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Role of IFI16 in DNA damage and checkpoint

Mutsuko Ouchi[1,2], Toru Ouchi[1,2,3]

[1] Department of Oncological Sciences, The Mount Sinai School of Medicine, New York University, New York, NY, [2] Department of Medicine, Evanston Northwestern Healthcare, Feinberg School of Medicine, Northwestern University, Evanston, IL, [3] Robert H. Lurie Comprehensive Cancer Center, Basic Sciences Research Division, Northwestern University, Chicago, IL

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1. ABSTRACT

IFI16 is a member of the HIN-200 family (hematopoietic interferon-inducible nuclear antigens with 200 amino acid repeat) that contains a DNA binding domain, a transcriptional regulatory domain, DAPIN/PAAD domain associated with interferon (IFN) response and a binding domain for BRCA1, breast cancer tumor suppressor protein. IFI16 has been identified as a target of IFNα and γ and is a member of the HIN-200 family (1). Although series of initial studies have demonstrated a potential activity of IFI16, a physiological role of the protein was largely unknown. A novel insight of the function of IFI16 stemmed from the observation that IFI16 constitutively binds to BRCA1 breast cancer tumor suppressor (2). Furthermore, it has been demonstrated that IFI16 is involved in p53-mediated regulation of cell growth and apoptosis (3,4). Immunocytochemical and immunohistological analyses of breast cancer cell lines and specimens revealed that levels of IFI16 are frequently decreased, supporting the notion that loss of IFI16 is closely associated with tumor development. Finally, siRNA-mediated depletion of IFI16 induces levels of NBS1, nijmegen breakage syndrome protein 1, leading to activation of DNA-PK (DNA-dependent kinase), phosphorylation of p53 Ser37 and accumulation of p21WAF1 (5, this issue). Localization of IFI16 is determined by the status of BRCA1 protein under conditions of DNA damage, such as ionizing radiation (IR) (2). More recently, it has been shown that levels of IFI16 are increased by oxidative stress (6). Together, these results illustrate that IFI16 is involved in DNA damage signaling and cell cycle checkpoint.

2. INTRODUCTION

IFI16 has been identified as a target of IFNα and γ, and is a member of the HIN-200 family (1,7). GAL4DBD-fused full length IFI16 acts as a potent transcription repressor when positioned in proximity to a promoter containing consensus GAL4DBD binding sequences (8). Each of the 200 amino acids repeats regions contain this transrepression activity independently, and the N-terminal can bind DNA (9). A nuclear function of IFI16 was postulated by the findings that IFI16 and its mouse homolog p202 interact with p53, Rb, E2F, AP1 and NF□B proteins (10-13). More recently, the PYRIN domain, which is commonly found among cell death-associated proteins such as PYRIN, ASC, and zebrafish caspase and is also known as the PAAD/DAPIN domain, has been found in the N-terminal of IFI16, suggesting that IFI16 has a role in the apoptosis pathway (14-18). Our recent studies further identified that the N-terminal PYRIN domain as BRCA1-binding region of the protein (2). Presumably, IFI16 regulates the activity of certain transcription factors and tumor suppressor(s) in the nucleus that are involved in the commitment to cell death.

It has recently been shown that IFI16 is detected in the nuclei of lymphocytes in the spleen, thymus, lymph nodes and palatine tonsil but is also found in epithelial cells in these tissues (19,20). Significantly, IFI16 protein was also expressed in non-lymphoid tissues including trachea, gastrointestinal tract, skin and testis. Thus, IFI16 expression is not restricted to cells of the immune system but is also found in epithelial cells. Given the selective expression of IFI16 in epithelial cells, it is likely that IFI16

may be important for some epithelial cell-specific functions. Importantly, levels of mRNA and/or protein of IFI16 is frequently reduced in many breast cancer cell lines and breast adenocarcinoma tissues, suggesting a potential link between loss of IFI16 and cancer development (3). However, whether IFI16 expression is directly linked to human diseases remains to be determined.

Inactivation of the p53 tumor suppressor gene occurs in more than half of all human cancers, indicating that loss of this gene represents a fundamentally important step in the pathogenesis of cancer (21). The p53 protein functions at least in part as a transcription factor and can transactivate cellular genes through sequence-specific binding to their promoters (22-24). Significantly, it was found that depletion of IFI16 by siRNA results in the decreased phosphorylation of p53 Ser15 under conditions of IR damage (3), however, phosphorylation of p53 Ser37 is increased under the same condition (5). These observations provide implication that IFI16 is involved in determination of the status of p53 activation under IR damage.

This review article describes recent progress of the function of IFI16 in DNA damage signaling and cell cycle checkpoint.

3. DISCUSSION

3.1. Regulation of p53 pathway

We first identified IFI16 as a BRCA1-associated protein (2). It has been demonstrated previously that BRCA1 plays a role in regulating p53-mediated gene transcription (25), therefore roles of IFI16 in p53 pathway has been extensively studied. Tetracycline (tet)-inducible system of IFI16 in p53-negative EJ cells (human bladder carcinoma cell line) was developed, in which IFI16 was induced by removing tet from cell culture. When tet was removed, cells were infected with the p53 adenovirus. Cells were further treated with actinomycin D (Act D). Without IFI16 induction, neither p53 nor Act D induced apoptosis in EJ cells. However, when IFI16 was induced, p53 expression strongly induced apoptosis after Act D treatment (2). These results indicate that IFI16 collaborates with p53 activated by genotoxic reagents to induce apoptosis.

Several results have been reported regarding the functional interaction between p53 and IFI16. On the basis of the observation that IFI16 enhances p53-mediated apoptosis under DNA damage, Fujiuchi *et al.* have characterized p53 pathway using human breast epithelial cell line, MCF10A, and human osteosarcome cell line, U2OS, in which endogenous p53 is wild type. The researchers transiently reduced levels of IFI16 by transfecting cells with siRNA for 48 h, and investigated signaling cascade from ATM to p53 under conditions of IR damage for the following 12 h (3). It has been well characterized that DNA damage induced by radiation and chemicals immediately induce autophosphorylation of ATM Ser1981, leading to dissociation of dimerized ATM proteins (26). Phosphorylation of ATM Ser1981 was similarly induced in both control cells and IFI16 siRNA cells, indicating that ATM activation by IR is not affected by loss of IFI16. p53 Ser15 is a well-characterized target of this ATM signaling (22). Interestingly, kinetics of p53 Ser15 phosphorylation are similar in both control and IFI16 siRNA-transfected MCF10A cells, however, this phosphorylation returned to the basal levels within 6 h after IR, although control cells retain high levels of phosphorylation, at least, 12 h after IR treatment. Lower levels of p53 Ser15 phosphorylation was also observed when IFI16 was knocked down in U2OS cells. These results demonstrate that IFI16 is necessary for the efficient phosphorylation of p53 Ser15 when cells were DNA-damaged. Consistent with this, IFI16 enhances apoptosis of MCF7 cells expressing wild type p53, when cells were treated with neocarcinostatin (NCS).

A potential link between IFI16 and p53 was also reported by Kwak *et al.* (4). Although the researchers again used U2OS cells to knock down endogenous IFI16 by siRNA, biochemical and biological phenotypes were studied without genotoxic stimuli for the following 96 h. They discovered that depletion of IFI16 is sufficient to induce levels of p21WAF1, leading to accumulation of cells in both G0-G1 and G2-M phase. They found that this induction of p21WAF1 by depletion of IFI16 is p53-dependent, although it remains unclear how loss of IFI16 immediately induces phosphorylation of several residues of p53 that are important to determine the p53 stability, or how p53 accumulates without modification of the protein in the absence of IFI16.

Taken together with studies by Fujiuchi *et al.*, these results provide us with a quite interesting model of how IFI16 regulates p53 activation. Depletion of IFI16 causes accumulation of p21WAF1 through p53 within 24 to 48 h, leading to weak inhibition of cell growth. However, when these cells were DNA-damaged by IR at that time point, they cannot fully increase p53 Ser15 phosphorylation, although IFI16-positive cells show maximal activation of p53. Thus, these IFI16-negative cells do not retain the machinery to produce fully activated p53.

3.2. Phosphorylation of p53 Ser37 and induction of p21WAF1 after IR stress

Do the cells that have lost IFI16 no longer contain intact checkpoint under conditions of DNA damage? Tawara *et al.* first observed that U2OS cells in which IFI16 is depleted grow much slower than those of control cells. They also observed that IR treatment further inhibits growth of IFI16-depleted cells (5). These observations raised a question of why IFI16-depleted cells grow slowly in spite of their insufficient phosphorylation of p53 Ser15. The researchers have discovered that phosphorylation of p53 Ser37 is enhanced in IFI16-siRNA cells after IR damage, which is possibly a target of DNA-PK (27,28). Taken together, these results suggest that a concentration of Ser37-phosphorylated p53 is increased in a pool of total p53. Consistent with this growth inhibition, p21WAF1 is strongly induced in IFI16-siRNA cells after IR treatment. It is not clear whether this accumulation of p21WAF1 is due to transcriptional regulation, or increased protein stability. In addition, since the genomic promoter of

p21WAF1 contains many sequence elements that are recognized by diverse transcription factors (29), decreased IFI16 may stimulate the p21WAF1 promoter through other transcription factors.

Interesting observation was that NBS1 tumor suppressor is increased in IFI16-siRNA cells (5). Recent studies have demonstrated that overexpression of NBS1 contributes to cell transformation (30,31). Given the observation that overexpression of NBS1 is sufficient for induction of both p53 Ser37 phosphorylation and p21WAF1 without DNA damage, it is intriguing to speculate that the IFI16-negative cells in which NBS1 is increased initially activate p53-checkpoint, but some of these cells escape from this checkpoint, leading to cancer development.

3.3. Oxidative stress and IFI16

Another line of evidence showing that IFI16 is involved in cell stress signaling has been illustrated in cells stimulated with oxidative stress (6). In these experiments, Gugliesi *et al.* have found that H_2O_2, S-nitroso-N-acetylpenicillamice (SNAP) and tert-butyl hydroperoxiside (TBHP) can induce levels of IFI16 in human umbilical vein endothelial cells (HUVEC). This is due to a prolonged half-life of the protein, not by increased mRNA. Significantly, basal levels of IFI16 are already high in HUVEC expressing papillomavirus proteins E6 and E7, and are not further enhanced by oxidative stress. Thus, induction of IFI16 by these chemical treatments is p53-independent. Mechanisms of induction of IFI16 under oxidative stress remain to be elucidated.

4. FUTURE COURSE

Unique aspects of IFI16 pathway in DNA damage was first demonstrated by Aglipay *et al.*, where IFI16 was identified as an interacting protein of BRCA1, breast cancer tumor suppressor. Immunocytochemical analysis revealed that IFI16 is in both nucleoplasm and nucleoli. Nucleoplasm IFI16 disappears after IR treatment, and it returns to the nucleoplasm later (2). This translocation is not observed in BRCA1-mutated HCC1937 breast cancer cell line, however, reexpression of BRCA1 in these cells restored IFI16 re-localization. Exact mechanism and a biological role of this re-localization of IFI16 remain unclear, but these results provide us with a novel insight that IFI16 and perhaps IFN pathway are crucial for tumor suppression, at least under conditions of genotoxic stimuli. In that sense, recent studies support this notion; the first, Pang *et al.* have illustrated that the Fanconi anemia protein type C (FANCC), which is known to be involved in repair of the damaged DNA, is required for STAT1 activation after IFNγ stimulation (32), and the second, Townsend *et al.* have demonstrated that STAT1 is required for phosphorylation of Chk2, NBS1 and p53, not for BRCA1 and γH2AX, after IR stress (33). Although mechanisms underlying these observations still need to be extensively addressed, potential cross-talk between IFN pathway and DNA damage checkpoint will provide us with novel insight of tumor development.

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Abbreviations: ATM: ataxia telangiectasia mutated; NBS1: Nijmegen breakage syndrome 1; IR: ionizing radiation; siRNA: small interfering RNA; tet: tetracycline; HUVEC: human umbilical vein endothelial cells: FANCC: Fanconi anemia protein type C; SNAP: S-nitroso-N-acetylpenicillamice; TBHP: tert-butyl hydroperoxiside; NCS: neocarcinostatin

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Send correspondence to: Dr. Toru Ouchi, 1001 University Place, Evanston, IL 60201, Tel: 224-364-7687, Fax: 224-364-7402, E-mail: t-ouchi@northwestern.edu

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