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Mini review

Mislocalization of the interferon inducible protein IFI16 by environmental insults: Implications in autoimmunity

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ABSTRACT

The nuclear DNA sensor IFI16, a member of PYHIN family of proteins, was previously studied for its role in cell cycle regulation, tumor suppression, apoptosis and DNA damage signaling. Autoantibodies against IFI16 are prevalent in the sera of patients with systemic autoimmunity, thus depicting physiological significance as an autoantigen. At present, the nuclear IFI16 protein has been thoroughly investigated for its role as an innate immune sensor involved in inflammasome signaling and viral restriction. While the sub-cellular localization of IFI16 during such events has been known, very little knowledge about its presence and significance in the extracellular space is available. Recently our group has discovered the presence of circulating IFI16 in the sera from systemic autoimmune patients indicating that in this setting it may be mislocalized form its nuclear site and secreted in the extracellular milieu. In this review, we will discuss the leakage of endogenous IFI16 that has been experimentally proved using in vivo and in vitro models. Also we will comment on the significance of mislocalized inflammasome components in the extracellular space and how it can be responsible for chronic inflammation.

1. Introduction: the interferon inducible 16 (IFI16) protein

The IFI16 protein was discovered two decades ago as a protein constitutively expressed in the nucleus and nucleoli of lymphoid cells that can be induced by Interferons in myeloid cells [1–3]. Such interferon-induced expression of IFI16 is transcriptionally controlled by AP-1 [4]. It is naturally present in 3 isoforms (A, B and C) that arise due to differential mRNA splicing; of which, the 729 amino acid IFI16-B isoform is most abundant [5]. While previously IFI16 expression was considered to be limited to hematopoietic cells, later it was discovered also in vascular endothelial cells and keratinocytes [6]. Primarily, IFI16 is associated with myeloid cell differentiation [7], p53 mediated control of tumorigenicity [8,9], transcriptional regulation [10], inhibition of cell proliferation [11] and DNA damage signaling [12,13]. Overexpression of IFI16 drives early steps of an inflammatory response through NF-κB mediated secretion of proinflammatory molecules such as ICAM1, E-selectin, IL-8 and MCP-1 [14,15]. Autoantibodies against IFI16 are present in the sera of patients with systemic sclerosis (SSc) [16], systemic lupus erythematosus (SLE) [17,18], rheumatoid arthritis (RA) [19,20] and Sjögren's Syndrome (SS) [19]. Recently, it has also been discovered as serum circulating protein in the same autoimmune diseases [21]. Overexpression of the IFI16 autoantigen in the epidermis and inflammatory dermal infiltrates is quite evident in skin biopsies from SLE and SSc patients, while the expression levels of anti-IFI16 autoantibodies can also be found and being exploited to diagnose and differentiate between limited and diffuse cutaneous SSc [16,22]. In a statistical study, 29% SSc and 63% SLE population tested positive for significantly higher anti-IFI16 autoantibodies, while this data could be correlated to disease characteristics [22,23]. The exact involvement of IFI16 in the development of autoimmunity remains obscure, but several data implicate its role in onset of inflammation when overexpressed in endothelial cells including its role in apoptosis through the NF-κB mediated activation of caspase2 and caspase3 [24].

IFI16 is an Absent in Melanoma (AIM2)-like receptor (ALR) [25,26] which belongs to the PYHIN family of proteins, a group of Interferon-Inducible proteins which also includes AIM2, MNDA (Myeloid cell nuclear differentiation antigen), PYHIN1 (PYRIN + -HIN-1) and POP3 (PYRIN domain only protein 3) encoded by the PYHIN gene cluster on chromosome 1q23 [25,27–29]. These

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proteins consist of a common PYRIN or PYD domain at the N-terminal region, which is followed by either one or two consecutive 200-amino acid DNA binding HIN domains [30,31]. The pathogenic-foreign or -self DNA binding and recognition ability of HIN-200 domain in these proteins classify them as pattern recognition receptors (PRRs) and innate immune sensors [27,30], forming center stage of inflammasome signaling. Moreover, the PYRIN domain is known to bind the pro-apoptotic speck protein ASC, which induces the formation of large cytoplasmic "specks" in cells and activates procaspase-1 which during pathogen DNA sensing is cleaved to caspase-1, leading to the production and secretion of IL-1β from pro-IL-1β. The innate immune sensing activity of IFI16 was first identified when it was found to be associated with IFN-β-inducing vaccinia viral DNA motifs in an experimental setup. While this complex further recruits STING mediated activation of IFN genes, the absence of IFI16 prevented HSV-1 mediated activation of IRF3 and NF-κB transcription factors [26]. This explained the DNA sensing activity of IFI16 residing in the cytoplasm like other inflammasome components including its putative murine counterpart p204. It is now clear that IFI16, in its nuclear location, can also sense KSHV DNA within the nucleus leading to inflammasome activation [32]. During this scenario, IFI16 interacts with ASC and translocate to the cytosol leading to the activation of caspase-1, which processes pro-IL-1β to active IL-1β.

2. IFI16 as pattern recognition receptor

The innate immune system monitors the presence of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) by germline-encoded pattern recognition receptors (PRRs) that comprise of Toll-like receptors (TLRs), the retinoic acid inducible gene-like receptors (RLRs) and the nucleotide oligomerisation domain-like receptors (NLRs) [33–35]. New discoveries related to the functional aspects of intracellular DNA sensing have led to the formation of new group of PRRs, namely the AIM2-like receptors, which includes AIM2 and IFI16 [26,35,36].

The pattern recognition aspect of IFI16 depends on whether it is able to sense and distinguish between self and non-self DNA at molecular level. IFI16 has been recognized as an innate immune sensor of dsDNA, sensing pathogenic dsDNA independent of sequence and GC content [26]. Such sequence-independent DNA binding is established by electrostatic forces between positively charged HIN domain residues and the dsDNA sugar phosphate backbone which allows IFI16 to sense dsDNA from Kaposi's sarcoma-related herpesvirus (KSHV), Epstein Barr virus (EBV), herpes simplex virus 1 (HSV-1) and human immunodeficiency virus (HIV-1) [32,37–39]. The consecutive HIN domains of IFI16, especially HIN-b domain with stronger DNA binding affinity [40], together play an important role in viral DNA recognition in a length dependent manner, comprised of 150 bp fragments eventually forming an optimal binding cluster of approximately ten IFI16 protomers [41]. Recent study describes that IFI16 cooperatively assembles into filaments on dsDNA, which has been proposed as an integral mechanism to detect foreign DNA [41]. Such assembly and filament formation is mediated by non-DNA binding PYD-domain of IFI16. Moreover, this study also suggests that IFI16 clusters into signaling foci in a switch-like manner and is capable of using the size of naked dsDNA as a molecular ruler to distinguish self from non-self [41]. Such recognition later leads to the activation of STING-TBK1 pathway leading to the production of IFN-β [41]. While it has been known that DNA recognition by IFI16 occurs in both nucleus and cytoplasm, recently, it has also been discovered that acetylation at two different sites on the nuclear localization signal (NLS), situated on the N-terminal region of IFI16, regulates the nucleo-cytoplasmic localization of IFI16 [42]. The NLS of IFI16 is required for the nuclear localization that, when acetylated at lysine residues present at position 99 and 128, critically inhibits the nuclear import [42]. Such structural studies elucidating the DNA binding and oligomerisation functions of IFI16 have improved our understanding on non-self DNA sensing and IFI16 localization, but few more questions concerning the nuclear/cytosolic interaction of STING and IFI16 and activation of inflammasome remain unanswered.

3. Extracellular IFI16 as an 'alarmin'

By now, we know that the IFI16 protein is overexpressed in several systemic autoimmune diseases and its specific autoantibodies are prevalent in the sera of these patients, thus linking this autoantigen to the pathogenesis of autoimmune diseases. Despite that, the exact molecular mechanism underlying the role of IFI16 in the initiation and progression of autoimmune syndromes are still unclear. Numerous data indicate the disease specific mislocalization of nuclear IFI16 in an inflammatory setting, while our group has recently demonstrated the occurrence of free IFI16 protein in the sera of autoimmune patients [21]. IFI16 has already shown to activate numerous inflammatory responses when overexpressed inside the cells, which raises potential hypothesis on the activity of IFI16 also extracellularly. The N-terminal PYRIN domain of IFI16 is known to be involved in apoptosis, inflammation and protein interactions with tumor regulators including p53 and BRCA1 [43]. When such protein is exposed to the extracellular environment, it may have potential interactions with components of innate immune responses, especially the PRRs. Our recent work in this direction suggests that IFI16, when secreted extracellularly by endothelial cells, interacts with neighboring cells through high affinity membrane binding affecting primary endothelial cell function. This activity is suppressed when the PYRIN domain on IFI16 is masked by anti-IFI16-N-terminal antibodies [21]. Such behavior relates IFI16 to be a potential alarmin or damage causing endogenous mislocalized molecule, similar to DAMPs, which has major contribution in the development of chronic inflammation and autoimmunity.

4. Passive leakage of endogenous IFI16

Environmental factors such as viral infection, stress, injury, and UV light are sufficient to expose exogenous PAMPs and endogenous self-molecules DAMPs to the immune system through active/passive release. These molecules interact with PRRs such as the TLRs to activate NF-κB-like transcription factors leading to the secretion of multiple inflammatory cytokines resulting in chronic inflammation [44,45]. IFI16 has been known to compartmentalize between nucleus, cytoplasm and extracellular space. Here we discuss different physical insults resulting into IFI16 mislocalization as summarized in Fig. 1.

4.1. Viruses

The viral DNA sensing activity of IFI16 resides within the nucleus [32], while it has been demonstrated that during early and late stages of viral infection, IFI16 moves from the nucleus to cytoplasm as a consequence of viral defence mechanism to inactivate IFI16 restriction activity [46]. During KSHV inflammasome signaling, IFI16 senses viral DNA within the nucleus of latently infected cells, leading to the recruitment of adapter protein ASC and pro-caspase-1, while this inflammasome complex further translocates to the cytoplasm and leads to caspase-1 activation and cleavage of pro-IL-1β and pro-IL-18 to their mature forms. Later, cytoplasmic IFI16 and the active form of IL-1β are