions of ISG15 in its ISGylated versus non-ISGylated forms. The detrimental effect on replication forks has been reported to be ISGylation-independent [109], whereas the protective effect requires ISGylation [1, 108]. Thus, upregulation of total ISG15 may not uniformly translate into increased ISGylation, as the activity of the conjugation machinery can vary under different conditions. In this view, the overall impact of ISG15 on replication fork dynamics would be determined both by genetic context and by the balance between ISGylated and non-ISGylated ISG15.

Regardless of the precise mechanism, these observations support a model in which ISG15, a key ISG, functions as part of the genome surveillance system by modulating replication fork stability through both ISGylation-dependent and -independent mechanisms.

Other ISGs have also been implicated in the replication stress response. Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) was initially characterized as an ISG with potent deoxynucleotide triphosphate (dNTP) triphosphohydrolase activity. By depleting cellular dNTPs, SAMHD1 restricts early-stage HIV-1 replication in dendritic and myeloid cells, thereby exerting a well-established antiviral function [110–112]. Beyond its antiviral role, SAMHD1 is increasingly recognized for its importance in maintaining genome stability. SAMHD1 seems to promote the degradation of nascent DNA at stalled replication forks by stimulating the exonuclease activity of MRE11. This function facilitates ataxia telangiectasia-mutated and RAD3-like protein (ATR)–CHK1 checkpoint activation and promotes efficient replication fork restart, thereby limiting the duration and magnitude of DNA replication stress [113].

Another example of an ISG involved in DDR is RNase L, a ribonuclease known for its role in antiviral immunity [114]. Recent findings indicate that RNase L also contributes to the repair of DNA double-strand breaks (DSBs). It interacts with key components of the nonhomologous end joining (NHEJ) repair pathway, including X-ray repair cross complementing 4 (XRCC4) and DNA ligase 4 (LIG4), thereby promoting DSB repair and enhancing cell survival under genotoxic stress [115]. Further studies will be needed to verify its direct functional involvement and elucidate the underlying mechanism.

Although historically considered species-specific, accumulating evidence suggests that components of innate immune systems, including ISGs, are evolutionarily ancient and conserved across diverse domains of life. Immune modules such as pattern recognition receptors, ubiquitin-like modifiers, and nucleic acid sensors can be traced back to prokaryotic systems, where they serve in defense against phage infection [22, 116]. This conservation implies that fundamental immune mechanisms—especially those involved in detecting and responding to nucleic acid stress—predate the emergence of interferon signaling and have been retained and adapted in eukaryotic lineages. The evolutionary

persistence of these pathways underscores the possibility that innate immune-derived mechanisms may constitute a foundational layer of genome surveillance—a concept warranting deeper investigation.

6. Closing Remarks

This review has proposed a model in which nuclear innate immune sensors—traditionally understood as pathogen detectors—also perform a surveillance role at DNA replication forks (Figure 2). In this framework, sensors such as IFI204/IFI205 recognize aberrant DNA structures that arise transiently, infrequently, and inevitably during normal replication. This engagement triggers low-level tonic ISG transcription, culminating in ISG15 conjugation to fork-associated proteins. A key outcome of this pathway is the stabilization of replication fork–associated protein complexes under homeostatic conditions. In the context of genome instability, the engagement of these sensors is substantially more frequent and drives chronic ISG transcription and increased chromatin plasticity which appears to create a permissive state for oncogene driven tumorigenesis. Sustained ISG transcription may also modulate the tumor microenvironment, with significant implications for cancer therapy outcomes. Obviously, these events are unrelated to pathogenic infection, yet they mirror the logic of classical innate immune surveillance: detection of abnormal nucleic acid structures, initiation of signal transduction cascade, and execution of transcriptional programs.

Several central findings support this model. First, innate immune sensors are localized to replication forks and become enriched following replication stress [1]. Second, DNA binding by IFI205 is essential for the chronic ISG transcriptional phenotype and tumor susceptibility observed in $Mre11^{ATLD1/ATLD1}$ organoids [4]. Third, ISG15 facilitates the recruitment of the replication fork protection complex—Timeless, Tipin, Claspin, and TopBP1—and is thus required to maintain fork stability under normal conditions [1, 103].

This model carries important implications for cancer susceptibility. *IFI16*, a member of the human ALR family, is recurrently amplified across a range of human cancers—13.89% in cholangiocarcinoma, 9.14% in liver hepatocellular carcinoma, 7.84% in breast invasive carcinoma, 6.08% in bladder urothelial carcinoma, and 5.65% in lung adenocarcinoma (TCGA, PanCancer Atlas). Notably, while human IFI16 and mouse IFI204/IFI205 both belong to the ALR family, they are not strictly orthologues, and their functions may not be identical. Nevertheless, these observations raise the possibility that excess ISG transcription contributes to cancer development. Moreover, genetically unstable epithelial cells may initiate this chronic ISG transcriptional program early, and the resulting permissive chromatin landscape may further predispose these tissues to malignant progression in conjunction with underlying genomic instability.

Future studies should address the specificity of sensor–fork interactions, the structural basis of ISG15-mediated complex stabilization, and the role of chromatin remodeling in shaping epigenetic trajectories downstream of sustained ISG signaling. Ultimately, elucidating how nuclear innate immune pathways intersect with replication and chromatin programs may reveal new strategies for understanding—and therapeutically targeting—the earliest stages of cancer development.

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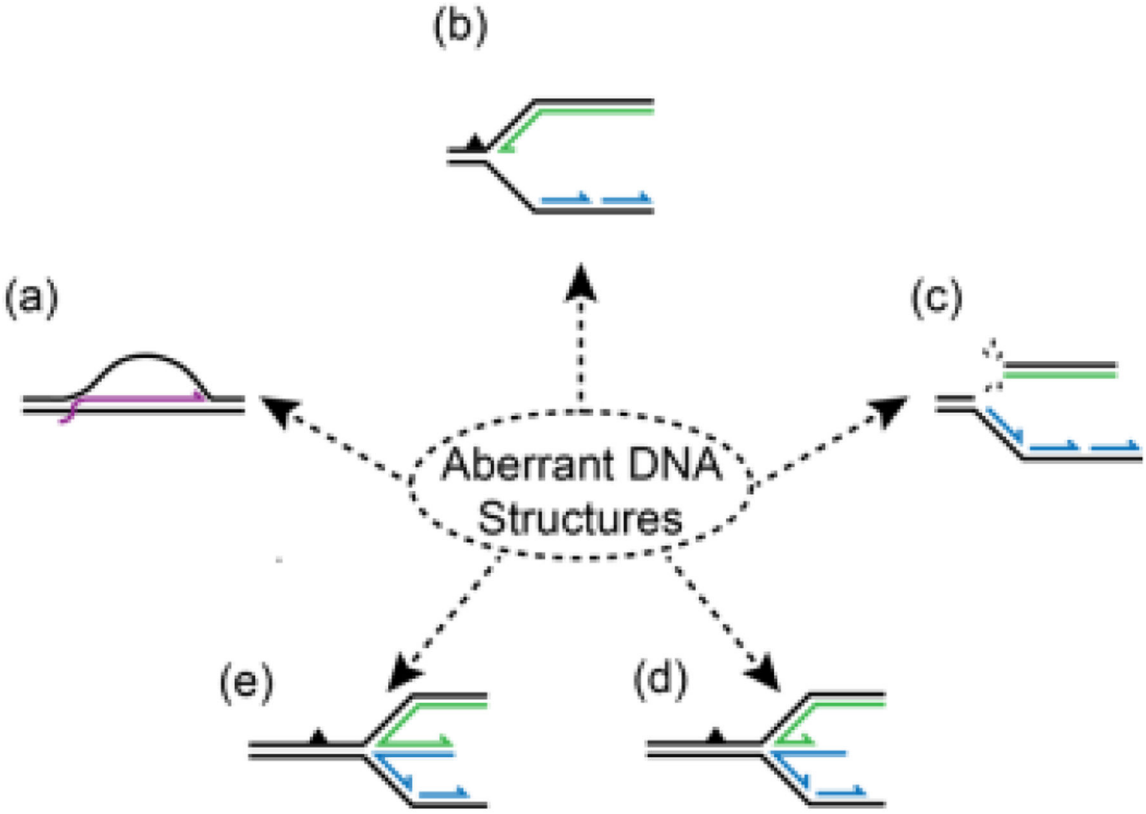

Figure 1. Aberrant DNA structures during replication stress.

Illustration of representative aberrant DNA intermediates generated during replication stress that may be recognized by innate immune sensors. (a) R-loop: RNA hybridization to the DNA template forms an RNA–DNA hybrid, displacing the non-template strand as ssDNA. (b) Extended ssDNA: Continued helicase unwinding without polymerase progression generates long stretches of single-stranded DNA. (c) Double-strand break (DSB): Collapsed or unrepaired forks can result in DNA double-strand breaks. (d, e) Fork reversal: Stalled forks may undergo reversal, in which the nascent leading and lagging strands anneal, generating a four-way junction (“chicken foot”). Black triangle, lesion on the leading-strand template; black, template strands; green, nascent leading strand; blue, nascent lagging strand; purple, transcribed mRNA.

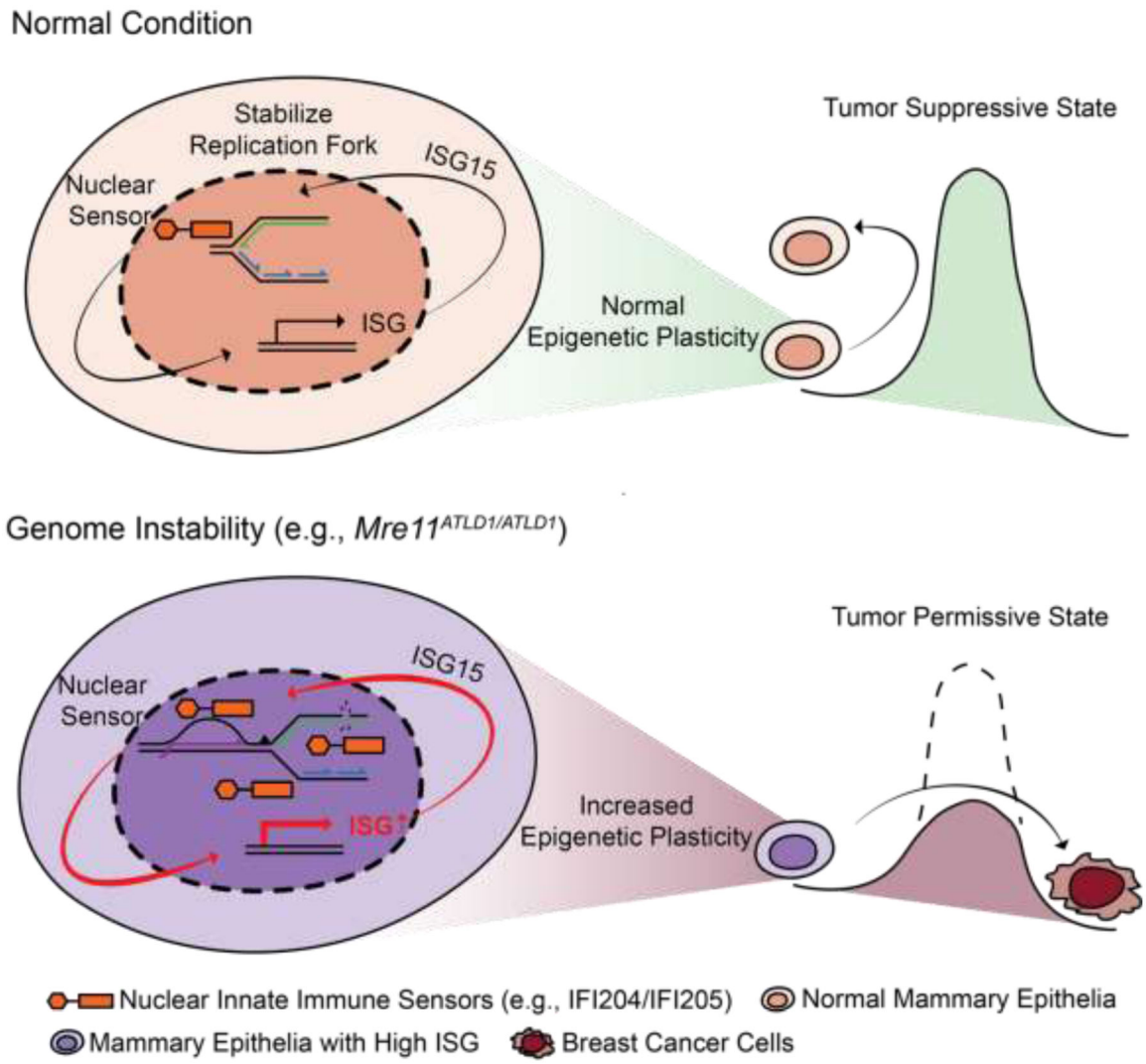

Figure 2. Schematic representation of the proposed model.

Nuclear innate immune DNA sensors, such as members of ALR (AIM2-like receptor) family, are constitutively associated with the DNA replication fork. These sensors surveil the genome and tonically activate a low-level interferon-stimulated gene (ISG) transcriptional program in response to rare, endogenous aberrant DNA structures that arise during normal DNA replication. A key output of this program is interferon-stimulated gene 15 (ISG15), which is conjugated to replication fork proteins and facilitates recruitment of the replication fork protection complex, thereby stabilizing replication forks and maintaining genome stability. Under conditions of genome instability, such as in *Mre11*$^{ATLD1/ATLD1}$ mutant mammary epithelia, substantially more frequent engagement of aberrant structures by nuclear sensors leads to chronic ISG transcription. This sustained signaling alters the chromatin landscape, increasing epigenetic plasticity and promoting oncogene-induced breast tumorigenesis.

Table 1.

Classification and characteristics of major pattern recognition receptor (PRR) families.

PRR Family	Localization	Ligands Detected	Key Members	Signaling Pathway / Outcome
Toll-like receptors (TLRs)	Membrane-bound (cell surface/ endosome)	Microbial lipids, lipoproteins, proteins (cell surface); nucleic acids (endosome)	TLR1–TLR10 (human), TLR1–TLR9, TLR11–TLR13 (mouse)	Signal via MyD88 or TRIF → activates NF-κB and IRFs → cytokines and type I IFNs
NOD-like receptors (NLRs)	Cytosolic	Bacterial products (e.g., diaminopimelic acid, muramyl dipeptide), viral RNA	NOD1, NOD2, NLRP3, NLRC4	NOD1/2: activate NF-κB, MAPK; NLRP3/NLRC4: assemble inflammasome, activate caspase-1
RIG-I-like receptors (RLRs)	Cytosolic	Viral RNA	RIG-I, MDA5, LGP2	Signal via MAVS → activate IRF3/7, NF-κB → type I IFN expression
C-type lectin receptors (CLRs)	Membrane-bound	Carbohydrate motifs on pathogens or damaged cells (Ca^{2+}-dependent)	Mannose receptor (Group I), Dectin-1/ CLEC7A, DCIR/ CLEC4A (Group II)	Signal via Syk, CARD9 → pro-inflammatory cytokines
AIM2-like receptors (ALRs)	Cytosolic and nuclear	Nucleic acids	AIM2, IFI16	IFI16*: signals via STING → ISGs; AIM2/IFI16: activates inflammasome → IL-1β, IL-18
OAS-like receptors (OLRs)	Cytosolic	Nucleic acids	OAS1–3, cGAS	Synthesize 2'–5' linked second messenger molecules → activate antiviral pathways

*IFI16**, human IFI16 is related to mouse IFI204/205; all are members of ALR family. *MyD88*, myeloid differentiation primary response 88. *TRIF*, TIR-domain-containing adapter-inducing interferon-β. *NF-κB*, nuclear factor kappa-light-chain-enhancer of activated B cells. IRF, interferon regulatory factor. *IFN*, interferon. *NOD1/2*, nucleotide-binding oligomerization domain 1/2. *NLRP3*, NOD-like receptor pyrin domain containing 3. *NLRC4*, NOD-like receptor CARD domain containing 4. *MAPK*, mitogen-activated protein kinase. *RIG-I*, retinoic acid-inducible gene I. *MDA5*, melanoma differentiation-associated protein 5, *LGP2*, laboratory of genetics and physiology 2. *CLEC7A*, C-type lectin domain family 7 member A. *DCIR*, dendritic cell immunoreceptor. *CLEC4A*, C-type lectin domain family 4 member A. *Syk*, spleen tyrosine kinase. *CARD9*, caspase recruitment domain-containing protein 9. *AIM2*, absent in melanoma 2. *IFI16*, interferon-inducible protein 16. *STING*, stimulator of interferon genes. *ISG*, interferon-stimulated gene. *IL-1β*, interleukin-1 beta. *IL-18*, interleukin-18. *OAS1–3*, 2'−5'-oligoadenylate synthetase 1–3. *cGAS*, cyclic GMP-AMP synthase.