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Interferon-inducible IFI16 protein in human cancers and autoimmune diseases

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1. ABSTRACT

Interferon-inducible IFI16 protein (encoded by *IFI16* gene located at 1q21 region) is a member of the p200-protein family. The family includes structurally and functionally-related mouse (for example, p202, p203, and p204 proteins) and human (for example, MNDA, AIM2, and IFIX) proteins. Increased expression of p200-family proteins in a variety of cells is known to inhibit cell cycle progression and modulate cell survival. Consistent with this role of p200-family proteins, increased expression of IFI16 protein in normal human diploid fibroblasts and prostate epithelial cells is associated with cellular senescence-associated permanent cell growth arrest. Furthermore, reduced or loss of IFI16 expression in cells is associated with the development of certain cancers, such as breast and prostate cancer. Interestingly, recent studies have provided evidence that the constitutive and interferon-induced expression of the IFI16 gene varies among individuals and may depend on the race. These studies raise the possibility that alterations (increases or decreases) in the expression of IFI16 protein may contribute to the development of human diseases. In this review, we discuss how our understanding of the regulation of IFI16 expression and its role in cell growth regulation will help elucidate the molecular mechanisms that contribute to the development of various human diseases.

2. INTRODUCTION

Interferons (IFNs), a family of cytokines, exhibit multiple biological activities both *in vitro* and *in vivo* (1, 2). These biological activities include well-characterized antiviral and immunomodulatory activities, and relatively less understood cell growth-regulatory activities, such as inhibition of cell proliferation, modulation of cell survival, promotion of cellular senescence and differentiation, and inhibition of angiogenesis (1-4).

The IFN family includes Type-I (IFN-α and IFN-β) and Type-II (IFN-γ) IFNs, among others (1). Infection of cells with certain viruses results in production of IFNs by the infected cells as a part of anti-viral response (1, 5). Therefore, it is not surprising that certain viruses have evolved mechanisms to overcome IFN-mediated antiviral actions, thus, resulting in viral infections (5).

Binding of an IFN to the corresponding cell surface receptor results in activation of the Janus family of tyrosine kinases (1, 4). The kinase that is used by both IFN-γ and IFN-α/β receptors to activate signal transducer and activator of transcription (STAT) proteins is JAK1 (1, 4). Importantly, transcriptional activation of IFN-activatable genes by activated STATs results in induction of IFN-inducible proteins that mediate the biological activities of IFNs (6, 7).

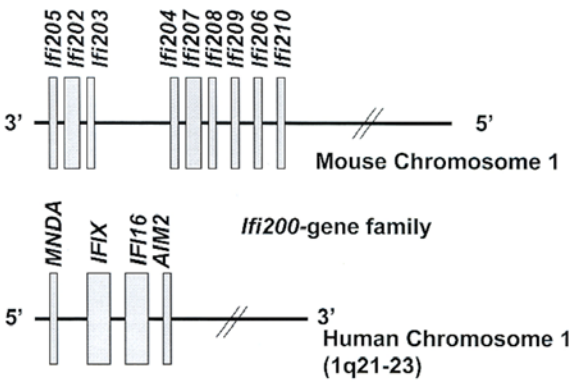

Figure 1. The relative location of Ifi200-family genes in mice (upper panel) and humans (lower panel). The mouse genes map between the erythroid α-spectrin and the amyloid P-component loci on the long arm of chromosome 1. The human genes form a cluster in the 1q21-23 region.

Earlier studies had revealed that interferon-α treatment of highly IFN-responsive cells, such as Daudi, results in cell growth arrest in the G_0/G_1 phase of cell cycle (8, 9). Interestingly, cell growth arrest was associated with inhibition of phosphorylation of retinoblastoma protein (pRb) (10), inhibition of E2F-mediated transcription (11), and down-regulation of c-Myc and cyclin A expression (8, 9). Although, these studies indicated that IFN-mediated growth suppression pathways involve pRb, c-Myc, and cyclin A, it remains unclear which IFN-inducible proteins contribute to cell growth suppression.

Increased serum levels of interferon-α are associated with certain autoimmune diseases (for example, SLE) (12-14). Moreover, defects in interferon signaling, which result in the lack of expression of IFN-inducible proteins, are associated with immortalization of human cells (15-18) and development of certain human cancers (15, 16). Interestingly, prolonged treatment of human diploid fibroblasts with IFN-β is known to induce cellular senescence (19). Because IFN-inducible proteins mediate the biological activities of IFNs (6, 7), we consider it is important to address the following questions: (i) how increased expression of IFN-inducible proteins in certain cell types contributes to the development of autoimmune diseases?; (ii) how defects in the expression of IFN-inducible proteins contribute to the development of certain human cancers?

One family of structurally-related IFN-inducible proteins is the p200-family (20-23). The family is encoded by IFN-stimulated mouse (for example, *Ifi202a*, *Ifi202b*, *Ifi203*, *Ifi204* and *Ifi205*) and human (for example, *IFI16*, *MNDA*, *AIM2*, and *IFIX*) genes (Figure 1). The human genes form a cluster in the 1q21-23 region (16). Importantly, genetic alterations in this region of human chromosome are linked to the development of certain cancers, including breast (24) and prostate (25) cancer. Moreover, this chromosomal region is predicted to harbor multiple autoimmunity susceptibility genes (26, 27).

The p200-family proteins share at least one of the following two partially conserved types of repeat motifs with 200-amino acids: *A*-type or *B*-type (20). Of note, each 200-amino acid repeat contains a conserved motif (MFHATVAT; 20); and substitution of His residue, the only charged amino acid residue in the motif, with a Phe in p202 protein (encoded by the *Ifi202a* gene) abrogates the inhibitory effect of p202 on the transcriptional activity of AP-1 and E2F1 (28). Additionally, some of the proteins, including the IFI16 protein, in the family also share a DAPIN/PYRIN domain in their N-terminus (29, 30).

The PYRIN domain was identified as putative protein-protein interaction domain at the N-termini of several proteins that are thought to function in apoptotic and inflammatory signaling pathways (30). It has been proposed that the domain serves as a sensor of changes in cellular environment (29, 30). Importantly, this domain can bind indirectly to at least two proteins important in inflammation: pro-caspase-1 and the IB kinase (IKK) complex, resulting in either activation or inhibition of these enzymes (30).

Sequence analysis of the p200-family proteins has revealed that each 200-amino acids repeat consists of two consecutive OB (oligonucleotide/ oligosaccharide binding)-folds commonly found in telomeric single strand DNA-binding proteins (31). The presence of the OB-fold in the p200-family proteins could provide a structural basis for their role in cell growth regulation. The p200-family proteins do not have any known enzymatic activity (32, 33). Therefore, based on their known interactions with other proteins, they are thought to act as scaffolds to assemble protein complexes that regulate transcription of genes (33).

There are excellent reviews concerning the role and regulation of p200-family proteins in the regulation of cell growth (21-23). Therefore, in this review, we focus on the recent advances in our understanding of the role and regulation of IFI16 protein in human diseases, such as lupus and cancers. We hope that these recent studies on IFI16 will serve basis to advance our understanding of the role of IFI16 protein in the development of various human diseases.

3. REGULATION OF IFI16 EXPRESSION

Based on earlier studies (21, 34), expression of IFI16 protein was thought to be restricted to hematopoietic cells. However, recent studies have provided evidence that the expression of IFI16 is detectable in cells of non-hematopoietic origin (35, 36). For example, expression of IFI16 is detectable by immunohistochemistry in epithelial cells of urogenital tract and glands and ducts of breast tissues, gastrointestinal tract, and skin (35, 36). In contrast, expression of IFI16 protein is not detectable in heart and brain tissues (35, 36).

Expression of IFI16 protein is induced by IFNs (α, β, or γ) in a variety of cells (21). Although, the mechanisms by which IFNs activate the transcription of

IFI16 gene remain to be elucidated, it is evident that other transcriptional modulatory proteins that remodel chromatin, such as Brahma-related gene 1 (BRG1), also contribute to the regulation of *IFI16* expression (37).

Interestingly, IFN-induced expression of IFI16 depends on the type of IFN and the cell type (38-41). Furthermore, as expected (because infection of cells with a virus results in interferon production), infection of human cells with rotavirus (41) or reovirus (43) results in induction of IFI16 mRNA. In the case of induction of IFI16 by reovirus, the induction depends on activation of NF-κB transcription factor by the virus (43). Moreover, expression of hepatitis C virus encoded NS5A protein in cells inhibits IFN-mediated up-regulation of IFI16 expression (44). Furthermore, it has been reported (45) that the members of IRF-family, such as IRF-5 and IRF-7, differentially regulate the expression of IFI16 mRNA in BJAB cells after infection with the NDV virus: the IRF-5 induces more than the IRF-7.

We recently found that the androgens through the androgen receptor (AR) up-regulate the expression of IFI16 in prostate epithelial cells (46). Although levels of IFI16 mRNA and protein increase in older populations of HDFs (47) and prostate epithelial cells (48), it remains unclear how the expression of *IFI16* gene is up-regulated in these cells. Because immortalization of HDFs with hTERT or SV40 large T-antigen results in reduced expression levels of IFI16 protein (47), these observations raise the possibility that immortalization-associated inactivation of the Rb/E2F and p53 pathways, down-regulates the expression of *IFI16* gene. Consistent with this idea, it has been reported that p53 induces the expression of *IFI16* in human HT-29 cells (49).

Based on the presence of higher densities of long interspersed nuclear element (LINE)-1 transposon sequence, fewer CpG islands, and fewer base-pairs of short interspersed nuclear elements (SINEs) sequence, the *IFI16* gene is a candidate for monoallelic expression (expressed from only one of the two alleles) (50). Consequently, it is likely that silencing of one the *IFI16* allele is sufficient to completely silence the expression of *IFI16* gene in human cancer cells.

3.1. The extent of IFI16 expression varies among individuals and may depend on the race

Studies based on different strains of mice have revealed that the expression of *Ifi202* gene varies among various strains of mice (51). This variation in the expression of *Ifi202* gene was in part associated with promoter polymorphisms (51). Consistent with the above observations, it has been reported that the expression of *IFI16* gene varies in peripheral blood mononuclear cells (PBMCs) among individuals in response to IFN-α treatment (39). Because the constitutive expression levels of *IFI16* mRNA depend on the cell type and the extent of IFN-α-mediated induction of *IFI16* expression in cells depends on the type of IFN (38-41), together, these observations provide support for the idea that promoter polymorphisms in *IFI16* gene and/or the cell type-specific factors may contribute to variations in the constitutive and the IFN-induced expression levels of IFI16 among individuals.

It has been reported (52) that African-American patients who have Hepatitis C virus infection exhibit a stronger interferon response than Caucasian patients. Consistent with this observation, the IFN-induced levels of IFI16 mRNA were higher in PBMCs from African-American patients than Caucasian patients (52). This observation raises the possibility that the extent of IFI16 induction by IFN treatment of PBMCs varies among individuals and may depend on the race.

3.2. Transcriptional as well as post-transcriptional mechanisms regulate the IFI16 protein levels

Studies have indicated that the expression of *IFI16* gene in a variety of cells is primarily regulated at the transcription level (21). However, it has been reported (53) that levels of IFI16 protein increase in primary human endothelial cells after their exposure to oxidative stress (caused by sub-lethal concentrations of H_2O_2) by a mechanism independent of transcription. Moreover, it was noted that the increase in the levels of IFI16 protein were in part due to increases in the half-life of IFI16 protein (from >30 min in IFN-β treated cells to 120 min in H_2O_2 treated cells). However, it remains unclear how the oxidative stress increase the half-life of IFI16 protein in endothelial cells. Notably, we found (Xin *et al*, Unpublished data) that the level of IFI16 protein did not increase measurably in young human diploid fibroblasts after treatment with increasing concentrations of hydrogen peroxide. Together, these observations suggest that the oxidative-stress-mediated increases in levels of IFI16 protein are cell type-dependent.

3.3. Interferon-independent regulation of IFI16 expression

Many studies have provided evidence for the involvement of signaling pathways in regulating the expression of *IFI16* gene (see Figure 2). For example, treatment of human adenocarcinoma cell line SW13 with IL-6 up-regulates the expression of IFI16 in STAT-3-dependent manner (54). Similarly, in human aortic endothelial cells (HAECs), hypoxia is shown to up-regulate the expression of IFI16 (55). Interestingly, the induction of IFI16 by hypoxia in HAECs appears to be a late (after 16 h) response. Moreover, it has been reported that treatment of human breast cancer cell line MCF-7 with TRAIL/APO-2L also activates the transcription of *IFI16* gene (56).

Studies have provided evidence that expression of IFI16 is also regulated by hormones (46, 57). For example, we found that androgens through the androgen receptor activate the transcription of *IFI16* gene in prostate epithelial cells (46). In contrast, treatment of T47D-Y ER^+ human breast cancer cells with estrogen is reported to result in down-regulation of IFI16 expression (57). Furthermore, expression profiling of genes between syndromic versus sporadic Wilms tumors has provided evidence that the expression of *IFI16* gene is up-regulated by Wilms tumor gene (*WT1)* (58; Figure 3). Similarly, overexpression of BRCA-1 in human prostate cancer cell line DU-145 up-

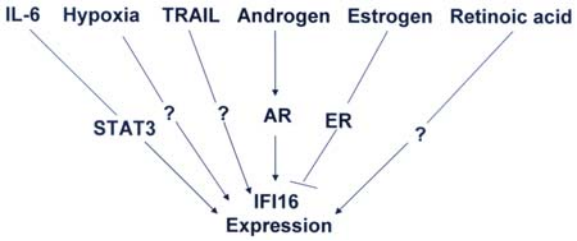

Figure 2. Interferon-independent signaling pathways that are activated by treatment of cells with IL-6, hypoxia, TRAIL, androgens, estrogens, or retinoic acid also regulate the expression of *IFI16* gene.

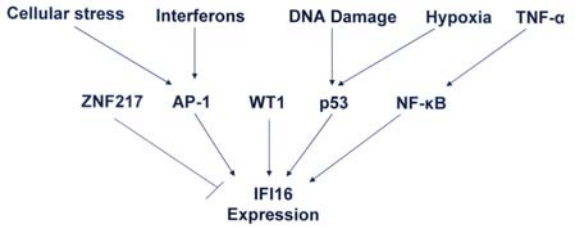

Figure 3. Transcriptional modulators that are known to regulate the transcription of *IFI16* gene include oncoprotein ZNF217 and the transcription factors, such as AP-1, WT1, p53, and NF-κB. Some of these transcription factors, such as AP-1 and p53, are activated by various signaling pathways that regulate cell proliferation and survival.

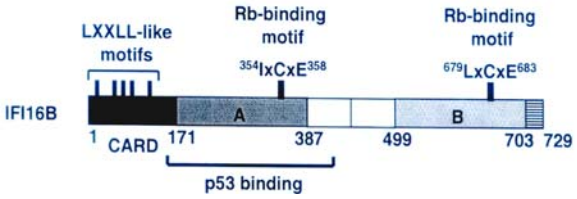

Figure 4. Schematic presentation of various structural domains and motifs in the IFI16 protein. The N-terminus of IFI16 protein contains five LXXLL-like motifs that present in proteins, which bind to the androgen receptor. The IFI16 protein also contains two LXCXE-like pRb-binding motifs. The p53-binding domain in IFI16B is also shown.

regulated the expression of IFI16 (59). However, overexpression of BRCA-1 in BRCA1-null ovarian cancer cell line UWB1.289 followed by ionizing radiation (IR) exposure to cells resulted in down-regulation (about 17-fold) of IFI16 expression (60). Interestingly, this down-regulation was independent of IFN-α or γ expression by these cells. Moreover, treatment of acute promyelocytic leukemia cell line NB4 with retinoic acid and arsenic trioxide (ATO) synergistically activated transcription of *IFI16* gene (61). In contrast to transcriptional activation of *IFI16* gene by the above signaling pathways, it has been reported that ZNF217 oncogene represses the transcription of *IFI16* gene (62; Figure 3).

4. THE IFI16 PROTEIN AS A SCAFFOLD PROTEIN

The *IFI16* gene is known to encode three isoforms (A, B, and C) of IFI16 protein through an alternative splicing of mRNA (21). The B form of IFI16 protein is the predominant form in normal HDFs (47) and normal human prostate epithelial cells (48). Like some other members of the family, IFI16 protein contains a bipartite nuclear localization signal (NLS) (21, 23). Moreover, consistent with the presence of a classical NLS in IFI16 protein, it is primarily detected in the nucleus in normal prostate (48) and breast (63) epithelial cells. In the nucleus, IFI16 protein is not excluded from the nucleolus (48, 63).

Expression of IFI16 protein is detectable by immunohistochemistry in a variety of normal human tissues and organs (35, 36). The protein IFI16 is detected primarily in the nucleus of normal human HDFs and prostate epithelial cells (47, 48). However, a polymorphic form of IFI16 protein, which is expressed in a human prostate cancer cell line (PC-3 cell line), was detected primarily in the cytoplasm (48).

Although IFN-inducible IFI16 protein remains a poorly characterized protein in the p200-protein family with respect to posttranslational modifications and structure-function analysis, there are indications that IFI16 protein is phosphorylated (64). Moreover, the amino acid sequence of IFI16 contains at least four S/TQ motifs [Thr-149, Thr-235, Thr-237, and Ser-624; the amino acid numbers are indicated for IFI16B protein], which could serve as phosphorylation sites for the ATM kinase.

The protein IFI16 contains two LXCXE-like motifs (Figure 4) that are found in several Rb-binding proteins (65). Consistent with the presence of these potential Rb-binding motifs in the IFI16 protein, the protein can bind to Rb in GST-pull down assays (48). Moreover, IFI16 protein also contains at least five LXXLL-like motifs in the N-terminus (Figure 4). These motifs are found in several AR-binding proteins (66). Consistent with the presence of these motifs in IFI16 protein, it can bind to AR in GST-pull down assays (46). However, further work is needed to determine whether the binding of IFI16 protein to Rb or AR depends on the presence of LXCXE or LXXLL-like motifs, respectively. Moreover, consistent with a role for IFI16 as a scaffold protein, IFI16 protein can also bind to several other proteins, such as p53 (67), E2F1 (48), and BRCA1 (68).

5. ROLE OF IFI16 IN CELL GROWTH REGULATION

Studies using human primary cells and cell lines have indicated that IFI16 protein is a negative regulator of cell proliferation (22, 23, 47, 48). However, molecular mechanisms by which increased levels of IFI16 protein inhibit cell proliferation remain unclear.

The A-type repeat in IFI16 protein binds to the C-terminus (amino acids 362-393) of p53 (67; Figure 4). Interestingly, p53, which is phosphorylated on Ser-15 residue, appears to bind IFI16 *in vivo* in normal primary human umbilical vein endothelial cells (HUVEC) (69).

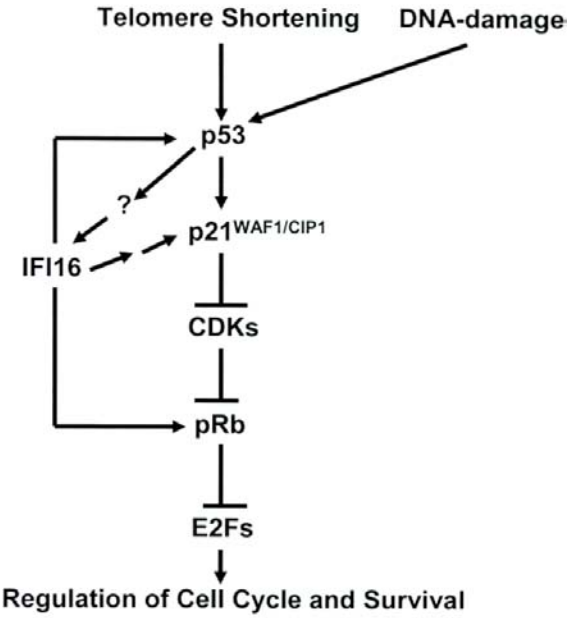

Figure 5. Role of IFI16 protein in the p53/p21^{CIP1} and Rb/E2F cell growth-regulatory pathways. The IFI16 protein physically and functionally interacts with the p53/p21^{CIP1} and the Rb/E2F pathways. The functional interactions of IFI16 protein with these two cell growth-regulatory pathways contribute to the regulation of cell growth by IFI16.

Overexpression of IFI16 appears to up-regulate p53 levels and stimulate p53-mediated transcription (69). Conversely, knockdown of IFI16 expression in a breast cancer cell line results in down-regulation of p53 levels (63). Notably, overexpression of IFI16 in: (i) immortal cell lines results in up-regulation of p21^{CIP1} expression (48); (ii) normal HAECs, but not in HPV16 E6/E7 immortalized cells, results in up-regulation of p53 and p21^{CIP1} (69); and (iii) medullary thyroid carcinoma cells results in down-regulation of E2F1, cyclin D1, and up-regulation of p21^{CIP1} (70). Furthermore, knockdown of IFI16 in WI-38 HDFs resulted in down-regulation of p21^{CIP1} levels and an inhibition of the activity of the p21-luc-reporter (47). Significantly, increased expression of IFI16 protein inhibits E2F1-stimulated transcription (48). These observations provide support for the idea that IFI16 protein inhibits cell proliferation in part through the Rb/E2F and p53/p21^{CIP1} pathways (Figure 5).

Our recent studies have suggested that increased expression of IFI16 protein in LNCaP prostate cancer cells down-regulates the AR expression and inhibits AR-mediated functions (46). Because increased expression of AR in prostate cancer cells and cell lines is associated with increased cell proliferation and transition of cells to an androgen independence (71), our observations will serve basis to determine whether the loss of IFI16 expression (or function) in prostate intraepithelial neoplasia (PIN) or localized prostate cancers contributes to their progression to a metastatic androgen depletion independent prostate cancers.

5.1. Role of IFI16 in cellular senescence

Increased expression of IFI16 protein in older (versus younger) populations of normal human diploid fibroblasts (47) and in normal human prostate epithelial cells (48) is associated with cellular senescence-associated cell growth arrest. Moreover, immortalization of normal human diploid fibroblasts is associated with down-regulation of IFI16 mRNA and protein (47). Additionally, DNA-methylation has been shown to silence the expression of *IFI16* gene in human breast cancer cell lines and tumors (63), immortalized human diploid fibroblasts (47), and human prostate cancer cell lines (72). Consistent with a potential role of IFI16 in cellular senescence, human prostate cancer cell lines either do not express IFI16 protein or express a variant form (48), which is primarily detected in the cytoplasm of prostate cancer cells and not in the nucleus. Moreover, overexpression of functional IFI16 in human prostate cancer cell lines inhibits colony formation (48). Additionally, ectopic expression of IFI16 protein in the PC-3 clonal prostate cancer cell lines is associated with a senescence-like phenotype, production of senescence-associated β-galactosidase (a biochemical marker for cellular senescence), and a reduction in the S-phase cells in culture (48). Importantly, up-regulation of p21^{WAF1}, and inhibition of E2F-stimulated transcription accompanied inhibition of cell growth by IFI16 in prostate cancer cell lines. Consistent with a role for IFI16 protein in cellular senescence, slow-dividing fraction among human hematopoietic progenitor cells is associated with 4-fold increases in IFI16 expression (73). Together, these observations suggest that increased expression of IFI16 protein in older populations of cells contributes to cellular senescence-associated cell growth arrest in part through the Rb/E2F and p53/p21^{CIP1} pathways.

5.2. How does IFI16 protein inhibit cell proliferation?

Binding of IFI16 protein to p53 in the C-terminus is reported to stimulate the transcription of p53-responsive reporter plasmids (67). Consistent with this observation, increased expression of IFI16 in MCF-7 breast cancer cell line resulted in increased transcriptional activity of p53 as determined by increased expression of the p53 target genes, such as p21^{CIP1}, Hdm2, and Bax (63). More importantly, increased forced expression of IFI16 in MCF-7 cells resulted in increased susceptibility to apoptosis following ionizing radiation treatment of cells. Conversely, knockdown of IFI16 expression in cells resulted in perturbations of p53 activation following IR treatment of cells (63). Together these observations provide support for the idea that IFI16-mediated inhibition of cell proliferation in part depends on the functional status of p53 (see Figure 5).

Interestingly, IFI16-mediated up-regulation of p21^{CIP1} expression in PC-3 clonal cell lines was independent of p53 function (48). Moreover, binding of IFI16 protein to Rb and E2F1 is correlated with inhibition of E2F1-mediated transcription of target genes (44).

Knockdown of IFI16 protein in medullary thyroid carcinoma (MTC) cell line resulted in increases in levels of

Table 1. Alterations in IFI16 expression in human cancers

Cancer Type	Increased or Decreased Expression of IFI16	Changes in the Treatment Outcome or the Phenotype	Ref
Invasive intraductal papillary mucinous neoplasms of the pancreas	Increased	Not known	71
Hepatocellualr carcinoma	Decreased	Increased metastasis	72
Ovarian carcinoma (xenografts)	Increased	Responsive to paclitaxel treatment	73
Ovarian cancer	Decreased	Carboplatin resistance	74
Ovarian cancer	Increased	Platinum resistance	75
Acute Myeloid Leukemia	Increased	Not known	76
Prostate cancer	Decreased	Not known	44
Breast cancer	Decreased	Not known	59

Ref: reference

E2F1, cyclin D1, and decreases in the levels of $p21^{CIP1}$ (70). Furthermore, induction of IFI16 in this MTC cell line by expression of Raf or treatment of cells with the leukemia inhibitor factor (LIF) revealed that IFI16 is necessary and sufficient downstream effector and needed for cell growth inhibition (70). Because binding of IFI16 protein to androgen receptor (AR) in the conserved region was correlated with inhibition of AR-mediated transcription of AR-responsive reporter genes and down-regulation of PSA expression (46), a known transcriptional target of AR, these observations also provide support for the idea that in prostate epithelial cells IFI16 inhibits cell proliferation through inhibiting AR-mediated functions.

5.3. Modulation of cell survival by IFI16

Studies have provided evidence that increased expression of IFI16 protein in MCF-7 cells increases the susceptibility of cells to ionizing radiation (IR)-induced apoptosis (63). Similarly, we have noted that increased expression of IFI16 in human prostate cancer cell line LNCaP increased the susceptibility to apoptosis induced by etoposide (72). Interestingly, this increase in the apoptosis was associated with down-regulation of AR expression (72). Because LNCaP cells express a wild-type p53 (74), together these observations provide support for the idea that IFI16 potentiates p53-mediated apoptosis in response to certain genotoxic stress.

Studies involving gene expression analyses have indicated that alterations in IFI16 expression in a variety of cancer cells *in vitro* or *in vivo* are associated with the modulation of cell survival in response to treatment with various anticancer agents (75-80). Because IFI16 could modulate cell survival through p53, it remains to be seen whether the modulation of cell survival by the increased (or decreased) levels of IFI16 protein is p53-dependent.

Expression of NUP98-HOXA9, the chimeric protein resulting from the t (7; 11) (p15; p15) chromosomal translocation commonly found in myelodysplastic syndromes and acute myeloid leukemia, in $CD34^+$ cells results in up-regulation of IFI16 protein (80). Importantly, increased expression of IFI16 in $CD34^+$ cells was associated with long-term cell proliferation and inhibition of differentiation.

5.4. Regulation of cell differentiation

The p200-family proteins are known to play an important role in differentiation of certain cell types *in vitro* (22, 23). The ability these p200-family proteins to modulate cell differentiation depends on their ability to bind and modulate the transcriptional activity of particular cell type-specific transcription factor. For example, p204 protein is essential for differentiation of myoblasts to mytubes *in vitro* (81). The p204 protein promotes differentiation of myblasts to myotubes in part by binding to inhibitors of differentiation proteins (Id proteins) (82), resulting in transcriptional activation by MyoD, which is inhibited by the Id proteins. Consistent with a role for the p200-family proteins in cell differentiation, it has been reported (80) that treatment of malignant B cells (CLL cells) with bryostatin-1, a differentiation-inducing agent, results in up-regulation of IFI16 expression. In contrast to the above observation, it has been reported that, in medullary thyroid carcinoma cell differentiation model system, IFI16 protein was not essential for differentiation of cells by leukemia inhibitor factor (70). Because AR plays an important role in differentiation of prostate epithelial cells (71), it will be important to investigate whether binding of IFI16 protein to AR has any effect on differentiation of prostate epithelial cells.

5.5. How expression or function of IFI16 is lost in cancer cells?

Studies have provided support for the idea that increased levels of IFI16 protein in cells negatively regulate cell proliferation (22, 23, 47, 48, 63). Moreover, it has been reported (83) that expression of IFI16 is down-regulated 2-5-fold in follicular lymphoma cells with the t(14;18) translocation. Therefore, we and others have investigated how the expression or function of IFI16 is lost in cancer cells. These studies have revealed that the expression of IFI16 appears to be reduced or lost in human breast cancer cell lines (63) and human diploid fibroblasts (47) by DNA-methylation. There is also evidence that the expression of IFI16 is reduced in human prostate cancer cell lines by mechanisms involving deacetylation of proteins by histone deacetylases (72). Moreover, it is likely that defects in localization of IFI16 protein in the nucleus of cells also contribute to the loss of IFI16 function in certain prostate cancer cells (48).

6. IFI16 IN HUMAN CANCERS

Human primary normal mammary epithelial cells express detectable levels of IFI16 protein (63). However, most human breast cancer cell lines that were examined expressed decreased mRNA and protein levels of IFI16 as compared to normal mammary epithelial cells. Moreover, immunohistochemistry analyses of tissues from 25 breast cancer patients revealed that carcinoma cells showed

negative or weaker staining for IFI16 protein compared with positive nuclear staining in normal mammary duct epithelium (63). These observations provide support for the idea that reduced or loss of IFI16 expression in breast epithelial cells contributes to the development of breast cancer.

Our studies have indicated that most human prostate cancer cell lines and prostate cancer tumors either do not express IFI16 mRNA and protein or express at a reduced levels as compared to cultured normal human prostate epithelial cells (48). Furthermore, we found that forced expression of IFI16 in human prostate cancer cell lines (LNCaP, DU-145, and PC-3) results in inhibition of colony formation and a senescence-like phenotype in PC-3 cells (48). Importantly, increased expression of IFI16 in older populations of primary prostate epithelial cells is associated with cellular senescence (48). Together, these observations provided support for the idea that the loss of IFI16 expression or function in prostate epithelial cells contributes to the development of prostate cancer.

Gene expression analyses in plasma cells from healthy donors and high-risk multiple myeloma patients have revealed that increased expression of IFI16 is correlated with high risk (84). Because gains of the long arm of chromosome 1 (1q) are one of the most common genetic abnormalities in myeloma (84), it is likely that increased expression of IFI16 protein (and possibly other p200-family proteins) in plasma cells contributes to increased cell survival.

7. DOES IFI16 HAVE A ROLE IN AUTOIMMUNE DISEASES?

As stated above, increased serum levels of interferon-α are associated with the development of systemic lupus erythematosus (SLE) in about 50% patients (12-14). Moreover, PBMCs from these patients exhibit interferon gene expression signature: expression levels of mRNAs encoded by the interferon-stimulated genes are up-regulated. Because increased levels of p202 protein in B6.Nba2 congenic mice are associated with development of autoantibodies (51), it is conceivable that IFI16 protein plays a role in the development of SLE. Consistent with this prediction, up to 29% SLE patients develop high titer autoantibodies to IFI16 protein (85). However, studies (12, 14) failed to detect increased levels of IFI16 mRNA in PBMCs isolated from SLE patients. Because the expression levels of the IFI16 protein could be regulated by both transcriptional and post-transcriptional levels (53), further work will be needed to determine whether PBMCs from SLE patients have higher levels of IFI16 protein as compared to PBMCs from normal donors. Moreover, it will be interesting to determine whether polymorphisms in the *IFI16* gene are associated with alterations in its expression levels in cells and/or localization of IFI16 protein in a particular cellular compartment, such as cytoplasm versus nucleus.

Consistent with a role for IFI16 protein in immune regulation, a recent study has suggested a role for IFI16 protein in T-cell development (86). Consistent with this idea, the naïve $CD8^+$ T cells express relatively low levels of IFI16 mRNA. However, memory $CD8^+$ cells express intermediate levels of IFI16 mRNA whereas the effector $CD8^+$ cells express the highest levels of IFI16 mRNA (87). These observations provide support for the idea that IFI16 may have role in differentiation of naïve $CD8^+$ cells. Although, these observations are consistent with a role for IFI16 protein in the immune functions and certain autoimmune diseases, further studies are needed to examine the role of IFI16 protein in immune cells and in the development of SLE.

8. ROLE FOR IFI16 IN OTHER HUMAN DISEASES

Gene expression studies have provided evidence that increased expression of IFI16 in quadriceps muscle (skeletal muscles) samples isolated from children under 2-years of age is associated with the development of Duchenne muscular dystrophy (DMD) at high frequency (88). Because the DMD molecular signature is characterized by co-coordinated induction of genes involved in the inflammatory responses, extracellular matrix (ECM) remodeling, and muscle regeneration, it will be important to understand the role of IFI16 in the development of DMD.

Increases in airway smooth muscle (ASM) mass are thought to contribute to the pathology of patients with severe asthma (89). Therefore, understanding of the molecular mechanisms that inhibit cell mitogenesis in ASM cells are important. Interestingly, our studies (89) have revealed that treatment of human ASM cells with IFN-γ resulted in inhibition of cell proliferation through the Rb/E2F pathway. Importantly, the inhibition was associated with up-regulation of IFI16 expression. Therefore, further studies are needed to examine whether decreases in IFI16 expression in ASM cells are associated with the development of asthma.

9. FUTURE PERSPECTIVES

Among the p200-family proteins, the IFI16 protein remains relatively poorly characterized. Therefore, further studies are needed to examine the structure-function relationship for IFI16 protein. In particular, which protein-protein interaction domain(s) and amino acids in IFI16 protein are needed for its interactions with other proteins, such as pRb, p53, and AR.

Although, studies using the primary cells and cancer cell lines have provided support for the idea that the IFI16 protein inhibits cell proliferation and modulates cell survival through the $p53/p21^{CIP1}$ and the Rb/E2F pathways, further studies are needed to understand the role of IFI16 protein in cellular senescence and immune functions. Because a decline or lack of IFI16 expression is observed in breast and prostate cancers, and increased levels of IFI16 protein in cells are associated with cellular senescence in cultured cells, studies are also needed to determine whether polymorphisms in the *IFI16* gene contribute to differential basal and IFN-induced expression of IFI16 in certain

populations of individuals. These polymorphisms in the *IFI16* gene are predicted to predispose individuals to the development of various diseases, including certain cancers and lupus.

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