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Review

Interferon-inducible p200-family protein IFI16, an innate immune sensor for cytosolic and nuclear double-stranded DNA: Regulation of subcellular localization

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ABSTRACT

The interferon (IFN)-inducible p200-protein family includes structurally related murine (for example, p202a, p202b, p204, and Aim2) and human (for example, AIM2 and IFI16) proteins. All proteins in the family share a partially conserved repeat of 200-amino acid residues (also called HIN-200 domain) in the C-terminus. Additionally, most proteins (except the p202a and p202b proteins) also share a protein–protein interaction pyrin domain (PYD) in the N-terminus. The HIN-200 domain contains two consecutive oligosaccharide/oligonucleotide binding folds (OB-folds) to bind double stranded DNA (dsDNA). The PYD domain in proteins allows interactions with the family members and an adaptor protein ASC. Upon sensing cytosolic dsDNA, Aim2, p204, and AIM2 proteins recruit ASC protein to form an inflammasome, resulting in increased production of proinflammatory cytokines. However, IFI16 protein can sense cytosolic as well as nuclear dsDNA. Interestingly, the IFI16 protein contains a nuclear localization signal (NLS). Accordingly, the initial studies had indicated that the endogenous IFI16 protein is detected in the nucleus and within the nucleus in the nucleolus. However, several recent reports suggest that subcellular localization of IFI16 protein in nuclear versus cytoplasmic (or both) compartment depends on cell type. Given that the IFI16 protein can sense cytosolic as well as nuclear dsDNA and can initiate different innate immune responses (production of IFN-β versus proinflammatory cytokines), here we evaluate the experimental evidence for the regulation of subcellular localization of IFI16 protein in various cell types. We conclude that further studies are needed to understand the molecular mechanisms that regulate the subcellular localization of IFI16 protein.

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1. The p200-family protein IFI16

Interferon (IFN)-inducible p200 family proteins in humans include IFI16, MNDA, IFIX, and AIM2 (encoded by the *IFI16*, *MNDA*, *IFIX*, and *AIM2* genes) (Asefa et al., 2004; Choubey et al., 2008; Johnstone and Trapani, 1999; Ludlow et al., 2005; Mondini et al., 2010; Ouchi and Ouchi, 2008). These proteins share a partially conserved repeat of 200-amino acid residues (the HIN-200 domain) towards the C-terminus, which allows these proteins to bind dsDNA (Dawson and Trapani, 1995b; Yan et al., 2008). Most p200-family proteins (except the murine p202a and p202b proteins) also contain a homotypic protein–protein interaction PYRIN domain (PYD) in the N-terminus.

The *IFI16* gene encodes three isoforms (A, B, and C) of the IFI16 protein through an alternative splicing of mRNA (Johnstone and Trapani, 1999; Choubey et al., 2008). The B form of IFI16 protein is the predominant form that is detected in human normal prostate epithelial cells and fibroblasts. The IFI16 protein contains two repeats (the repeat A and B) of 200-amino acid residues (or HIN-200 domain) and a serine–threonine–proline (S/T/P)-rich spacer region separates the two repeats (Fig. 1). The size of the spacer region in the IFI16 protein is regulated by mRNA splicing and can contain one, two, or three copies of highly conserved 56-amino acids S/T/P domain encoded by distinct axons. The N-terminus of IFI 16 protein contains a PYD (Choubey et al., 2010).

Accumulated experimental evidence has attributed diverse functions to the p200-family proteins ranging from transcriptional regulation, apoptosis, cell growth regulation, autoimmunity, viral resistance, inflammasome assembly in response to cytosolic dsDNA, and cell differentiation (Johnstone and Trapani, 1999;

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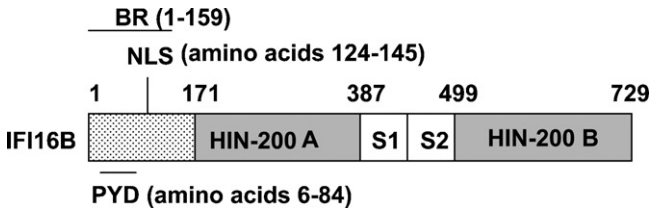

Fig. 1. Schematic representation of structural and functional domains in IFI16 protein. Light dotted area in the amino terminus includes the basic region (BR; amino acid residues 1-159), which is sufficient to bind dsDNA *in vitro*, a PYD domain (amino acid residues 6-84), and a nuclear localization signal (NLS; amino acid residues 124-145). The dark gray boxes in the IFI16 protein denote a type-A and a type-B 200-AA repeat (or HIN-200 domain), respectively (Ludlow et al., 2005). White boxes (the S1 and S2) between the two repeats denote two spacer regions in the IFI16B protein.

Ludlow et al., 2005; Choubey et al., 2008; Ouchi and Ouchi, 2008; Mondini et al., 2010). Interestingly, the PYD domain of the AIM2 protein has been shown to heterodimerize with an adaptor protein ASC in response to cytoplasmic dsDNA to form the AIM2 inflammasome (Choubey et al., 2010; Fernandes-Alnemri et al., 2009). Moreover, upon sensing cytosolic dsDNA, the IFI16 protein has been reported to recruit the *st*imulator of *in*terferon genes (STING) protein to stimulate the expression of IFN-β through the activation of the transcriptional activity of IRF3 and NF-κB (Unterholzner et al., 2010). Accordingly, a recent study noted that IFI16 protein may play a role in human dendritic cell activation by dsDNA and in the subsequent activation of the adaptive immune system (Kis-Toth et al., 2011). However, during the Kaposi Sarcoma-associated herpesvirus (KSHV) infection of human endothelial cells, IFI16 protein is shown to interact with the ASC protein through the PYD to form a functional IFI16-ASC inflammasome (Kerur et al., 2011). This protein complex containing the IFI16 protein was initially detected in the nucleus and then in the perinuclear area. Given that ASC adaptor protein is detected primarily in the nucleus in resting human monocytes/macrophages (Bryan et al., 2009) and, upon infection, it redistributes to the cytosol (Bryan et al., 2009), it is likely that interactions between ASC and IFI16 proteins through the PYD in the nucleus contribute to the translocation of IFI16 protein to the cytoplasm.

The HIN-200 domain in the p200-family proteins consists of two oligonucleotide/oligosaccharide binding folds (OB-folds), which recognizes nucleic acids (Albrecht et al., 2005). Accordingly, the AIM2 protein requires this domain to sense cytosolic dsDNA and to assemble an inflammasome (Choubey et al., 2010). Similarly, the IFI16 protein can bind both single and double-stranded DNA *in vitro* (Dawson and Trapani, 1995b; Yan et al., 2008).

Expression of IFI16 protein is detectable in epithelial cells, fibroblasts, endothelial cells, and cells of the hematopoietic origin (Choubey et al., 2008; Johnstone and Trapani, 1999; Ouchi and Ouchi, 2008; Kis-Toth et al., 2011). Subcellular localization of IFI16 protein has been examined in different cell types (primary cells and cancer cell lines) and the primary tissues (Johnstone and Trapani, 1999). In contrast to the exclusive nuclear or cytoplasmic localization pattern of the other 200-family proteins, the IFI16 protein has been detected in the nucleus (within nucleus, both in the nucleolus and nucleoplasm), cytoplasm, or both (Table 1). As the subcellular localization of IFI16 protein is likely to determine the nature of an innate immune response (production of IFN-β versus activation of an inflammasome) following sensing of dsDNA, we decided to review the experimental evidence for the regulation of subcellular localization of IFI16 protein. Here we evaluate the experimental evidence for the regulation of subcellular localization of IFI16 protein. Additionally, we discuss various factors that are likely to regulate the subcellular localization of the IFI16 protein, thus, its functions.

2. Subcellular localization of IFI16 protein

As noted above, the amino terminus of IFI16 protein contains a bi-partite *n*uclear *l*ocalization signal (NLS; Briggs et al., 2001). Accordingly, a study noted that nuclear localization signal in IFI16 protein is sufficient to drive the nuclear localization of the β-Gal fusion protein (Briggs et al., 2001). However, the study noted the following deviations from the conventional nuclear import mechanisms: (i) the lack of strong binding of IFI16 NLS-fusion proteins with the importin heterodimers; (ii) the requirement for ATP, but not the cytosolic factors, for the nuclear import; and (iii) the Ran-independent import of the IFI16NLS-fusion proteins in the nucleus. Additionally, the study noted that the IFI16NLS fusion protein interacted with the Casein kinase 2 (CK2) and the CK2 phosphorylation site in the IFI16 protein also regulated the extent of the nuclear accumulation as well as the nuclear retention of

Table 1
Subcellular localization of IFI16 protein in various cell types.

Localization	Approach	Cell line (s)/tissue	Protein detected	Reference
Nuclear	CF[a]	HL-60 (a leukemia line) treated with IFN-γ	Endogenous	(Dawson and Trapani, 1995a)
Nuclear	IHC	Peripheral blood leukocytes	Endogenous	(Dawson and Trapani, 1995a)
Nuclear (nucleolar)	IIF	Peripheral blood mononuclear cells	Endogenous	(Dawson and Trapani, 1995b)
Nuclear	IIF	HL-60 (a leukemia line) treated with IFN-γ	Endogenous	(Dawson and Trapani, 1995b)
Nucleolus	CF	Daudi (a lymphoma line)	Endogenous	(Dawson and Trapani, 1995b)
Cytoplasmic and nuclear	IIF	HTC rat hepatoma tissue-culture line	Overexpressed fusion protein	(Briggs et al., 2001)
Nuclear (nucleolar)	IIF	HCC1937 (a breast cancer line with BRCA1 mutation)	Endogenous	(Aglipay et al., 2003)
Cytoplasmic and nuclear	IIF	PC-3 (a prostate cancer line) and PrECs	Endogenous	(Xin et al., 2003)
Cytoplasmic and nuclear	IIF	A431 (a skin carcinoma line)	Endogenous	(Barbe et al., 2008)
Nuclear and cytoplasmic	IIF	U2OS (an osteosarcoma line)	Endogenous	(Barbe et al., 2008)
Nuclear and nucleolus	IIF	U-251 MG (a glioma cell line)	Endogenous	(Barbe et al., 2008)
Nuclear	IIF	293 T (a human kidney transformed cell line)	Overexpressed fusion protein	(Hornung et al., 2009)
Nuclear	IIF	HeLa (a cervical cancer line with HPV-18)	Overexpressed fusion protein	(Burckstummer et al., 2009)
Nuclear and nucleolus	IIF	Primary human foreskin fibroblasts	Endogenous	(Cristea et al., 2010)
Cytoplasmic and nuclear	IIF	HeLa	Endogenous and overexpressed	(Berry et al., 2010)
Cytoplasmic and nuclear	IHC	Lung tissue sections	Endogenous	(Berry et al., 2010)
Cytoplasmic and nuclear	IIF	Primary skin keratinocytes	Endogenous	(Costa et al., 2011)
Cytoplasmic and nuclear	CF	Primary lung fibroblasts (WI-38)	Endogenous	(Duan et al., 2011)
Cytoplasmic	CF	THP-1	Endogenous	(Veeranki et al., 2011)

A summary of subcellular localization of IFI16 protein using the indicated approaches (cell fractionation, indirect immunofluorescence, or immunohistochemistry) in a variety of cell types (normal and transformed). The table illustrates the fact that the subcellular localization of the IFI16 protein in the cytoplasm, nucleus, or both varies with the cell type (myeloid versus epithelial) and the phenotype of cells (normal versus transformed). Additionally, factors, such as levels of the IFI16 protein (endogenous or ectopically expressed) and IFN-treatment of the IFN-responsive cells also seem to affect the subcellular localization.

[a] CF, Cell fractionation; IHC, Immunohistochemistry; IIF, Indirect immunofluorescence; PrECs, Prostate epithelial cells.