REVIEW

BRCA1-regulated nuclear innate sensing of herpesviral genome by IFI16 and IFI16's acetylation is critical for its cytoplasmic trafficking and induction of innate responses

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Sensing of invading DNA virus genomes appear to be triggered by a number of host cell DNA sensors depending on their subcellular localization which stimulate innate anti-viral responses such as the induction of type-I interferons (IFNs) and/or activation of inflammasomes resulting in the production of inflammatory IL-1β and IL-18 cytokines. With growing understanding of diverse identities whether these proteins function alone or with other host cell molecules and the post-translational modifications affecting their functions are under intense investigations. Nuclear resident IFI16 have been shown to sense the episomal DNA genomes of herpes viruses resulting in the induction of IFI16-inflammasome and/or interferon responses. Here, we highlight our recent finding regarding the role of cellular BRCA1, a transcription factor and DNA damage response protein, forming a distinct complex with IFI16 to regulate the nuclear innate sensing of herpes viral DNA and subsequent IFI16-ASC-procaspase-1 inflammasome complex formation and distribution to the cytoplasm leading into caspase-1 and IL-1β production. BRCA1 is also responsible for the cytoplasmic IFI16-STING signalosome activation and induction of IFN-β during *de novo* KSHV and HSV-1 infection. Our concurrent studies have also revealed that the histone acetyl transferase p300 mediated acetylation of nuclear IFI16 is a dynamic post-genome recognition event responsible for Ran dependent nuclear to cytoplasmic trafficking of IFI16 during herpesvirus infection. This post-translational modification is essential for IFI16-ASC interaction and inflammasome activation as well as for the association with STING in the cytoplasm resulting in IRF-3 phosphorylation, nuclear pIRF-3 localization and interferon-β production. Collectively, these comprehensive studies highlight that BRCA1 plays a hitherto unidentified immunomodulatory role to facilitate the anti-viral functions of IFI16 and acetylation of nuclear IFI16 is a necessary post-translational modification for innate responses during herpesvirus infection. These studies open up a new understanding of virus-host interplay, viral genome sensing and host innate anti-viral defense mechanisms.

Abbreviations: IFNs, interferons; PAMPs, Pathogen Associated Molecular patterns; DAMPs, Damage Associated Molecular patterns; PRRs, pattern recognition receptors; TLR9, endosomal membrane bound; HIN, hematopoietic expression, interferon-inducible nature and nuclear localization; IFI16, interferon-inducible protein 16; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus type-1; HCMV, human cytomegalovirus; NLS, nuclear localization signal; DDR, DNA damage response; PTM, post-translational modification; HMVEC-d, human microvascular dermal endothelial; PEL, primary effusion B-cell lymphoma; STING, stimulator of interferon genes; HFF, human foreskin fibroblast; wt, wild type; P/DAMP, pathogen/danger associated molecular pattern; BRCA1, breast cancer tumor suppressor protein; CHX, cycloheximide; GAPs, GTPase-activating proteins.

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Introduction

The Innate immune system is the front line of host defense that detects pathogens via the exogenous PAMPs (Pathogen Associated Molecular patterns) and Damaged-self through endogenous DAMPs (Damage Associated Molecular patterns) to elicit the inflammatory and/or protective responses. Detection of PAMPs and/or DAMPs are achieved via the germ line encoded cellular surveillance pattern recognition receptors (PRRs). A remarkable feature of mammalian cells is that they can distinguish self from non-self despite the similarities between the PAMPs and equivalent cellular molecules. Viral DNA or RNA genomes are recognized by a diverse number of host sensors depending upon their subcellular localizations and consequently induce DNA- or RNA- driven antiviral immune reactions including the induction of Type-I interferons (IFNs) and/or inflammasomes, production of inflammatory cytokines or induction of cell death [1, 2].

There is a wealth of information available regarding the sensors of viral RNA such as RIG-I, MDA5, TLR3, TLR7, PKR, RNase L and LGP2. Similarly, significant progress is made regarding the sensors of viral DNA and studies have demonstrated the presence of a number of viral DNA sensors in the cytoplasm as well as in the nucleus which includes TLR9 (endosomal membrane bound), ZBP1/DAI, AIM2, DDX-41, DDX-60, DNA-PK, DHX-9, DHX-36, IFI16, RNA-polymerase III, LRRFIP1 and cGAS (Table 1) which bind to DNA in a sequence-specific or non-sequence-specific manner.

Several *in vivo* and *in vitro* studies have demonstrated that some of these proteins, known as HIN (hematopoietic expression, interferon-inducible nature and nuclear localization)-family proteins, play roles in the activation and regulation of cellular signaling, culminating in the production of IFNs plus IL-1β and IL-18, upon recognition of foreign viral DNA via the 200-amino acid HIN domain [3-6]. In addition to the HIN domain, the majority of these proteins also have a pyrin domain (PYD) and have been grouped in the PYHIN family and are also known as absent in melanoma 2-like receptors (ALRs). So far, four homologs from humans (absent in melanoma 2 [AIM2], gamma interferon-inducible protein IFI-16 [IFI16], myeloid cell nuclear differentiation antigen [MNDA], and interferon-inducible protein X [IFIX]) and 13 homologs from mice belonging to the PYHIN family have been identified [7, 8].

Herpesviruses establish a latent infection in the nucleus of specific cells and reactivation results in the viral dsDNA replication and infectious virus production. Similar to the important roles played by the cytoplasmic PYHIN proteins in intrinsic immunity, nucleus associated interferon-inducible protein 16 (IFI16) has emerged as an innate sensor of nuclear episomal genomes of herpesviruses such as Kaposi's sarcoma-associated herpesvirus (KSHV), Epstein-Barr virus (EBV), herpes simplex virus type-1 (HSV-1), and human cytomegalovirus (HCMV) resulting in the induction of inflammatory cytokines and IFN production, as well as a restriction factor against viral transcription and replication [9-22].

In this research highlight we summarize our key recent findings of a) the novel role of cellular BRCA1 protein, a DDR repair sensor and transcription regulator, in IFI16's ability to sense the nuclear herpes viral DNA and subsequent induction of innate responses, and b) the role of nuclear acetylation of IFI16 after viral genome recognition in trafficking to the cytoplasm during herpesvirus infection [23, 24].

IFI16

IFI16 has the following molecular architecture (Figure 1): (1) An N-terminal PYD domain involved in homotypic protein-protein interactions and/or heterotypic interactions with several other cellular proteins including p53, Rb, BRCA1, ASC, SP1 and STING [9, 10, 11, 25, 26]. (2) Four nuclear localization signal (NLS) motifs. Motif 1 (aa 96-100) and motif 2 (aa 127-138) are attributed to nuclear entry after synthesis in the cytoplasm [27]. (3) Two DNA binding HIN domains (HIN-A and HIN-B). HIN-A is reported to possess greater affinity towards DNA than HIN-B. HIN-B can also interact with oligonucleotides and oligosaccharides via an oligonucleotide-oligosaccharide binding fold (OB-fold) which contains two tandem β-barrels [28]. (4) Region in between the HIN-A and HIN-B domains consisting of several serines, threonines and prolines. The length of this region is regulated by alternative mRNA splicing and produces three IFI16 isoforms (A-C) [29]. The B-isoform is most abundantly expressed in human non-immune (endothelial, fibroblasts, epithelial cells) as well as in immune (macrophages, B and T cells) cells.

IFI16 has been shown to participate in a spectrum of functions ranging from the DNA damage response (DDR), induction of apoptosis and senescence, regulation of cell growth and cell differentiation, and as a secreted autoantigen in the pathogenesis of autoimmune systemic lupus erythematosus disease [30-36].

Although IFI16 is predominantly a nuclear protein, studies such as immunofluorescence, cell fractionation or DNA-protein pull downs have shown that a portion of the IFI16 pool localizes in the cytoplasm during bacterial or viral

Table 1. Viral DNA sensors

Sensors	localization	DNA type	Virus	Immune response pathway	References
TLR9	Endosome	Unmethylated CpG motif	ADV, HSV1, HSV-2, CMV, EBV	IFN-α, IFN-β via MYD88	[48, 49, 50, 51]
DAI/ZBP-1	Cytoplasm	ds DNA	HSV-1, CMV	IFN-β via STING; Necrosis via RIP3	[52, 53]
DHX 36	Cytoplasm	Class A CpG motif	HSV-1	IFN-α, via MYD88	[54]
DHX 9	Cytoplasm	Class B CpG motif	HSV-1	IFN-α, via MYD88	[54]
DDX41	Cytoplasm	ds DNA	ADV, HSV-1	IFN-α, IFN-β via STING	[55, 56]
DDX60	Cytoplasm	ds DNA	HSV-1	IFN-β via RIG-I	[57]
RNA POL III	Cytoplasm	AT rich ds DNA	ADV, HSV-1, EBV	IFN-β via RIG-I	[58, 59]
DNA-PK	Cytoplasm	ds DNA	VACV, HSV-1, HSV-2	IFN-β, IFN-γ, IL6 via STING	[60, 61, 62]
AIM2	Cytoplasm	ds DNA	ADV, CMV, HBV, HPV-16, VACV	IL-1β, IL-18 via inflammasome	[63, 64, 65, 66]
IFI16	Nucleus, Cytoplasm	ds DNA, ss DNA	HSV-1, KSHV, EBV, CMV, HIV, VACV 70 mer DNA	IL-1β, IL-18, IL-33, via inflammasome; IFN-β, CXCL-5, CXCL-10, CXCL-20 via STING	[12, 13, 14, 16, 23, 24,27, 67]
IFIX	Nucleus, Cytoplasm	ds DNA	HSV-1, VACV 70 mer DNA	unknown	[68]
c-GAS	Nucleus, Cytoplasm	ds DNA	ADV, HSV-1, MAV/VACV (modified vaccinia virus)	IFN-α, IFN-β via STING	[46, 69, 70]

ADV: Adeno virus; CMV: Cytomegalo virus; HBV: Hepatitis B virus; HPV-16: Human papilloma virus -16; HSV-1 and 2: Herpes simplex virus-1 and 2; VACV: Vaccinia virus; EBV: Epstein Barr virus; KSHV: Kaposi's Sarcoma-associated herpes virus.

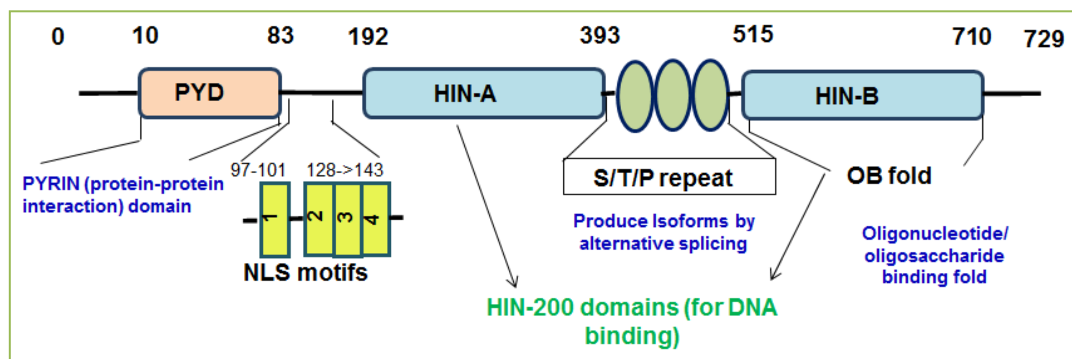

Figure 1. Schematic representation of human IFI16 structure. Residues 10-83 constitute the N-terminal PYRIN domain. 97->143 contain NLS motifs. The solid-blue boxes denote the HIN-A (residues 192-393) and the HIN-B (residues 515-710) domains. Oval regions indicate the S/T/P-rich seven amino acid repeat motifs between the two HIN domains, resulting from alternative splicing of the gene. The HIN-B domain also contains an oligonucleotide/oligosaccharide binding fold. Numbers located above the diagram represent amino acid positions.

infection, *in vitro* DNA transfection or UV-light treatment [9, 18, 34, 37, 38]. Interestingly, IFI16-DNA binding may result in the activation of different signaling pathways depending on the crucial subcellular localization of IFI16 that is influenced by interaction with other proteins. Intracellular distribution of IFI16 is likely to be associated with its post-translational modification (PTM) or primary sequence since treatment of IFN-α have been shown to distribute the phosphorylated form of p202 and p204 (murine variant of HIN-family protein) towards the cytoplasm, nucleoplasm and nucleolus [39] . IFI16, which exhibited a predominant cytoplasmic localization in a prostate cancer epithelial cell line, has been shown to have sequence variability than the predominant nuclear IFI16 observed in primary epithelial cell [30]. Acetylation at a nuclear localization signal of IFI16 has also been shown to restrict it to the cytoplasm instead of nucleus [27]. However there is a dearth of knowledge on the kinases, acetyl transferases or acetylases and other cellular modulators targeting IFI16 for PTMs particularly during virus infection and it is therefore important to define how these proteins influence IFI16 localization and function.

Role of IFI16 in sensing herpes viral DNA and innate responses

Whether innate responses recognize and respond to the presence of foreign episomal genomes of herpesviruses as well as other DNA viruses in the infected cell nuclei leading to the induction of inflammatory responses was not known until 2011. Our studies revealed that *de novo* KSHV infection of primary human microvascular dermal endothelial (HMVEC-d) cells induces IFI16-ASC-procaspase-1

inflammasome formation in the nucleus and its redistribution to the cytoplasm [10]. Colocalization of IFI16 with viral genome in the infected endothelial cell nucleus, induction of IFI16-ASC inflammasomes by UV-inactivated KSHV and the absence of induction by lentivirus vectors expressing KSHV genes demonstrated that a) KSHV genes individually do not play a role in IFI16-inflammasome activation, b) the IFI16-inflammasome is not induced against linear integrated foreign DNA, and c) episomal KSHV genome is required for IFI16-inflammasome activation.

Our subsequent studies demonstrated that only the IFI16-inflammasome is constitutively induced in KSHV latently infected endothelial and primary effusion B-cell lymphoma (PEL) cells [12], as well as in B-lymphoma, epithelial and lymphoblastoid cells latently infected with EBV [13]. Colocalization of IFI16 with the latent KSHV and EBV genome in the nuclei suggested that continuous sensing of latent genome could be a reason for the constitutive inflammasome induction and cytoplasmic relocalization. However, the mechanism behind the redistribution was not known. In addition, IFI16 has also been shown to interact with STING (stimulator of interferon genes) leading to phosphorylation and nuclear translocation of IRF3 via the IFI16-STING-TBK signaling axis, resulting in IFN-β production during HSV-1 infection [9, 16].

Our studies also revealed that *de novo* infection of primary human foreskin fibroblast (HFF) cells by HSV-1 also induces IFI16-ASC-procaspase-1 inflammasome formation in the nucleus and its redistribution to the cytoplasm, and the association of IFI16 with ASC was limited to 4 h p.i. after infection with 1 PFU/cell [14]. IFI16 colocalization with the HSV-1 genome at early times post-infection suggested that recognition of the viral DNA is probably the driving force for IFI16 inflammasome assembly. Consistent with an earlier report [16], HSV-1 infection of HFF cells induced the proteasomal degradation of IFI16 via HSV-1 immediate early ICP0 protein. Although studies by Orzalli *et al*., 2012 showed the degradation of IFI16 during HSV-1 infection in an ICP0-dependent manner, they did not observe any cytoplasmic IFI16 after 4 h of infection. However, experiments with fractionated cells infected with wild type (wt) HSV-1 were not carried out. Our immunofluorescence studies show that IFI16 exited the nucleus of cells infected with mutant d106 and d109 as well as wt HSV-1 but translocation was lesser with the mutant viruses than with wt HSV-1, which may account for the discrepancy. In addition, Orzalli *et al*., 2012 used a significantly higher MOI of 10 to 50 PFU/cell than the 1 PFU/cell in our studies, which could likely lead to some differences in the kinetics of IFI16 translocation and not detecting cytoplasmic IFI16 at their chosen time points, as at this high MOI, we observed the degradation of IFI16 before 4 hrs in the wt HSV-1 infected cells.

Cytosolic sensors like AIM2 and NLRP3 have been shown to activate inflammasomes in response to infection by bacteria, DNA and RNA viruses. However during latency, KSHV and EBV genomes persist in the infected cell nuclei without being exposed to these cytosolic sensors. Therefore, induction of the inflammasome by latent KSHV suggests existence of a pathogen/danger associated molecular pattern (P/DAMP) recognition system within the nucleus and our studies identify IFI16 as one of the nuclear danger sensing receptors. Our studies clearly established that IFI16 forms a functional inflammasome in response to KSHV/EBV and HSV-1 infection. Silencing IFI16 inhibited inflammasome activation without effecting AIM2 or NLRP3 pathways. Additionally, results of our immunofluorescence and co-IP studies in the cell culture system were strongly supported by the colocalization of IFI16 and ASC in KSHV infected PEL patient samples [12].

Failure to detect interaction between ASC and IFI16 by some earlier studies could be due to these studies being aimed at identifying a protein that can detect cytosolic DNA, they used the transfected cytosolic DNA as a trigger to examine ASC interaction with IFI16 and other HIN-200 members. However, none of the stimuli used were located in the nucleus, and therefore it is not surprising that nuclear IFI16 or other nuclear HIN-200 proteins did not have any role in this context. Our studies suggest the requirement for an appropriate stimulus in the nucleus.

Role of IFI16 as a restriction factor for herpesviruses

IFI16 has been described as a restriction factor for herpes viral lytic replication [11, 15, 17]. It restricts HCMV replication by displacing transcription factors on E and L but not IE gene promoters [11] and restricts HSV-1 replication particularly replication of ICP0-deficient HSV-1 [15, 17]. We have also recently demonstrated that, independent of its innate immune response, IFI16 inhibited HSV-1 replication by repressing viral gene expression via its binding to the transcription start sites of viral genes, reducing the association of transcription factors to these sites and by promoting global histone modifications on the viral genome [22]. However, questions such as whether pathogen DNA is recognized by IFI16 directly or in association with other host proteins and how IFI16 differentiates host vs. pathogen DNA remain unknown.

BRCA1 is a component of IFI16-inflammasomes induced in herpesvirus (KSHV, EBV, and HSV-1) infected cells

Upon entry into the nucleus, the linear epigenetically naïve virion-associated herpesviral dsDNA genome circularizes, associates with histones and nucleosome proteins leading to epigenetic control [40]. The host cell DDR acts in a signal cascade event that includes the phosphorylation of repair mediators (H2AX, BRCA1, 53BP1, and Mdc1) and effectors of the checkpoint responses [CHK1 (Checkpoint kinase 1) and CHK2 (Checkpoint kinase 2)]. The DDR may also recognize exogenous genomes of nuclear DNA viruses which manipulate the DDR for their advantage [41]. During *de novo* infection, entry of KSHV DNA into the nuclei of endothelial cells induces an immediate DDR response of ATM kinase, H2AX, CHK1, CHK2 and BRCA1 (breast cancer tumor suppressor protein) activation [42]. Since IFI16 is believed to be a part of the large BRCA1-associated genome surveillance DDR complex (BASC) [43] and is also known to form a functional IFI16-ASC-procaspase-1 inflammasome during KSHV, EBV and HSV-1 infection [10, 12, 13, 14], we hypothesized that IFI16 forms complexes with different proteins to mediate different functions, and that one or more of these IFI16 complexes recognizes the KSHV DNA to induce an innate inflammasome or interferon response.

Herpesvirus infection induced increased association between IFI16 and BRCA1 was observed from our coimmunoprecipitation (co-IP) and proximity ligation assay (PLA) analyses; however, IFI16 was not associated with DDR proteins during virus infection and even in bleomycin induced DDR. Hence this interaction is probably distinct from the complexes formed by BRCA1, H2AX and CHK2 proteins during DDR and the IFI16-BRCA1 complex may have some different roles during herpesvirus infection.

We, therefore, asked the questions what is the functional implication of this IFI16-BRCA1 association? Does this association lead to regulation of IFI16-inflammasome activation and/ or innate interferon response during herpes infection?

Interestingly, we did find the association of BRCA1-ASC, BRCA1-Caspase-1, BRCA1-IFI16 and IFI16-ASC and their cytoplasmic distribution during *de novo* infection by KSHV (HMVEC-d, HFF and peripheral blood monocyte cell [PBMC]), HSV-1 (HFF) and EBV (PBMC) as well as latent KSHV [TIVE-LTC (Telomere immortalized vascular endothelial-latently infected cell) and PEL (BCBL-1) and EBV [LCL (Lymphoblastoid cell line)] infection in our co-IP and PLA studies. In addition, our double sequential PLA also demonstrated that BRCA1, IFI16, ASC are present in the same molecular complex as we found colocalization of some of the BRCA1-IFI16 complex with IFI16-ASC complex and some did not colocalize but remained very close to each other or were separated. This certainly gives the indication that BRCA1 probably is a component of herpesvirus induced IFI16-inflammasome complex.

Since BRCA1 lacks a PYD domain to interact directly with the PYD domain of ASC, the similarities of the BRCA1 association with ASC to that of IFI16 with ASC prompted us to investigate whether the BRCA1-ASC interaction requires the presence of IFI16. Although ASC was found in BRCA1 immunoprecipitates from control si-RNA treated KSHV infected cells, in contrast, little or no ASC was co-IP-ed with BRCA1 in si-IFI16 treated virus infected cells. This result suggests that KSHV induced association of BRCA1 (with no PYD domain) with inflammasome adaptor ASC (with PYD domain) relies on the presence of IFI16. Interestingly, BRCA1 was not co-IPed with AIM2 or NLRP3 in uninfected or KSHV infected cells which not only demonstrates the specific interaction of BRCA1 with IFI16 but also ruled out its association with other cytoplasmic inflammasome sensors under physiological conditions as well as during KSHV infection.

In contrast to the nuclear replicating herpesvirus infected cells, in cells infected with vaccinia virus replicating its dsDNA in the cytoplasm of infected cells, we observed the activation of the cytoplasmic AIM2-ASC inflammasome instead of the IFI16-ASC inflammasome and no significant change in the association of BRCA1 and IFI16 which suggests that the presence of nuclear viral DNA is probably necessary for increased BRCA1-IFI16 interactions, association with ASC and their cytoplasmic redistribution.

BRCA1 is essential for IFI16-inflammasome induction and cytoplasmic distribution during herpesvirus (KSHV, EBV, and HSV-1) infection

To understand the role of BRCA1 in IFI16-inflammasome activation, we assessed the association of IFI16 (sensor), ASC (adaptor) and caspase-1 (executor) in BRCA1 knockdown (by si-RNA) or BRCA1 null cells (HCC-1937) upon virus (KSHV and HSV-1) infection. Surprisingly, in virus infected si-control or BRCA1 positive cells (184b5) interaction among IFI16, ASC and caspase-1 and their cytoplasmic distribution was prominent but very little or no association and cytoplasmic distribution was observed in the absence of BRCA1 from our co-IP and PLA analyses. As expected, cleaved caspase-1 and mature IL-1β and secreted IL-1β were observed in BRCA1 positive cells but not present in detectable levels or at very low levels in BRCA1 knockdown conditions or the absence of BRCA1. Additionally, when we transduced $BRCA1^-$ HCC cells with lentiviruses expressing BRCA1 or control lentiviruses the presence of cleaved caspase-1 and mature IL-1β was clearly

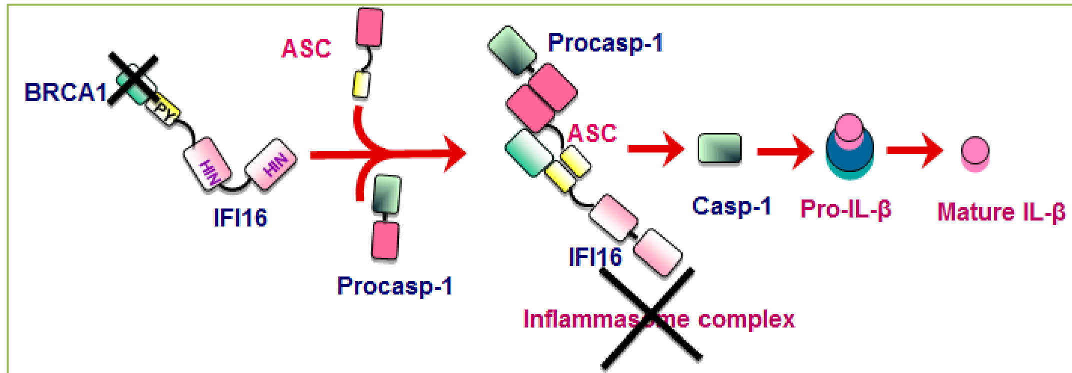

Figure 2. BRCA1 is necessary for herpes virus infection induced IFI16 inflammasome assembly and activation. BRCA1 knockdown or absence abrogates IFI16-mediated recognition of herpes virus genome and subsequent IFI16-ASC-Caspase-1 inflammasome assembly, its cytoplasmic translocation and Il-1β production during herpes virus infection.

seen in BRCA1 expressing but not in BRCA1 negative cells during either the early or late period post-KSHV infection. Together, these results demonstrate the active participation of BRCA1 in the assembly and cytoplasmic trafficking of IFI16-ASC-caspase-1 inflammasomes and formation of functionally active caspase-1 and mature IL-1β during KSHV and HSV-1 infection (Figure 2).

BRCA1 is necessary for IFI16-STING mediated Type-I interferon response during herpesvirus infection

Because a fraction of IFI16-BRCA1 complexes did not colocalize with IFI16-ASC in our sequential PLA experiments we set out to investigate the role of BRCA1 in the other arm of the IFI16-mediated innate response namely type-I interferon production. Compared to the si-control or BRCA1 positive cells where there was a gradual increase in IFN-β secretion (via IFN-β ELISA) and then a decrease during KSHV or HSV-1 course of infections, BRCA1 knockdown or absence resulted in significant decrease in secreted IFN-β levels even during virus infection. The reason behind this observation was revealed from the studies where association of IFI16 with STING (from PLA analysis), responsible for the initial signal cascade for IFN-β, was found to be abrogated in the absence of BRCA1 and was subsequently reflected from the very low level of phosphorylation of downstream signal molecule IRF3 followed by its lower nuclear translocation (from western blot and IFA analysis). Together, these results clearly highlighted the essential role of BRCA1 in the regulation of IFI16 mediated host innate responses of induction of IFN-β during herpesvirus infection (Figure 3).

BRCA1 is involved in herpesvirus genome recognition by IFI16 during *de novo* infection

From our observations that (a) BRCA1 associates with IFI16 and ASC and undergoes cytoplasmic translocation as a part of the IFI16 inflammasome complex during *de novo* KSHV, EBV, HSV-1 as well as latent KSHV and EBV infections, (b) presence of nuclear viral genome is required for increased BRCA-IFI16 and IFI16-ASC interactions, and (c) absence of BRCA1 impairs IFI16 cytoplasmic translocation, IFI16 inflammasome assembly and activation and IFI16-STING signalosome dependent IFN-β production, we hypothesized that IFI16, although, reported to directly bind DNA in an artificial system [5], in the dynamic nuclear environment during virus infection probably requires BRCA1 for sensing/recognizing nuclear viral DNA followed by the subsequent innate response. Indeed from the PLA-IFA studies we observed a considerable level of IFI16 or BRCA-IFI16 complex colocalized with EdU (5' Ethynyl, 2' deoxy uridine)-labelled KSHV and HSV-1 genomes. In contrast, BRCA1 knockdown or absence resulted in a notable decrease in IFI16-viral DNA colocalization and its cytoplasmic distribution. Further biochemical analysis following EdU-HSV-1 infection in si-control and si-BRCA1 pretreated HFF cells, protein-DNA cross-linking, chromatin shearing, biotin-TEG azide tagging to EdU via Click reaction and capturing of biotin-tagged Edu-chromatin fragments of HSV-1 genome on streptavidin beads, the presence of virus genome associated IFI16 was observed from eluted proteins of streptavidin captured genome from si-control cells. In contrast, significantly less IFI16 was detected from the EdU genome pull down fraction from si-BRCA1 treated virus infected cells. Overall, these data clearly demonstrated the involvement of BRCA1 in herpesvirus nuclear genome recognition by IFI16 and subsequent cytoplasmic distribution.

Differential subcellular distribution of IFI16 upon herpes virus infection

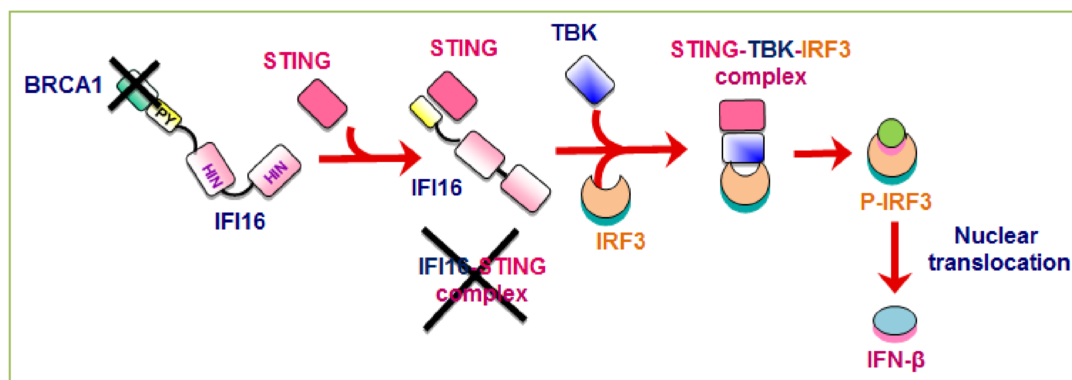

Figure 3. BRCA1 is necessary for IFI16-STING mediated Interferon response. BRCA1 knockdown or absence decreases IFI16-STING signalosome activation, phosphorylation of IRF3 and its nuclear translocation and IFN-β production during herpes virus infection.

Although IFI16, a predominantly nuclear protein, acts as a nuclear DNA sensor of KSHV, EBV, HSV-1 and HCMV1 DNA genome and undergoes (some fraction of total pool) relocalization to the cytoplasm for the induction of innate or intrinsic antiviral responses, the molecular mechanism regulating this intracellular distribution was unknown. It could likely be associated with its PTMs, since, the presence of phosphorylated IFI16 has been observed in the cytoplasm early during HCMV infection [38] and acetylation at nuclear localization signal of IFI16 (Lys 99 and Lys 128) has been shown to restrict it to the cytoplasm instead of the nucleus [27]. However information regarding any kinases, acetyl transferases or acetylases and other cellular modulator proteins responsible for IFI16 PTMs particularly during virus infection was lacking. In our concurrent studies, we for the first time have demonstrated that the presence of KSHV genome in the nucleus induces p300 mediated acetylation of nuclear IFI16 followed by its Ran-GTPase assisted cytoplasmic translocation and this acetylation is required for IFI16-ASC inflammasome assembly and IFI16-STING signalosome activation. We theorized that acetylation of IFI16 could be one of the reasons for cytoplasmic transport since acetylation of HMGB-1 (high-mobility group protein B1) protein involved in transcription/ chromatin bending has been shown to result in HMGB-1's translocation into the cytoplasm [44]. However, the fate of IFI16 during nuclear DNA sensing was not studied.

Acetylation inhibitor interfere with IFI16's nucleus to cytoplasmic distribution during herpesvirus infection

Analysis of the kinetics of IFI16 acetylation by IP-d with anti-acetyl lysine antibody followed by western blotting in the nuclear and cytoplasmic fraction of KSHV infected HMVEC-d cells demonstrated the presence of acetylated cytoplasmic IFI16 as early as 30 min p.i. which steadily increased during the observed 24 h p.i.. Based on the earlier report that the cellular transcriptional coactivator protein p300, which functions as a histone acetyltransferase, has been involved in the cytoplasmic acetylation of IFI16's NLS domains [27], we used p300 competitive inhibitor C-646 (with least toxic 1μM concentration that did not interfere viral entry or nuclear viral genome delivery) and analyzed IFI16 acetylation and nuclear/cytoplasmic distribution during *de novo* KSHV and HSV-1 infected primary cells as well as KSHV and EBV latently infected cell lines via co-IP and PLA. A Significant increase in IFI16 acetylation was observed in KSHV, HSV-1 *de novo* infected HMVEC-d and HFF cells respectively as well as KSHV latently infected BCBL-1 and EBV latently infected Raji (latency-I) and LCL (latency-III) cell lines than uninfected or KSHV negative BCBL-1 or EBV negative Ramos cell lines. In contrast, treatment with C-646 substantially decreased the IFI16 acetylation and cytoplasmic distribution. However, use of vaccinia virus infection (replicating dsDNA exclusively in the cytoplasm) did not significantly increase IFI16 acetylation and cytoplasmic localization and replication incompetent UV-treated KSHV (UV-KSHV) infection which also delivers the viral DNA to the nucleus also induced IFI16-acetylation and cytoplasmic distribution. These results suggested that p300 mediated acetylation of IFI16 may drive its cytoplasmic localization during herpes virus infection and it requires the presence of nuclear herpesvirus genome but not necessary for the virus to be replication competent.

The p300 HAT assay and HDAC assay performed with nuclear and cytoplasmic fractions of KSHV infected HMVEC-d cells and treatment with their respective specific inhibitors revealed increased p300 activity in the nucleus but not in the cytoplasm, thus supporting our conclusion that p300 acetylates IFI16 in the nucleus after viral genome entry into the nucleus. Simultaneously, the increased activity of HDAC, further demonstrates that the increase in acetylation was not due to the activity of HDACs but due to p300. Interestingly, from the PLA study with anti-BrdU and ant-IFI16 antibodies to check nuclear colocalization between

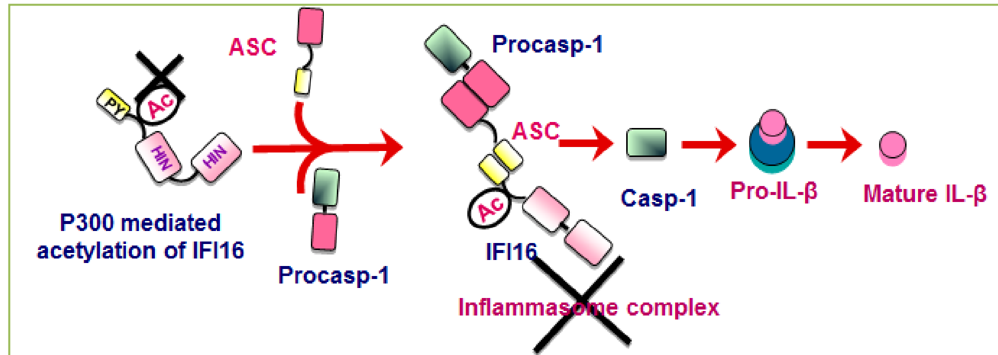

Figure 4. Acetylation of IFI16 is critical for IFI16-inflammasome assembly and activation. Inhibition of IFI16 acetylation restricts formation of the IFI16-ASC-Caspase-1 inflammasome complex and subsequent production of IL-1β.

KSHV/HSV-1 BrdU labelled genome and IFI16 demonstrated no significant changes in IFI16-genome colocalization even in the presence of C-646. Similarly, IFI16-CHIP assay of KSHV positive BCBL-1 cells with or without C-646 showed no major differences in binding of IFI16 with KSHV genome. These results suggested that a) IFI16 directly associates with KSHV and HSV-1 genomes, and b) the acetylation of IFI16 is not obligatory for genome recognition.

Acetylation inhibitor impedes Ran-GTP dependent IFI16 transport from nucleus to cytoplasm and leptomycin hinders nuclear export of IFI16

The dynamic process of exporting molecules of >50-kDa from the nucleus is initiated by exportins binding to cargo and Ran-GTP protein. The guanine-nucleotide exchange factor (GEF) of Ran that converts Ran-GDP to the GTP form is in the nucleus and GTPase-activating proteins (GAPs) for Ran-GTPase are present in the cytoplasm as well as on the cytoplasmic face of the nuclear pore. To determine whether Ran is responsible for IFI16 transport from the nucleus to the cytoplasm and the role of acetyl transferase inhibitor C-646 on this event we performed co-IP or PLA to analyze IFI16-Ran association in the presence or absence of C-646 in KSHV infected HMVEC-d cells (4h). Both studies showed increased association of the IFI16 with Ran which was significantly decreased during C-646 treatment suggesting that acetylation enhances the association of IFI16 with Ran-GTP during virus infection which could be facilitating IFI16 cytoplasmic transport.

Previous *in vitro* studies suggested that acetylation of IFI16 NLS domain stabilizes its cytoplasmic retention and restricts its nuclear movement [27]. However, to determine whether the cytoplasmic IFI16 detected during KSHV *de novo* infection and latency represents newly synthesized IFI16 or redistributed from the nucleus, we used 50nM Leptomycin B (LPT) to block nuclear export to the cytoplasm. Indeed, LPT treatment abolished KSHV induced IFI16 cytoplasmic distribution although blocking of nuclear IFI16 export by LPT did not affect nuclear IFI16 acetylation or IFI16-inflammasome formation since no detectable changes were observed in the level of acetylated-IFI16 or IFI16-ASC association or caspase-1 cleavage. Interestingly, in the presence of cycloheximide (CHX), that blocks protein synthesis, a similar level of cytoplasmic IFI16 was seen in virus infected cells which ruled out the possibility that detection of acetylated IFI16 is due to accumulation of newly synthesized IFI16 in the cytoplasm. Taken together, these results demonstrated that a) blocking nuclear export by LPT did not interfere with the acetylation of IFI16, formation of IFI16-ASC complex or activation of caspase-1, b) blocking protein synthesis by CHX did not affect the cytoplasmic distribution of IFI16 from the nucleus, and c) the increased level of IFI16 in the cytoplasm in the infected cells was due to its redistribution from the nucleus and not due to newly translated cytoplasmic IFI16.

Inhibition of IFI16 acetylation impairs induction of the IFI16 innate inflammasome and interferon response during herpesvirus infection

Keeping in view of the similar pattern between acetylated IFI16 distribution and inflammasome activation in virus infected cells, we determined the correlation of IFI16 acetylation and inflammasome activation during *de novo* KSHV infection as well as KSHV latently infected cells. Both PLA and co-IP analysis detected prominent association between inflammasome sensor IFI16 and adaptor ASC in untreated virus infected cells, whereas, C-646 treatment significantly reduced such interaction. In addition, infection induced aggregation of IFI16, a trigger to the initiation of IFI16 inflammasome activation, was also compromised due to treatment of acetylation inhibitor C-646. Effect of acetylation inhibitor was further reflected to the downstream

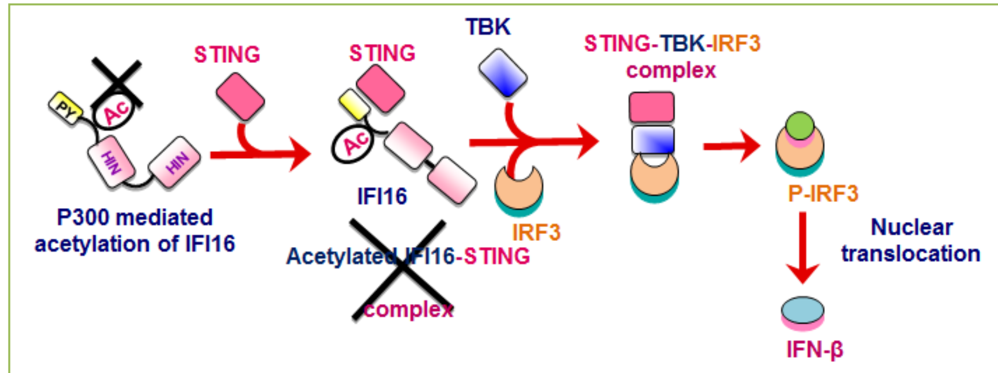

Figure 5. Acetylation of IFI16 is essential for IFI16-STING mediated Interferon beta response. Inhibition of IFI16 acetylation hinders IFI16-STING association, IRF3 phosphorylation and its nuclear translocation to produce IFN-β during herpes virus infection.

signal as we observed a very low level of cleaved caspase-1 and cleaved/mature IL-1β and IL-33 in KSHV infected cells compared to untreated virus infected cells. A Similar effect was also observed in p300 knockdown (si-RNA mediated) cells.

These results confirmed that acetylation of IFI16 promotes functional inflammasome assembly and activation (Figure 4). However, ASC knockdown did not result in detectable changes in overall IFI16 acetylation, although the cytoplasmic fraction of acetylated IFI16 was slightly reduced suggesting that ASC is dispensable for IFI16 acetylation which probably takes place prior to inflammasome formation and the IFI16-ASC inflammasome contributes to a portion of the IFI16 detected in the cytoplasm but inflammasome independent cytoplasmic distribution of IFI16 may be attributed to export alone or in complex with other protein(s).

With little or no information regarding post-translational modifications of IFI16 (such as acetylation) regulating virus induced interferon (IFN) response, we further analyzed the role of IFI16 acetylation in IFI16-STING-signalosome mediated IFN-β production during KSHV and HSV-1 infection although earlier reports suggest moderate IFN-β induction during *de novo* KSHV and HSV-1 infection and early lytic and/or latent gene products interfere with this response at latter times post infection [9, 16, 18, 22, 45]. At 4h post KSHV or 6h post HSV-1 *de novo* infection, IFN-β was detected by ELISA from the infected cell supernatant, which was significantly (> 3-4 folds) reduced upon C-646 treatment. Furthermore, IP, WB and IFA analysis showed the abrogation of IFI16-STING association, subsequent significant reduction of p-IRF3 and its nuclear localization as a result of treatment of acetylation inhibitor. However, STING knockdown had no effect on IFI16 acetylation implying that IFI16 acetylation is upstream to STING signalosome activation. Collectively, these studies unequivocally demonstrate that IFI16-acetylation is crucial for host antiviral IFI16-STING mediated IFN-β induction during herpesvirus early infection (Figure 5).

Overall, these comprehensive studies shed light into the so far unresolved question and highlight that (a) IFI16 acetylation occurs as a dynamic post-genome recognition event, and (b) post-acetylation, IFI16 probably moves away from the genome for the formation of its complexes and eventually leading to its cytoplasmic translocation.

Conclusions

The interesting and exciting avenues of viral-DNA sensing and the appearance of a number of viral DNA sensors with their associated host defense pathways have certainly expanded the emerging field; however mechanisms of many of their functions are still to be elucidated. For example, recent studies suggested that IFI16 and cGAS interact with each other and work together in the same signaling cascade during HSV-1 infection where IFI16 appears to be stabilized by cGAS although IFI16 seems to be the primary DNA sensor [46]. However, whether this interaction occurs in the nucleus or in the cytoplasm and the kinetics of such association after nuclear viral genome recognition remains to be determined. In addition, how the message of nuclear viral genome sensing by IFI16 in the presence of nuclear cGAS is transmitted to the cytoplasm for STING-signalosome activation is not clear.

Similar to AIM2, where an auto inhibited intramolecular complex state mediated by PYD and HIN domain interaction is liberated upon DNA binding, it could be possible that BRCA1 association to the PYD domain of IFI16 [26] could be liberating its auto inhibited complex state facilitating the HIN domains to sense viral genomes. The protective immune response triggered by the detection of microbial effectors termed “effector triggered immunity (ETI)” enables the host to distinguish pathogens from non-pathogen [2, 47] and this is

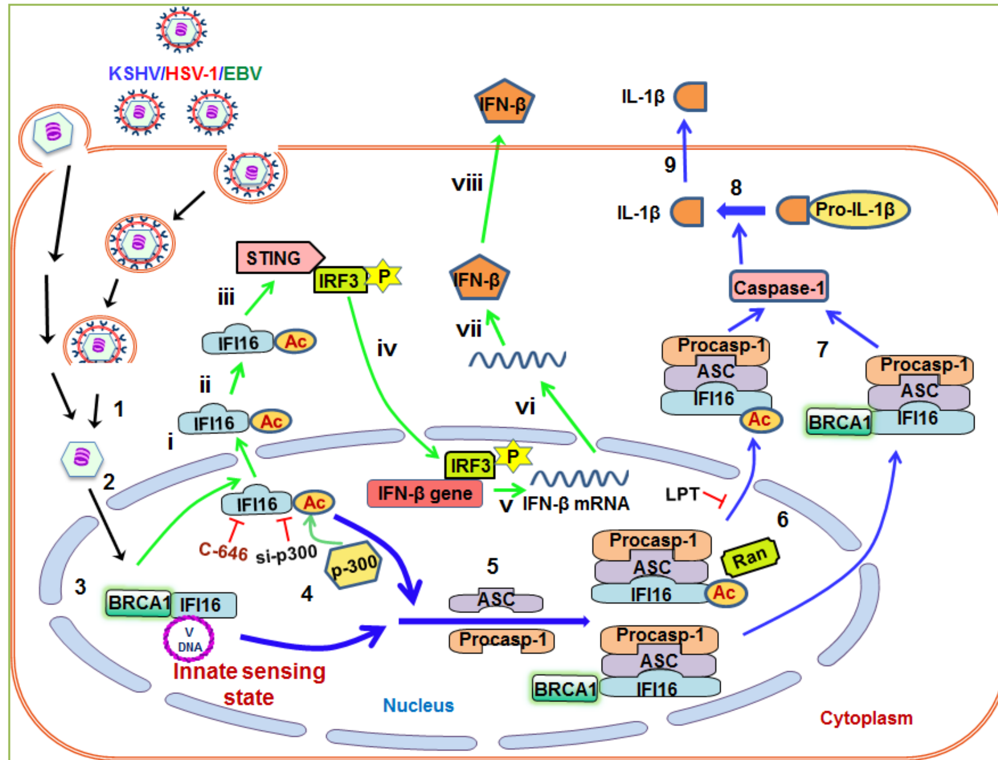

Figure 6. **Schematic model for the proposed BRCA1-mediated herpes viral DNA genome sensing by IFI16 and post-genome recognition IFI16 acetylation in innate inflammasome as well as interferon responses**. Following viral DNA entry into the nucleus (1 and 2) sequence-independent sensing of viral DNA by the DNA binding HIN-domain of IFI16 likely needs a pre-existing complex with BRCA1 via association with the PYD domain of IFI16, which predisposes IFI16 as a dedicated viral DNA sensor (innate sensing state), leading to IFI16 mediated innate immune activation, one arm of which results in IFI16-ASC-Caspase-1 inflammasome assembly and activation (3–7) while the other arm initiates cytoplasmic IFI16-STING-immune signaling, IRF3 phosphorylation and nuclear translocation resulting in IFN-β/type I IFN induction (i-viii). Nuclear IFI16 also undergoes post-genome recognition dependent acetylation by histone acetyl transferase p300 (4), facilitating ASC-caspase-1 association for inflammasome complex formation (5) and subsequent cytoplasmic translocation via Ran-GTP (6). Cytoplasmic distribution of acetylated IFI16 in one way induces inflammasomes by caspase-1 activation (7) followed by mature IL-1β production (8-9) and in the other way activates STING-signalosome dependent IFN-β production (i- viii). Use of p300 inhibitor C-646 and knockdown of p300 does not inhibit the ability of IFI16 to recognize the episomal viral DNA but impairs the acetylation of IFI16. Leptomycin B (LPT) treatment abrogates acetylated IFI16 translocation to the cytoplasm.

probably of relevance in non-professional immune cells too, such as epithelial, endothelial and other cells that come in contact with pathogens at different *in vivo* sites, including at the portal of pathogen entry. Our studies demonstrating that IFI16-BRCA1 functions as an innate sensor of KSHV, EBV and HSV-1 genomes in non-immune professional cells, such as endothelial, epithelial and fibroblast cells, and in B-lymphoma cell lines as well as the IFI16 and ASC interactions in tissue sections from PEL and KS patients [12], clearly suggest that these cells have evolved to respond to danger signals.

In addition, (i) association of IFI16 with latent chromatinized KSHV and EBV genomes, (ii) continuation of viral latent gene expression in the presence of IFI16 and IFI16-inflammasomes [12, 13], (iii) IFI16's ability to mediate global differences in HSV-1 genome chromatin modifications [17, 22], and (iv) the binding of IFI16 to the KSHV gene promoters in latently infected cells as detected in our ongoing studies, we theorize that IFI16 has very complex roles in gene regulation and there may be other factors involved in IFI16's ability to discriminate foreign vs. host DNA. Similar to the IFI16-BRCA1 complex involved in pathogen DNA recognition, inflammasome induction and IFN-β production shown here, it is possible that IFI16 could be forming distinct complexes with different proteins and each complex could be mediating distinct functions, such as transcription and other responses, which may differ between various virus nuclear lytic and latent infections and as per host cell types. Determining these possibilities and

deciphering whether BRCA1 plays a role in IFI16's ability to influence viral promoters and in the nuclear life cycles of DNA viruses requires further extensive studies.

Furthermore, there is limited information regarding the role of PTMs and the mediators of PTMs on the activation or inhibition of protein-DNA and protein-protein interactions and downstream immune signaling pathways during virus infection. Our concomitant study on IFI16-PTM suggests that post-genome recognition IFI16 acetylation in the nucleus by histone acetyl transferase p300 is necessary for IFI16-mediated innate response during herpes virus infection. However, why the IFI16-p300 interaction increases in the presence of herpes viral DNA and whether IFI16 recruits p300 directly or via its interaction with other proteins needs to be evaluated further. Although phosphorylation, acetylation and ubiquitination have been reported to modulate DNA sensing pathways, other PTMs are likely to play critical roles in the immune response and which of the site/s or domain/s undergo modifications during viral genome recognition needs to be examined further. Since IFI16 has also been suggested to act as a viral transcriptional repressor during HCMV and HSV-1 infection whether the regulatory functions of these genes are dependent or independent of acetylation or other PTMs need to be determined.

Given the ubiquitous expression of many of these DNA binding proteins and the significant potential for endogenous DNA to engage these molecules, it is important that DNA recognition is tightly regulated. It becomes increasingly necessary to understand their possible coordinated or redundant functions and how these immune signaling pathways are regulated depending upon cell type variability where one or multiple pathways could be active. In view of our concurrent studies which enlighten the crucial role of BRCA1 as a cofactor regulating viral DNA sensing of IFI16 and p300 mediated IFI16 acetylation as a post-genome recognition event (Figure 6) a broader impact could be envisioned in terms of other DNA sensors and their possible PTMs in other host-virus interactions and subsequent innate or intrinsic responses. Additional studies with increasing implementation of modern new technologies will certainly provide better understanding of the molecular mechanisms of BRCA1 and IFI16 along with other DNA sensors and the role of PTMs in the fundamental relation between DNA sensing and antiviral response, viral and host genome regulation and the dynamic nature of protein interactions during infection for future viral intervention strategies.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Takeuchi O and Akira S. Pattern recognition receptors and inflammation. Cell 2010; 140: 805-20.
2. Blander JM and Sander LE. Beyond pattern recognition: five immune checkpoints for scaling the microbial threat. Nat Rev Immunol 2012; 12: 215-225.
3. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, *et al*. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science 2009; 323:1057-1060.
4. Barbalat R, Ewald SE, Mouchess ML, Barton GM. Nucleic acid recognition by the innate immune system. Annu Rev Immunol 2011; 29:185- 214.
5. Jin T, Perry A, Jiang J, Smith P, Curry J, Unterholzner L, *et al*. Structures of the HIN domain: DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. Immunity 2012; 36: 561-571.
6. Ru H, Ni X, Zhao L, Crowley C, Ding W, Hung L-W, *et al*. Structural basis for termination of AIM2-mediated signaling by p202. Cell Res 2013; 23: 855-858.
7. Brunette RL, Young JM, Whitley DG, Brodsky IE, Malik HS, Stetson DB. Extensive evolutionary and functional diversity among mammalian Aim2-like receptors. J Exp Med 2012; 209:1969-1983.
8. Atianand MK and Fitzgerald KA. Molecular basis of DNA recognition in the immune system. J Immunol 2013; 190: 1911-1918.
9. Unterholzner L, Keating SE, Baran M, Horan KA, Jens en SB, Sharma S, *et al*. IFI16 is an innate immunesensor for intracellular DNA. Nat Immunol 2010; 11: 997-1004.
10. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, *et al*. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma associated herpesvirus infection. Cell Host Microbe 2011; 9: 363-375.
11. Gariano GR, Dell'Oste V, Bronzini M, Gatti D, Luganini A, De Andrea M, *et al*. The intracellular DNA sensor IFI16 gene acts as restriction factor for human Cytomegalovirus replication. PLoS Pathog 2012; 8: e1002498.
12. Singh VV, Kerur N, Bottero V, Dutta S, Chakraborty S, Ansari MA, *et al*. Kaposi's Sarcoma-Associated Herpesvirus latency in endothelial and B Cells activates gammainterferon-inducible protein 16-mediated inflammasomes. J Virol 2013; 87: 4417-4431.
13. Ansari MA, Singh VV, Dutta S, ValiaVettil M, Dutta D, Chikoti L, *et al*. Constitutive interferon-inducible protein 16-inflammasome activation during Epstein-Barr virus latency I, II,and III in B and epithelial cells. J Virol 2013; 87: 8606-9623.
14. Johnson KE, Chikoti L, Chandran B. Herpes Simplex Virus 1 infection induces activation andsubsequent inhibition of the IFI16 and NLRP3 inflammasomes. J Virol 2013; 87: 5005-5018.
15. Cuchet-Lourenco D, Anderson G, Solan E, Orr A, Evert RD. The viral ubiquitin ligase ICP0 is neither sufficient nor necessary for degradation the cellular DNA sensor IFI16 during herpex simplex virus 1 infection. J Virol 2013; 87: 13422-13432.
16. Orzalli MH, DeLuca NA, Knipe DM. Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. Proc Natl Acad Sci U S A 2012; 109:E3008-17.
17. Orzalli MH, Conwell SE, Berrios C, Decaprio JA, Knipe DM. Nuclear Interferon-Inducible protein 16 promotes silencing of herpesviral and transfected DNA Proc Natl Acad Sci U S A 2013; 110: E4492-4501.

18. Horan KA, Hansen K, Jakobsen MR, Holm CK, Soby S, Unterholzner L, *et al*. Proteasomal degradation of Herpes Simplex Virus capsids in macrophages releases DNA to the cytosol for recognition by DNA sensors. J Immunol 2013; 190:2311-2319.
19. Orzalli MH and Knipe DM. Cellular sensing of viral DNA and viral evasion mechanisms. Annu Rev Microbiol 2014; 68: 477-492.
20. Connolly DJ and Bowie AG. The emerging role of human PYHIN proteins in innate immunity: implications for health and disease. Biochem Pharmacol 2014; pii: S0006-2952(14)00521-8.
21. Jakobsen MR and Paludan SR. IFI16: at the interphase between innate DNA sensing and genome regulation. Cytokine Growth Factor Rev 2014; pii: S1359-6101(14)00058-6.
22. Johnson KE, Bottero V, Flaherty S, Dutta S, Singh VV, *et al*. IFI16 Restricts HSV-1 replication by accumulating on the HSV-1 genome, repressing HSV-1 gene expression, and directly or indirectly modulating histone modifications. PLoS Pathog 2014; 10: e1004503.
23. Dutta D, Dutta S, Valiya Veettil M, Roy A, Ansari MA, Iqbal J, *et al*. BRCA1 Regulates IFI16 Mediated Nuclear Innate Sensing of Herpes Viral DNA and Subsequent Induction of the Innate Inflammasome and Interferon-β Responses. PLoS Pathog 2015; 11(6): e1005030.
24. Ansari MA, Dutta S, Valiya Veettil M, Dutta D, Iqbal J, Kumar B, *et al*. Herpesvirus Genome Recognition Induced Acetylation of Nuclear IFI16 Is Essential for Its Cytoplasmic Translocation, Inflammasome and IFN-β Responses. PLoS Pathog 2015; 11(7): e1005019.
25. Johnstone RW, Wei W, Greenway A, Trapani JA. Functional interaction between p53 and the Interferon-Inducible nucleoprotein IFI 16. Oncogene 2000; 19: 6033-6042.
26. Aglipay JA, Lee SW, Okada S, Fujiuchi N, Ohtsuka T, Kwak JC, *et al*. A member of the Pyrin family, IFI16, is a novel Brca1-associated protein involved in the p53-mediated apoptosis pathway. Oncogene 2003; 22: 8931-8938.
27. Li T, Diner BA, Chen J, Cristea IM. Acetylation modulates cellular distribution and DNA sensing ability of interferon inducible protein IFI16. Proc Natl Acad Sci U S A 2012; 109: 10558-10563.
28. Albrecht M, Choubey D, Lengauer T. The HIN domain of IFI-200 proteins consists of two OB folds. Biochem Biophys Res Commun 2005; 327:679-676.
29. Johnstone RW, Kershaw MH, Trapani JA. Isotypic variants of the interferon inducible transcriptional repressor IFI 16 arise through differential mRNA splicing. Biochemistry 1998; 37:11924-11931.
30. Xin H, Curry J, Johnstone RW, Nickoloff BJ, Choubey D. Role of IFI16, a member of the Interferon-inducible p200-protein family, in prostate epithelial cellular senescence. Oncogene 2003; 22: 4831-4840.
31. Zhang Y, Howell RD, Alfonso DT, Yu J, Kong L, Wittig JC, *et al*. IFI16 inhibits tumorigenicity and cell proliferation of bone and cartilage tumor cells. Front. Biosci 2007; 12: 4855-4863.
32. Gugliesi F, De Andrea M, Mondini M, Cappello P, Giovarelli M, Shoenfeld Y, *et al*. The proapoptotic activity of the interferon-inducible gene IFI16 provides new insights into its etiopathogenetic role in autoimmunity. J Autoimmun 2010; 35: 114-123.
33. Song LL, Ponomareva L, Shen H, Duan X, Alimirah F, Choubey D. Interferon-Inducible IFI16, a negative regulator of cell growth, down-regulates expression of human telomerase reverse transcriptase (hTERT) Gene. PLoS One 2010; 5: e8569.
34. Costa S, Borgogna C, Mondini M, De Andrea M, Meroni PL, Berti E, *et al*. Redistribution of the nuclear protein IFI16 into the cytoplasm of ultraviolet B exposed keratinocytes as a mechanism of autoantigen processing. Br J Dermatol 2011; 164: 282-290.
35. Gariglio M, Mondini M, De Andrea M, Landolfo S. The multifaceted Interferon-inducible P200 family proteins: from cell biology to human pathology. J Interferon Cytokine Res 2011; 31: 159-172.
36. Caneparo V, Cena T, De Andrea M, Dell'Oste V, Stratta P, Quaglia M, *et al*. Anti-IFI16 antibodies and their relation to disease characteristics in Systemic Lupus Erythematosus. Lupus. 2013; 22: 607-613.
37. Hansen K, Prabakaran T, Laustsen A, Jorgensen SE, Rahbek SH, Jensen SB, *et al*. Listeria monocytogenes induces IFNb expression through an IFI16, cGAS, and STING dependent pathway. EMBO J 2014; pii: e201488029.
38. Dell'Oste V, Gatti D, Gugliesi F, De AM, Bawadekar M, Lo CI, *et al*. Innate nuclear sensor IFI16 translocates into the cytoplasm during the early stage of in vitro human cytomegalovirus infection and is entrapped in the egressing virions during the late stage. J Virol 2014: 88: 6970-6982.
39. Choubey D and Lengyel P. Interferon action: nucleolar and nucleoplasmic localization of the interferon-inducible 72-kDa protein that is encoded by the Ifi 204gene from the gene 200 cluster. J Cell Biol 1992; 116: 1333-1341.
40. Knipe DM, Lieberman PM, Jung JU, McBride AA, Morris KV, Ott M, Margolis D, Nieto A, Nevels M,Parks RJ, Kristie TM. Snapshots: chromatin control of viral infection. Virology 2013; 435:141-156.
41. Turnell AS and Grand RJ. DNA viruses and the cellular DNA-damage response. J Gen Virol 2012; 93: 2076-2097.
42. Singh VV, Dutta D, Ansari MA, Dutta S, Chandran B. Kaposi's Sarcoma Associated Herpesvirus induces the ATM and H2AX DNA damage response early during de novo infection of primary endothelial cells, which play roles in latency establishment. J Virol 2014; 88: 2821-2834.
43. Ouchi M and Ouchi T. Role of IFI16 in DNA damage and checkpoint. Front Biosci 2008; 13: 236-239.
44. Lu B, Wang H, Andersson U, Tracey KJ. Regulation of HMGB1 release by inflammasomes. Protein Cell 2013; 4: 163-167.
45. Sathish N and Yuan Y. Evasion and subversion of interferon-mediated antiviral immunity by Kaposi's sarcoma-associated herpesvirus: an overview. J Virol 2011; 85: 10934-10944.
46. Orzalli MH, Broekema NM, Diner BA, Hancks DC, Elde NC, Cristea IM, *et al*. cGAS-mediated stabilization of IFI16 promotes innate signaling during herpes simplex virus infection. Proc Natl Acad Sci U S A 2015; E1773-E1781.
47. Vance RE, Isberg RR, Portnoy DA. Patterns of pathogenesis: discrimination of pathogenic and non-pathogenic microbes by innate immune system. Cell Host Microbe 2009; 6: 10-21.
48. Tabeta K, Georgel P, Janssen E, Du X, Hoebe K, Crozat K, *et al*. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc Natl Acad Sci U S A 2004; 101:3516-3521.
49. Sato A, Linehan MM, Iwasaki A. Dual recognition of herpes simplex viruses by TLR2 and TLR9 in dendritic cells. Proc Natl Acad Sci 2006; 103:17343-17348.
50. Zhu J, Huang X, Yang Y. Innate immuneresponse to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. J Virol 2007; 81: 3170-80.

51. Rasmussen SB, Jensen SB, Nielsen C, Quartin E, Kato H, Chen ZJ, *et al*. Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene- like receptors, which synergize to induce type I interferon production. J Gen Virol 2009; 90: 74-78.
52. DeFilippis VR, Alvarado D, Sali T, Rothenburg S, Früh K. Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. J Virol 2010; 84:585-598.
53. Upton Jason W, Kaiser William J, Mocarski Edward S. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virusinduced programmed necrosis that is targeted by murine cytomegalovirus vIRA. Cell Host Microbe 2012; 11: 290-297.
54. Kim T, Pazhoor S, Bao M, Zhang Z, Hanabuchi S, Facchinetti V, *et al*. Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells. Proc Natl Acad Sci 2010; 107:15181-15186.
55. Stein SC, Falck-Pedersen E. Sensing adenovirus infection: activation of interferon regulatory factor 3 in RAW 264.7 cells. J Virol 2012; 86: 4527-4537.
56. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu Y-J. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nat Immunol 2011; 12: 959-965.
57. Miyashita M, Oshiumi H, Matsumoto M, Seya T. DDX60, a DEXD/H box helicase, is a novel antiviral factor promoting RIG-I-like receptor-mediated signaling. Mol Cell Biol 2011; 31: 3802-3819.
58. Chiu Y-H, MacMillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell 2009; 138:576-591.
59. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat Immunol 2009; 10:1065-1072.
60. Peters NE, Ferguson BJ, Mazzon M, Fahy AS, Krysztofinska E, Arribas-Bosacoma R, *et al*. A mechanism for the inhibition of DNA-PK-mediated DNA sensing by a virus. PLoS Pathog 2013; 9:e1003649
61. Zhang X, Brann TW, Zhou M, Yang J, Oguariri RM, Lidie KB, *et al*. Cutting edge: Ku70 is a novel cytosolic DNA sensor that induces type III rather than type I IFN. J Immunol 2011; 186: 4541-4545.
62. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. In: Medzhitov R, editor. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. eLife 2012; 1: e00047.
63. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 2009; 458:509-513.
64. Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, *et al*. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat Immunol 2010; 11:395-402.
65. Reinholz M, Kawakami Y, Salzer S, Kreuter A, Dombrowski Y, Koglin S, *et al*. HPV16 activates the AIM2 inflammasome in keratinocytes. Arch Dermatol Res 2013; 305:723-732.
66. Zhen J, Zhang L, Pan J, MaS, Yu X, Li X, *et al*. AIM2 mediates inflammation-associated renal damage in hepatitis B virus associated glomerulonephritis by regulating caspase-1, IL-1β and IL-18. Mediators Inflamm 2014; 2014:190860.
67. Jin T, Latz E, Xiao TS, Fitzgerald KA, Paludan SR, Bowie AG. IFI16 is an innate immunesensor for intracellular DNA. Nat. Immunol 2010; 11: 997-1004.
68. Diner BA, Li T, Greco TM, Crow MS, Fuesler JA, Wang J, *et al*. The functional interactome of PYHIN immune regulators reveals IFIX is a sensor of viral DNA. Mol Syst Biol 2015; 11: 787.
69. Lam E, Stein S, Falck-Pedersen E. Adenovirus detection by the cGAS/STING/TBK1 DNA sensing cascade. J Virol 2013; 88:974-981.
70. Dai P, Wang W, Cao H, Avogadri F, Dai L, Drexler I, *et al*. Modified vaccinia virus Ankara triggers type I IFN production in murine conventional dendritic cells via a cGAS/STING mediated cytosolic DNA-sensing pathway. PLoS Pathog 2014; 10: e1003989.