Requirement of IFI16 for the Maximal Activation of p53 Induced by Ionizing Radiation*

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IF16 is a member of the PYRIN superfamily that has been implicated in BRCA1-mediated apoptosis and inflammation signaling pathways. Here we report that most breast cancer cell lines examined expressed decreased mRNA and protein levels of IFI16, although IFI16 is expressed in human primary normal mammary epithelial cells. Significantly, immunohistochemical analysis of tissues from 25 breast cancer patients demonstrated that carcinoma cells showed negative or weaker staining of IFI16 compared with positive nuclear staining in normal mammary duct epithelium. siRNA-mediated reduction of IFI16 resulted in perturbation of p53 activation when treated with ionizing radiation (IR). Expression of IFI16 enhanced p53 transcriptional activity in cells exposed to IR. Adenovirus expression of IFI16 in IFI16-deficient MCF7 induced apoptosis, which was enhanced by radiomimetic necrocinostatin treatment. Tetracycline-regulated IFI16 also induced apoptosis when coexpressed with p53 in p53-deficient EJ cells subjected to IR, suggesting that IFI16 is involved in p53-mediated transmission of apoptosis signaling. Consistent with these results, expression of IFI16 enhanced activation of the known p53 target genes, including p21, Hdm2, and bax in MCF7 cells. These results suggest that loss of IFI16 results in deregulation of p53-mediated apoptosis, leading to cancer development.

IFI16 has been identified as a target of interferon α and γ and is a member of the HIN-200 family (1, 2). GAL4DBD-fused full-length IFI16 acts as a potent transcription repressor when positioned in proximity to a promoter containing consensus GAL4DBD binding sequences (3). Each of the 200 amino acids repeat regions contains this transrepression activity independently, and the N-terminal can bind DNA (4). A nuclear function of IFI16 was postulated by the findings that IFI16 and its mouse homolog p202 interact with p53, Rb, E2F, AP1, and NFκB proteins (5–8). 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 (9–13). Presumably, IFI16 regulates the activity of certain transcription factors in the nucleus that are involved in the commitment to cell death.

Recent studies have demonstrated 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 (14, 15). 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. 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 (16). The p53 protein functions at least in part as a transcription factor and can transactivate cellular genes through sequence-specific binding to their promoters (17–19).

In the present study, we investigated a physiological function of IFI16 by means of siRNA-mediated suppression of endogenous IFI16, adenovirus IFI16, and tetracycline-inducible IFI16 and found that IFI16 is involved in p53-mediated apoptosis. Importantly, immunohistochemical analysis of breast tissues demonstrated that expression of IFI16 is frequently lost in breast carcinoma, suggesting a role of this protein in malignant transformation. Taken together, our studies suggest that IFI16 is involved in the regulation of apoptosis and that loss of IFI16 expression results in deregulated regulation of p53-mediated apoptosis, leading to cancer development.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary human normal mammary epithelial cells were established from reduction mammoplasties obtained through the Cooperative Human Tissue Network and designated 12N, 15N, and 17N. These cells were grown in DFCI-1 medium (D-complete) and were used at early to mid-passage, i.e. 5–10 population doublings (20). MCF7, MCF10A, T47D, U937, and MDA-MB-231 cells were obtained from ATCC. MDAMB-453, -468 and -468 breast cancer cell lines were obtained from Dr. Stuart Aaronson at the Mount Sinai School of Medicine. Tetracycline-regulatable IFI16 in EJ human bladder carcinoma cells was established by following previously described protocols (21). MCF10A cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 0.2% horse serum, 0.2 μg/ml of epidermal growth factor, 0.5 μg/ml of hydrocortisone, 10 μg/ml of insulin, and 0.1 μg/ml of cholera toxin. Other cells were cultured in Dulbecco’s
modified Eagle’s medium-10% fetal bovine serum. EJtet cells were maintained in the presence of tetracycline (1 μg/ml). Ionizing radiation was administered using a MARK2 IRRADIATOR (J. L. Stephen & Associate).

Western Blotting Analysis—Cell extracts were prepared in EBC buffer (50 mM Tris, pH 8, 120 mM NaCl, 0.5% Nonidet P-40 with the addition of 100 mM NaF, 0.2 mM sodium orthovanadate, 100 μg/ml polymethylsulfonyl fluoride, 2 μg/ml aprotinin, and 5 μg/ml leupeptin). Fifty micrograms of whole cell extract were loaded per lane by 7.5% SDS-PAGE. Transfer to nitrocellulose was performed using a semidyed transfer method (Trans-Blot; Bio-Rad) in 25 mM Tris base, 192 mM glycine, 10% methanol (for 1.5 h at 15 V). After blocking with 1% nonfat dried milk in phosphate-buffered saline (0.05% Tween 20), the primary antibody (anti-IFI16 (Santa Cruz Biotechnology)) was used at 1 μg/ml in phosphate-buffered saline/1% nonfat dried milk for 1 h at room temperature. The secondary antibody was peroxidase-conjugated goat anti-mouse IgG (H+L; Jackson ImmunoResearch) at 1:10,000 in 1% nonfat milk-Tris-buffered saline-T. Signals were developed by ECL (Amersham Biosciences). Antibodies used in these studies are ATM (GeneTex); ATM(ser1981) (Rockland); p53, Hdm2, p14ARF, and p21WAF1 (Calbiochem); p53Ser15 (Cell Signaling); p14ARF and Actin (Santa Cruz Biotechnology); and BAX (BD Biosciences).

Plasmids and Luciferase Assay—Full-length IFI16 cDNA was PCR-amplified using primers 5′-AAAGATTCTAGTTGAAGAGGT-GAAAAAAATAC-3′ and 5′-AGGCGGCGCTTAGAAGAAAAAGTCG-GTGAGACTTTCC-3′ from a HeLa cell cDNA library. An amplified fragment was subcloned into pcDNA3 (Invitrogen) and sequenced. p53-pcDNA3 was described previously (22). The bax promoter construct was from J. C. Reed (23). Cells at ~70% confluence in 60-mm plates were transfected using FuGene (Roche Applied Science). 1 μg of CAT reporter gene plasmid, 0.5 μg of PCMV-β-galactosidase plasmid, and 2 μg of p53-pcDNA3 and/or IFI16-pcDNA3. After 48 h, transfected cells were resuspended in 50 μl of 0.25 M Tris (pH 7.8), subjected to three freeze/thaw cycles, and centrifuged at 12,000 rpm for 5 min to obtain supernatants for measurements of CAT and β-galactosidase activity as described previously (24).

Adenovirus and Retrovirus—All adenoviral vectors were generated using the AdEasy System (kindly provided by B. Vogelstein). Briefly, Adp53 and Ad-IFI16 were constructed by subcloning wild type p53 and IFI16 cDNA into pAd-TRACK-CMV vector. After recombination with the wild type adenovirus DNA, all viruses were propagated, purified, and titrated. Adenovirus infections were carried out at multiplicity of infection (MOI) of 10–50. Retrovirus-expressing human papilloma virus E6 protein was produced in Phoenix packaging cells with amphotropic envelope (kindly provided by G. Nolan, Stanford University) by transfecting with p2E67B (25). MCF7 cells were infected with E6 virus and maintained in Dulbecco’s modified Eagle’s medium-10% fetal bovine serum containing 700 μg/ml G418 (Sigma).

Northern blot—Twenty micrograms of total RNA/sample were then denatured, subjected to electrophoresis through a 0.8% agarose-formaldehyde gel, and transferred by capillarity onto a nylon membrane. IFI16 and 36B4 probes were 32P-labeled using the random primed DNA labeling technique. Blots were exposed on x-ray film after washing.

Immunohistochemical Analysis—Immunohistochemical studies were done using paraffin blocks of breast tissues provided from the Cooperative Human Tissue Network. Briefly, deparaffinized samples were treated with 1mMEDTA (pH8.0) and microwaved for 5 min. After blocking with 1% nonfat milk in phosphate-buffered saline and 0.5 ml of trypan blue solution (0.4% w/v) was added. After 5 min, cells were counted with a hemocytometer. For Giemsa staining, cells on the plates were washed with phosphate-buffered saline and fixed with 70% ethanol and stained with Giemsa solution as described elsewhere (26).

RESULTS

Frequent Loss of IFI16 Expression in Breast Cancer Cells—Previous studies have demonstrated that IFI16 is involved in tumor-suppressive functions of both p53 and BRCA1 proteins, suggesting the roles of IFI16 in cancer development (22, 27, 28). We carried out an immunocytochemical analysis of IFI16 in human cancer and evaluated levels of IFI16 in breast cancer cell lines and mammary epithelial cells. Western and Northern blot analyses detected higher levels of IFI16 protein and mRNA in normal human mammary epithelial cells (12N, 15N, MCF10A), but most human breast cancer cell lines, including T47D, MCF7, 21PT, SKBR3, BT20, ZR75, and MDA-MB-468, -431, and -453 showed no level of expression (Fig. 1, A and B). Low-level protein expression was detected in such breast cancer cells as HCC1937, Ha578T, and 21MT2 (data not shown). These results indicate that expression of IFI16 is frequently lost in human breast cancer cells.

Frequent Loss of IFI16 Protein in Mammary Epithelial Carcinoma—Previous immunohistochemical analysis has shown that IFI16 expression is not restricted to the hematopoietic compartment but may take place in some epithelial cells, such as
as those of trachea, spleen, and thymus; expression in mammary epithelial cells has not been studied (14, 15). To further understand IFI16 roles in mammary carcinogenesis, immunohistochemical analyses of IFI-16 expression was performed on breast tissue from 25 patients (12 carcinomas, 10 normal samples, 3 benign lesions (fibroadenomas)); 5 matched normal and tumor samples were included. Normal mammary epithelium expressed nuclear IFI16 moderately to strongly; cytoplasmic expression, though variable, tended to be weaker than nuclear expression (Fig. 2, a and c). Autologous carcinoma cells showed negative or only weakly positive nuclei but weak to moderate cytoplasmic expression. (Fig. 2b). In this regard, benign fibroadenoma was like normal epithelial cells (Fig. 2d); other carcinoma samples showed reduced IFI16 expression (Fig. 2, e and f).

Thus, the IFI16 expression pattern differs in benign versus malignant breast epithelial cells, demonstrating loss of IFI16 from the nucleus in carcinoma cells. Accordingly, IFI16 may play a role in the normal mammary epithelial cell phenotype, but IFI16 function is lost in malignancy.

Loss of IFI16 Results in Reduced p53 Activation by Ionizing Radiation—We studied whether IFI16 is required for the maximal activation of p53 under conditions of DNA damage, because IFI16 is involved in p53-mediated regulation of transcription (22, 27, 28). Transfection of MCF10A cells with siRNA specific for down-regulation of the IFI16 gene resulted in significant reduction of IFI16 protein 48 h after transfection compared with the control si-RNA (Fig. 3, top). Cells were treated with ionizing radiation (10 Gy, time 0), and cell lysates were prepared at 0, 0.5, 1, 2, 4, 6, and 12 h after IR treatment. Recent studies have revealed that phosphorylation of ATM-Ser1981 is crucial for the activation of the catalytic activity of ATM when cells are damaged by IR (29). Phosphorylation of Ser-1981 was similarly induced in both control MCF10A cells and cells in which IFI16 is reduced, indicating that ATM activation by IR damage is not affected by loss of IFI16. Previous results have demonstrated that phosphorylation of p53Ser15 by ATM protein kinase results in increased stability of p53 (17). Consistent with the increased phosphorylation of ATM upon IR treatment, induction of both protein levels and phosphorylation of Ser-15 of p53 was similar in both control MCF10A cells and cells in which IFI16 is reduced, indicating that ATM activation by IR damage is not affected by loss of IFI16. After 6 h in IFI16-reduced cells, protein levels of p53 and phosphorylation of p53Ser15 were decreased, whereas they were sustained in control cells for 12 h. It has been demonstrated that p14ARF inhibits MDM2-mediated p53 degradation by sequestering MDM2 from p53 (30). In MCF10A cells transfected with IFI16 si-RNA, p14ARF was transiently de-
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IFI16 Enhances p53-mediated Apoptosis—Previous results demonstrated that the reporter construct containing the p53-binding element and the CAT gene is enhanced by ectopic expression of p53 and IFI16 (27). To elucidate the physiological roles of IFI16 in p53-mediated regulation of cell proliferation, we studied whether IFI16 can stimulate endogenous p53 activated by DNA damage. MCF7 cells expressing wild type p53 were transfected with the minimal reporter plasmid PGluc, which contained two copies of p53 binding sites cloned upstream of the luciferase gene (31), and reporter activity was examined with or without IFI16 under conditions of IR (10 Gy) treatment (Fig. 4A). IFI16 transfection and IR treatment activated PGluc 3- and 11-fold, respectively. When IFI16-transfected cells were irradiated, PGluc showed 19-fold induction. These results suggest that IFI16 is involved in the p53-mediated DNA damage pathway.

Next, we examined the biological activity of IFI16 by infecting MCF7 cells with adenovirus IFI16 under DNA damage (Fig. 4B). Subconfluent MCF7 cells were infected with GFP-Ad-TRACK or Ad-IFI16 (Ad-IFI16) adenovirus for 24 h and then subjected to DNA damage with neocarcinostatin (NCS). After 12 h, flow cytometric analysis of Annexin V staining was performed to study the apoptosis fraction counted in the lower-right quadrants of each panel. Whereas AdTRACK induced apoptosis with NCS treatment (from 3.82 to 9.42%), Ad-IFI16 alone induced significant apoptosis (24.4%), which was enhanced by NCS treatment (36.0%). These results suggest that IFI16 can potentially induce apoptosis. After induction of apoptosis of MCF7 cells infected with Ad-IFI16, the remaining cells did not grow as quickly as the control MCF7 cells infected with Ad-TRACK virus.

It has been shown that human papilloma virus protein E6 induces p53 degradation through the ubiquitin pathway (32). We studied apoptosis of MCF7 cells and MCF7-E6 cells that stably express retrovirus E6 to clarify the collaboration of IFI16 and p53 in p53-mediated DNA damage pathway in breast cancer cells (25). Reduced levels of p53 in MCF7-E6 cells were confirmed by immunoblot analysis (Fig. 4C). Both cell types (5 ¥ 10⁶ cells) were infected with Ad-TRACK or Ad-IFI16 and treated with NCS as described in Fig. 4B. After 12 h of NCS treatment, cell viability was studied by trypan blue staining (Fig. 4D, left). Consistent with results shown in Fig. 4B, MCF7 cells expressing IFI16 showed reduced viability (about 48% reduction) when treated with NCS compared with NCS alone or Ad-IFI16 alone (−20 and −25% reduction, respectively). Significant reduction of cell viability was not observed in MCF7-E6 cells. Cell survival was further studied by Giemsa staining. MCF7 and MCF7-E6 cells (1 ¥ 10⁶ cells) were infected with Ad-TRACK or Ad-IFI16 as described. After adenovirus infection, cells were maintained for 7 days in the presence of NCS. Cells were stained with Giemsa and survived colonies were counted (Fig. 4D, right). MCF7 cells treated with Ad-TRACK, Ad-IFI16, or AD-TRACK plus NCS showed similar numbers of colonies (−180–190 colonies); however, Ad-IFI16 plus NCS treatment markedly reduced the numbers of colonies (−60 colonies). Decreased cell survival was not observed in MCF7-E6 cells.

IFI16 induction of apoptosis was further examined by means of tetracycline-regulatable IFI16 in human bladder carcinoma EJ cells (Fig. 4E), which do not express endogenous p53. When tetracycline was removed, cells were infected with Ad-TRACK or Ad-p53 for 24 h followed by IR treatment (10 Gy). Cells undergoing apoptosis were studied with Annexin V staining as described above. As shown in Fig. 4F, Ad-p53 alone or Ad-p53 plus IR treatment did not induce apoptosis without IFI16 induction (2.5 and 3.8%, respectively). When IFI16 was induced, weak induction of apoptosis by Ad-p53 (4.2%) was strongly enhanced by IR treatment (29%). Ad-TRACK did not induce apoptosis in IFI16-induced or -uninduced cells. Expression of p53 and IFI16 was confirmed by immunoblot analysis (Fig. 4D, bottom). Taken together, these results demonstrate that IFI16 is involved in the p53-mediated apoptosis pathway induced by DNA damage.

IFI16 Enhances Expression of p53 Target Proteins—Increasing numbers of apoptosis- and cell cycle-related genes that are transcriptionally regulated by p53 have been identified. These are candidates for implementing p53 effector functions. Because our results suggest that IFI16 is involved in apoptosis pathway mediated by p53, we examined whether IFI16 affects p53-induced expression of the target proteins. A Bcl-2 family member, BAX, is a transcriptional target of p53 and one of the well characterized proapoptosis proteins (23, 24). To study the activity of IFI16 in p53-mediated gene expression, we examined bax promoter activation by coexpressing p53 and IFI16 (Fig. 5). MCF7 cells were transfected with p53 and/or IFI16 together with the reporter gene containing 370 bp of the human bax promoter subcloned upstream of the CAT gene (24). IFI16 and p53 alone activated the bax promoter ~5- and 8-fold, respectively. When both proteins were coexpressed, promoter activity was enhanced up to 17-fold. Immunoblot analysis indicated that MCF7 cells express an undetectable level of endogenous IFI16 (Fig. 1 and Fig. 5, lower panel) and confirmed increased levels of p53 when cells were transfected with p53 (Fig. 5, lower panel). Consistent with the results showing the collaboration of p53 and IFI16, IFI16 in transcription, endogenous levels of BAX, p21WAF1, and HDM2 were synergistically induced by expressing both proteins. These results demonstrate that IFI16 enhances expression of p53 target proteins.

DISCUSSION

In this study, we first evaluated the distribution of IFI16 immunohistochemically in 25 breast tissues. Although IFI16 was originally isolated from mRNA of a lymphocyte cell culture and is highly expressed in lymphocytes, our results showing that strong nuclear staining of IFI16 is also detected in breast epithelial cells demonstrate that expression of IFI16 is not restricted to cells of the immune system. It is noteworthy that expression levels of IFI16 are also reduced in 6 of 18 pancreatic cancer cell lines examined, suggesting that the low of IFI16 is associated not only with breast cancer development but also pancreatic cancer.²

Gene silencing that arises from methylation is an important epigenetic mechanism of gene inactivation (33, 34). Methylated CpGs are recognized by proteins that recruit histone deacetylases, leading to stable transcriptional repression, which can often be reversed by the methylation inhibitor 5aza2dC (5azaC, Ref. 35). To determine whether IFI16 is repressed by methylation, breast cancer cell lines (MCF7, MDAMB468, MDAMB431, and MDAMB453) were treated with 5 μM 5azaC

² N. Maehara, F. Siahin, and G. H. Su, manuscript in preparation.
for 2–3 population doublings and IFI16 expression was examined. Only in several cell lines did 5azaC produce a 3–5 fold increase in IFI16 protein (not shown). Although this indicates that transcriptional silencing by methylation may contribute to IFI16 inactivation in subsets of breast cancer, it is also possible that a transactivating regulator of IFI16 may be modulated by methylation or that methylation affects an enhancer or other regulatory element outside the IFI16 promoter because CpG

**FIG. 4.** IFI16 and IR synergistically activate p53 activity. A, human breast cancer MCF7 cells were transfected with IFI16 and p53 reporter plasmid, PGluC. Thirty-six hours later, cells were IR-treated (10 Gy) as indicated, and luciferase activity was measured after 12 h. Relative luciferase activity was normalized by cotransfection of β-galactosidase control plasmid. B, MCF7 cells were infected with GFP (Ad-TRACK) or IFI16 (Ad-IFI16) adenovirus for 24 h followed by neocarzinostatin (NCS) treatment (100 ng/ml, 12 h). Apoptosis detected by Annexin V staining was analyzed by FACSCalibur (BD Biosciences). Duplicate or triplicate experiments were performed, and means and S.D. values were indicated. C, immunoblot analysis of p53 in MCF7 and MCF7-E6 cells. D, left, MCF7 or MCF7-E6 cells (5 × 10⁶ cells) were infected with adenovirus as indicated for 24 h, followed by NCS treatment for 12 h as described above. Cells were trypsinized and stained with trypan blue. Viable cells were counted in hemocytometer. Experiments were performed in duplicate. Right, MCF7 or MCF7-E6 cells (1 × 10⁶ cells) were infected with adenovirus as indicated for 24 h. NCS was added to the cell culture, and cells were maintained for 7 days. Cell colonies were stained with Giemsa and counted. Experiments were performed in duplicate. E, expression of IFI16 in EJ cells was induced by removing tetracycline from the cell culture medium. Actin was the control immunoblot. F, IFI16-induced EJ cells were infected with adenovirus for 24 h, followed by IR treatment (10 Gy). Twelve hours later, apoptosis was determined by Annexin V staining as described in panel B. Cell extracts were also used for immunoblot analysis to measure the expression levels of induced IFI16 and Ad-p53. Actin was the control immunoblot.
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whether proapoptosis activity of IFI16 is specific to a certain type of DNA damage. We are currently studying proapoptosis activity of IFI16 under different conditions of DNA damage. More recently, we have found that loss of IFI16 resulted in accumulation of p53, leading to accumulation of p21WAF1 and growth suppression without DNA damage (28). These results and our current studies suggest a model that IFI16 is required for the immediate activation of p53 under conditions of DNA damage and that loss of IFI16 provides a stress to cells that also causes p53 activation. Although the mechanism of p53 activation induced by loss of IFI16 remains to be elucidated, Hdm2 might induce degradation of p53 when p14ARF is transiently reduced (Fig. 3).

Considering our findings in the whole, it is possible that functional interaction between p53 and IFI16 is relevant to p53 signaling to breast cancer. This model is reinforced by an observation showing increased apoptosis induced by DNA damage in cells expressing IFI16. Given that AIM2, a member of the PYRIN family of the protein, is a tumor-suppressor protein of human melanoma (36), loss of apoptosis regulation by IFI16 is likely closely associated with tumor malignancy.

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REFERENCES
1. Johnstone, R. W., and Trapani, J. A. (1999) Mol. Cell. Biol. 19, 5833–5838
2. Trapani, J. A., Browne, K. A., Dawson, M. J., Ramsay, R. G., Eddy, R. L., Show, T. B., White, P. C., and Dupont, B. (1992) Immunogenetics 36, 369–376
3. Johnstone, R. W., Kerr, J. A., and Trapani, J. A. (1996) J. Biol. Chem. 271, 17172–17177
4. Dawson, M. J., and Trapani, J. A. (1995) Biophys. Res. Commun. 214, 152–162
5. Datta, B., Li, B., Choubey, D., Nallur, G., and Lengyel, P. (1996) J. Biol. Chem. 271, 37544–37555
6. Choubey, D., and Lengyel, P. (1995) J. Biol. Chem. 270, 6134–6140
7. Choubey, D., Li, S.-J., Datta, B., Gutterman, J. U., and Lengyel, P. (1996) EMBO J. 15, 5668–5678
8. Min, W., Ghosh, S., and Lengyel, P. (1996) Mol. Cell. Biol. 16, 359–368
9. Aravind, L., Dixit, V. M., and Koonin, E. V. (2001) Science 291, 1279–1284
10. Staud, K., Dahl, E., and Rosenthal, A. (2001) Trends Biochem. Sci. 26, 83–85
11. Fairbrother, W. J., Gordon, N. C., Humke, E. W., O’Rourke, K. M., Starkovanskii, M. A., Yin, J. P., and Dixit, V. M. (2001) Protein Sci. 10, 1911–1918
12. Pawlikowski, K., Pin, F., Chu, Z., Reed, J. C., and Godzik, A. (2001) Trends Biochem. Sci. 26, 85–87
13. Martinon, F., Hofmannm, K., and Tschopp, J. (2001) Curr. Biol. 11, 118–120
14. Wei, W., Clarke, C. J. P., Sumner, G. R., Cresswell, K. S., Loveland, K. A., Rourke, K. M., Starostowicz, P., White, P. C., and Dupont, B. (1992) Oncogene 11, 45–54
15. Gariglio, M., Aranzimonti, B., Pagano, M., Palestro, G., Andrea, M. D., Valente, G., Voglino, G., Navine, L., and Landolfi, S. (2003) J. Interferon Cytokine Res. 23, 815–821
16. Vogelstein, B., and Kinzler, K. W. (1992) Cell 71, 523–526
17. Giaccia, A. J., and Kastan, M. B. (1998) Science 270, 2973–2983
18. Vousden, K. H. (2000) Cell 101, 691–694
19. Prives, C., and Manley, J. L. (2001) Cell 107, 815–818
20. Band, V., Zachowski, D., Kulesa, V., and Sager, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 463–467
21. Lee, S. W., Fang, L., Igarashi, M., Ouchi, T., Lu, K.-P., and Aarnonen, S. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8302–8305
22. Aglipay, J. A., Lee, S. W., Okada, S., Fujisuchi, N., Ohsuka, T., Kwak, J. C., Wang, Y., Johnstone, R. W., Deng, C. X., Qin, J., and Ouchi, T. (2003) Oncogene 22, 8931–8938
23. Reed, J. C. (1994) J. Cell Biol. 124, 1–6
24. Miyashita, T., and Reed, J. C. (1995) Cell 80, 293–299
25. Yutusudo, M., Okamoto, Y., and Hakura, A. (1988) Virology 166, 594–597
26. Watkins, S. (1993) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, R., eds) Vol. 1, pp. 14.5.1–14.5.2, Greene and Wiley-Interscience, New York
27. Johnstone, R. W., Wei, W., Greenway, A., and Trapani, J. A. (2000) Oncogene 19, 6033–6036
28. Kwak, J. C., Ongusaha, P. P., Ouchi, T., and Lee, S. W. (2003) J. Biol. Chem. 278, 40989–40994
29. Bakkenist, C. J., and Kastan, M. B. (2003) Nature 421, 499–506
30. Sherr, C. J., and Weber, J. D. (2000) Curr. Opin. Genet. Dev. 10, 3689–3695
31. Ouchi, T., Monteiro, A. N., August, A., Aarnonen, S. A., and Hanafusa, H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2302–2306
32. Howley, P. M. (1991) J. Virol. 65, 451–454
33. De Young, K. L., Ray, M. E., Su, Y. A., Anzick, S. L., Johnstone, R. W., and Trapani, J. A. (1997) Oncogene 15, 453–457

Fig. 5. IFI16 collaborates with p53 to activate the bax promoter. MCF7 cells were employed for reporter gene assays by transfecting 370 bp of the bax promoter upstream of the CAT gene with empty vector, p53-pcDNA3, and IFI16-pcDNA3 as indicated. CAT activity was measured 2 days later and was normalized relative to β-galactosidase. Cell extracts were also used for the immunoblot analysis to study the expression levels of transfected IFI16, p53, and endogenous p21, HDM2, and BAX proteins.
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