INTRODUCTION
Mammalian cells treated with ionizing radiation (IR) induces a stress response associated with an enhanced tolerance to the subsequent cytotoxicity of IR (1–3). The adaptive response is originally observed in cells pre-exposed to a low or very low dose of IR (4–7). Some tumor cells treated in vitro with relative high doses of IR, e.g., fractionated IR (FIR), also demonstrate a transient radiore sistance that appears to be transient during growth (8–10). The molecular mechanism underlying this kind of stress response that may be causally related to tumor response to radiotherapy has not been elucidated. Although many stress signaling genes are activated by IR (11), only a small fraction of genes, i.e., elements of cell cycle control, apoptosis/antia poptosis, and DNA repair (12, 13) are believed to play a key role in the signaling network of IR-induced phenotypic changes. Using cell lines derived from chronic exposure of FIR we have reported that stress-responsive transcription factors nuclear factor (NF)-κB, p53, and AP-1 are activated by FIR (10, 14, 15). The mechanism causing the activation of these transcription factors by chronic radiation have not been identified.

Two major phenotypic alterations, growth arrest and apoptosis, are linked with p53 activation after a variety of stress conditions including IR (16, 17). p53-induced growth arrest is believed due to the delay at G1-S or G2-M boundaries that is required for apoptosis (18–21). More than 100 genes of human genome have been identified as having binding sites for p53 (22), and many stress genes activated by IR are a result of p53 activation (23, 24). With a link to these IR effector genes, p53 activity has been shown to affect the anticancer efficiency of radiotherapy using FIR (25). Additional evidence suggest that p53 is very sensitive to IR-induced DNA damages (26), and DNA strand breaks induced by IR are believed to be the major source that trigger the p53-dependent repair system (27). However, it is unclear how p53 is regulated in cells that showed a transient tolerance to IR after exposure chronically to IR. This transient radioreistant phenotype in radiation-derived cells (8–10) presents a tolerance of tumor cells to radiotherapy. Both MDM2 functioning in p53 protein degradation and p14ARF functioning in MDM2 inhibition may be involved in the metabolic regulation of p53 activity (28, 29). IR-induced p53 initiates the signaling process that causes cell cycle arrest, which is accompanied by the ability for DNA repair and apoptosis (30, 31). This process can be actively regulated by expression of MDM2. Activation of p53 and MDM2 is found in cells arrested at G1-S phase or G2-M boundaries induced by IR (17). Interaction of MDM2 with the p53 NH2-terminal region has been shown to accelerate p53 degradation and to inhibit the capacity for p53-mediated gene transcription (28, 29). In addition, MDM2 is found actively involved in the process of p53 nuclear exclusion (32), and the sequence required for MDM2-mediated p53 degradation and nuclear export has been identified (33, 34). MDM2 is also shown to be a unique ubiquitin-protein ligase to ubiquitinate and degrade p53 (28, 29).

However, the inhibitory effect of MDM2 on p53 can be counteracted by p14ARF, a protein that directly binds to MDM2 and, as a result, inhibits p53 degradation by blocking both p53-MDM2 nuclear export (35, 36) and p53 ubiquitination (37–39). As such, the balance of MDM2 and p14ARF protein levels may play a critical role in the regulation of p53 causing cell phenotypic alterations after chronic IR. By pairwise analysis of radioresistant MCF+ FIR cells that were derived from chronic FIR with sham-FIR control MCF-7 cells, the present study was designed to determine whether p53 is regulated because of the alteration of p53-regulating proteins. Results of luciferase reporter and DNA microarray analysis demonstrated that p53 was activated and that transcripts of p21, MDM2, and p14ARF, as well as a group of stress-responsive genes, were up-regulated by chronic exposure to IR. However, time course analysis showed different protein levels induced by IR in sham-FIR control versus MCF+FIR cells. No difference was detected in p21 induction.

MDM2 was inhibited and, correspondingly, MDM2 inhibitor p14ARF was prominently activated in MCF+FIR cells. Compared with sham-FIR control cells, MCF+FIR cells showed an increased level of phosphorylated p53 (Thr 55) in the cytoplasm and the nucleus of MCF+FIR, indicating that MDM2 inhibition and p14ARF activation contribute to a high responsiveness of p53 in chronic IR-derived breast cancer cells.

MATERIALS AND METHODS
Analysis of IR-Derived MCF+FIR Cells. MCF-7 cells (starting at passage 168) were purchased from American Type Culture Collection. MCF+FIR
cells were obtained from MCF-7 cells by exposure to FIR with a total dose of 60 Gy γ-irradiation. Radiation was delivered at room temperature at a rate of 46 cGy/min (Theratron-80; 3N 140 Co-60 Unit; Atomic Energy of Canada Limited). MCF-7 cells, treated with the same procedure but without FIR, were maintained as sham-FIR control cells. Both sham-FIR control and MCF+FIR cells were cultured in DMEM, and experiments were performed with the MCF+FIR cells within 7–10 passages after the termination of FIR.

**Determining IR-Induced Apoptosis.** The terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL) method was used to detect apoptotic cells. Briefly, cells with or without 5 Gy of IR were incubated at 37°C, and, at times after IR, both attached and floating cells were harvested and mixed. After washing with PBS, cells were dropped on to slide glasses and were dried with cold air. All of the cells were fixed for 30 min at room temperature with 4% paraformaldehyde PBS. The slides were coded and evaluated using the ×40 objective with a photomicrography rectangle. Ten fields were evaluated in each slide to obtain a mean number for the presence of up to 2000 positive cells for each experimental data point. The fraction of apoptotic cells was expressed as the percentage of the total number of cells and was determined in three independent experiments for each time point.

**Cell Cycle Distribution after IR.** Sham-FIR control and MCF+FIR cells were growing in complete medium and were harvested at different times after exposure to a single dose of 5 Gy of IR and were fixed in 75% ethanol. The fixed cells were collected and resuspended in 1 ml of staining buffer, containing 0.1% sodium, 50 μg/ml propidium iodide, 0.1% Triton X-100, and were further incubated overnight at 4°C in the dark. Flow cytometry was performed to detect propidium iodide-stained nucleus with a the system of FACScan Plus (Becton Dickinson, San Jose, CA), and the data from 1 × 106 cells were collected and analyzed using Multicycle software.

**Reporter Transfection and Luciferase Assay.** Sham-FIR control and MCF+FIR cells were grown in 12-well culture flasks until they reached 70% confluence. For gene transfection, 0.3 μg of p53-controlled luciferase reporters (40) and 0.2 μg of β-galactosidase reporters were cotransfected using LipofectAMINE-plus Reagent (Life Technologies, Inc., Gaithersburg, MD). Both MCF+FIR and sham-FIR control MCF-7 cells were transfected for 3 h and were recovered in complete medium for 6 h. The luciferase activity was measured at different times after radiation with a single dose 5 Gy. For the control of reporter transfection efficiency, an aliquot of the same cell lysates that was prehybridized in the ExpressHyb Hybridization solution (Clontech) was used for the measurement of β-galactosidase activity (β-galactosidase Enzyme Assay system; Promega Inc. Madison, WI), and the luciferase activity was normalized to β-galactosidase activity.

**Purification of Cytoplasmic and Nuclear Proteins.** Cytoplasmic and nuclear proteins were extracted as described previously (41). Briefly, sham-FIR control MCF-7 and MCF+FIR cells were exposed to a single dose of 5 Gy of radiation, collected with trypsin, and washed three times with PBS. The cell pellets were then resuspended in 1 ml of cell lysis buffer containing: 50 mM KCl, 0.5% NP40, 25 mM HEPES (pH 7.8), 32 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 4 μg/ml aprotinin, and 100 μM DTT. The lysis mixture was then centrifuged at 4°C for 1 min. The supernatant was collected as cytoplasmic protein, and the pellets were washed once with 0.5 ml of washing buffer by centrifuging at 4°C in a roller. Cell nuclei were then resuspended in 100 μl of nuclear protein extraction buffer containing 500 mM KCl, 25 mM HEPES, and 10% glycerol and were rocked at 4°C for 15 min, and supernatants were saved as nuclear proteins.

**Gel Shift Analysis.** One to three μg of nuclear proteins were incubated on ice for 10 min in a total of 20 μl of DNA binding buffer containing binding buffer containing: 10 mM Tris-HCl (pH 7.5), 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM DTT, 5% glycerol, and 50 μg/ml polydeoxinosinic:polydeoxyctydilic acid strand DNA. They were then incubated for 20 min at room temperature with 5 × 104 cpm 32P-labeled p53 oligonucleotides probe (5′TACGGCATGTCTAGC- GATGTTAAGGCT-3′). The DNA-protein complexes were resolved on 10% native PAGE and visualized in X-ray film.

For supershift assay, nuclear extracts were incubated with antibodies at room temperature for 20 min before the addition of the radioactively-labeled DNA oligonucleotides.

**RNA Purification and DNA Microarray Analysis.** RNA preparation and DNA microarray analysis followed the protocol as described earlier (15). Briefly, total RNA was extracted from sham-FIR control and MCF+FIR cells using TRIzol Reagent (Life Technologies, Inc.). After confirmation of the integrity on an agarose gel, RNA was digested using RNase-free DNasel for 20 min, was extracted with phenol-chloroform, and then was precipitated with 2.5 volume of ethanol. Polyadenyllic acid + RNA was isolated using the Oligotex RNA kit (Qiagen Inc., Valencia, CA). Gene expression was analyzed using the Atlas Human Cancer cDNA Expression Array filter (588–1176 genes) from Clontech (Clontech Laboratories, Inc., Palo Alto, CA). For hybridization, 1 μg of polyadenyllic acid + RNA was transcribed with nucleotides containing [α-32P]dATP, and the labeled cDNA was purified, denatured, and added to 5 ml of ExpressHyb Hybridization solution (Clontech). The final probe with a concentration of 1 × 106 cpm/ml was freshly applied to the array membrane that was prehybridized in the ExpressHyb Hybridization solution (Clontech) for 30 min. Hybridization was allowed to proceed overnight at 68°C in a roller bottle.

The filters were then stringently washed with agitation for 20 min in 200 ml of prewarmed (68°C) solution 1 (2× SSC, 1% SDS) and twice with solution 2 (0.1× SSC, 0.5% SDS) before being exposed to X-ray film overnight at −80°C. The filters were also exposed to a Phosphor Screen overnight and scanned using a Storm 840 PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA), and signals of paired genes were quantified by ImageQuant software. Results represent the quantitation of one hybridization with two sets of fluorescence-labeled RNA probes, and changes less than 2-fold were not listed.

**Western Blot.** Ten to 20 μg of cytoplasmic or nuclear proteins were mixed with 50 μl of loading buffer, heated at 70°C for 10 min, size-separated in 12.5% acrylamide SDS-PAGE, and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). The membranes were then blocked at room temperature for 2 h in blocking solution (Pierce Co., Rockford, IL), washed with 0.01% Tween PBS, and incubated overnight at 4°C with antibodies to MDM2 (sc-5304), p21 (sc-817), and p14ARF (sc-8340) purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The blots were then incubated with horse-radish peroxidase-conjugated secondary antibody at a dilution of 1:3000. Protein bands were visualized using the ECL Plus detection system (Amer- sham Life Science, Arlington Heights, IL).

**RESULTS**

**FIR-Induced Radioresistance and Cell Cycle Distribution.** As we have demonstrated previously, MCF-7 cells derived from chronic IR MCF+FIR showed an increased tolerance to IR-induced cytotoxicity (15). To determine the apoptotic response of MCF+FIR cells, MCF+FIR and sham-FIR control cells were irradiated with a single dose of 5 Gy, and apoptotic cells were calculated at different times after IR.

Although no obvious change was found in basal level of apoptotic

![Fig. 1](https://example.com/fig1.png)
p53/DNA binding activity was estimated by densitometry (representative of three experiments; some error limits are hidden in the symbols). Gel shift analysis was performed with 32P-labeled p53 consensus oligonucleotide and 4 μg of nuclear proteins isolated from sham-FIR control MCF-7 and MCF-FIR cells. The sham-FIR control MCF-7 cells showed a low level (6.3-fold and 7.9-fold at 8 h and 20 h, respectively, in MCF-7 cells). The basal DNA binding activity was strikingly induced in MCF-FIR cells after 5 Gy of IR (2.0-fold and 3.3-fold at 8 h and 20 h, respectively, in the sham-FIR control cells; Fig. 2A). The basal DNA binding activity of p53 detected by gel shift assay was increased (~10-fold) in MCF-FIR cells, and little increase was induced after IR (Fig. 2, Lanes 6–8). The sham-FIR control MCF-7 cells showed a low basal DNA binding but predominant induction by IR (~12.3-fold at 24 h; Fig. 2B, Lane 4). These results demonstrate that DNA binding and p53-controlled luciferase transcription are differently regulated in IR-derived MCF-FIR cells.

Increased Phosphorylation and Nuclear Distribution of p53 in MCF-FIR Cells. Western blotting was applied to determine whether increased DNA binding activity of p53 is caused by phosphorylation and protein accumulation. The basal and 5-Gy-IR-induced p53 and Thr 55 phosphorylation was accessed in sham-FIR control MCF-7 and MCF-FIR cells. Total p53 in cytoplasm showed little difference except a slight increase in 5-Gy-treated MCF-FIR cells (Fig. 3A). In contrast, the basal and IR-induced p53 (Thr 55) was enhanced in both the cytoplasm and the nucleus of MCF-FIR cells. Interestingly, both total and phosphorylated p53 was slightly reduced in the control MCF-7 cells, which is accompanied by the increased DNA-binding of p53 (Fig. 2), suggesting an enhanced p53 degradation in control MCF-7 cells. This inhibitory function appears to be down-regulated in IR-derived MCF-FIR cells.

p53-Responsive Genes Detected by DNA Microarray Analysis. DNA microarray analysis was then applied to detect effector genes differently regulated in MCF-FIR versus sham-FIR control cells. We have reported a cluster of effector genes activated in the radioresistance induced by expression of MnSOD and/or FIR (15). The present study shows gene expression levels in MCF-FIR versus sham-FIR control cells using 588-1176 grouped human gene fragments (Clontech Atlas Human Cancer cDNA). Each gene was pair-wise analyzed by comparison of the expression level from different conditions.
MCF+FIR to the level of sham-FIR control MCF-7 cells. Genes found to be down-regulated (<2-fold decrease compared with MCF-7; Table 2) or up-regulated (>2-fold increase compared with MCF-7) were grouped by their functions and were ranked with gene expression levels (Table 3). A group of signaling elements associated with p53 regulation and signaling apoptosis and/or antiapoptosis including MD2 (3.8-fold), p21 (4.6-fold), INKA-ARF (3.9-fold), IAP3 (4.1-fold), Cyclin B1 (3.3-fold), Cyclin D1 (3.4-fold), and others were up-regulated in MCF+FIR cells (marked in the list of Table 3). p14ARF, an alternative gene product of INKARF (39) has been indicated to act as a negative control of MDM2 and thus controls MDM2-mediated p53 regulation.

Because p21, MDM2, and p14ARF are closely linked with p53 reg-

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* Results represent the quantitation of two hybridizations of two sets of fluorescence-labeled RNA probes, not including values with changes of less than 2-fold.

* MCF + FIR profiles were obtained by normalizing gene expression levels of MCF + FIR population to the level of sham-FIR treated MCF-7 cells.

* Underlining indicates the genes associated with p53 function and the time-course of protein expression (numbers 4, 7, and 8) was analyzed in MCF + FIR versus sham control MCF-7 cells.
To show equal loading of proteins (representative of three experiments).

Fig. 4. No difference in ionizing radiation (IR)-induced p21 protein expression and nuclear distribution. A, Western blotting was performed with 20 μg of cytoplasmic or nuclear proteins isolated from sham-fractionated IR (FIR) control MCF-7 and MCF+FIR cells at times indicated after 5 Gy of irradiation. The nonspecific bands (NS) were included to show equal loading of proteins (representative of three experiments). B, relative levels of cytoplasmic and nuclear p21 proteins estimated by the ratio of p21:NS bands measured with densitometry.

ulation, we further analyzed the time course changes of the protein levels of p21, MDM2, and p14ARF and their nuclear distribution after IR.

p21 Induced by IR in Both Sham-FIR Control and MCF+FIR Cells. To confirm the protein levels of p21 expression detected by microarray analysis, Western blot was performed using cytoplasmic and nuclear proteins of sham-FIR control and MCF+FIR cells with or without 5 Gy of IR (Fig. 4). Although the basal p21 protein expression was slightly increased (in agreement with up-regulated gene transcripts shown in Table 3) in the cytoplasm and nucleus of MCF+FIR cells (2-fold in cytoplasmic and 3-fold in nuclear proteins), no difference was detected in p21 induction and nuclear translocation after 5 Gy of IR (Fig. 4).

These results suggest that the p21 expression level may not act as a rate-limiting factor in signaling IR-derived radioresistance.

MDM2 Induction and Nuclear Distribution Was Inhibited in MCF+FIR Cells. Our microarray data showed an elevated MDM2 mRNA level in MCF+FIR cells (3.8-fold; Table 3). To get into the inside mechanisms underlying p53 activation in MCF+FIR cells, distribution of cytoplasmic and nuclear MDM2 as well as MDM2 in p53 DNA-binding complexes were analyzed. The basal MDM2 was detectable only in MCF+FIR cells but not in the sham-FIR control cells (Fig. 5A, Lane 5 normalized to the background of Lane 1). However, surprisingly, MDM2 immunoreactive protein was not induced during a time period of 3–24 h after IR (Fig. 5A, Lanes 5–8). In contrast, the cytoplasmic MDM2 was markedly enhanced (~7-fold at 3 h) in sham-FIR control cells (Fig. 5A, Lanes 1–4) and detected in the nuclear protein purified from sham-FIR control cells treated with IR. However, no MDM2 nuclear distribution was detected in MCF+FIR cells with or without IR (Fig. 5B).

We then asked the question of whether the nuclear-distributed MDM2 combines to p53/DNA complexes leading to a feed-back control of p53 activation. Supporting this concept, supershifting analysis with antibody to MDM2 demonstrated that MDM2 was present in the p53/DNA complex of the sham-FIR control cells 24 h after 5 Gy of IR (Fig. 5C, Lanes 1 and 2), and MDM2 was not detected in the p53/DNA complex of MCF+FIR cells with or without 5 Gy of IR (Fig. 5C, Lanes 3 and 4). These results demonstrate that MDM2/p53 binding occurs in the nucleus of control MCF-7 cells, indicating a feed-back control of p53 by MDM2 in IR-induced stress response. This regulation mechanism appears to be down-regulated in chronic IR-derived MCF+FIR cells. Lack of phosphorylated p53 (Thr 55) in control cells, but increased in MCF+FIR cells (Fig. 3), laid a support for this hypothesis.

Transcriptional and Protein Levels of MDM2 Inhibitor p14ARF Were Increased in MCF+FIR Cells. Two proteins have been reported by the splicing of the mRNA of INKARF: p16ARF (interaction with Rb protein), and p14ARF (interaction with MDM2; Ref. 38). Most recently, p14ARF has been linked to MDM2 protein degradation (38, 42). INKARF transcripts were up-regulated 3.8-fold in MCF+FIR cells (Table 3).

The increased p14ARF that reduces p53 degradation by MDM2, may contribute to the high p53 level of protein in MCF+FIR cells, p14ARF mRNA and protein levels were analyzed with or without 5 Gy of IR (Fig. 6). Using published primers of p14ARF (43), reverse transcription-PCR showed that basal p14ARF transcripts were very low but were enhanced by 5 Gy of IR (3-fold) in sham-FIR control cells (Fig. 6A, Lanes 1 and 2). However, basal p14ARF transcripts were much more highly increased (~9-fold) and were maintained at a similar level in MCF+FIR cells after 5 Gy of IR (Fig. 6A, Lanes 3 and 4). Consistent with the increased transcripts, p14ARF proteins that were detected in cytoplasmic and nuclear extracts were 6–8-fold higher in MCF+FIR cells compared with the levels of sham-FIR control cells (Fig. 6, B and C). These results suggest that the expression of p14ARF and the inhibition of MDM2 work together to maintain p53 protein in the nucleus causing a high responsiveness of p53 in chronic IR-derived radioresistant cells.

DISCUSSION

The present findings demonstrate that p53 is actively involved in human breast carcinoma MCF+FIR cells that showed a transient radioresistance after chronic exposure to IR. p53 transcriptional activity was increased with little recruitment of p53 in DNA binding:
and p53-modulating protein MDM2 was inhibited and MDM2-inhibitory protein p14ARF was activated in MCF+FIR cells. These results suggest that p53-regulating elements MDM2 and p14ARF play a key role in the up-regulation of the p53 activity under chronic stresses with IR.

p53 signaling network has been shown to play a central role in maintaining the genomic integrity and protecting cells against IR-induced damage (18, 26, 27). The present results indicate an elevated “constitutive” DNA binding and transcriptional activity of p53 in IR-derived MCF+FIR cells that showed an enhanced resistance to IR-induced apoptosis. It has been well accepted that activation of p53 is able to adjust the cell cycle time to allow cells to repair the damaged DNA induced by IR (44). IR-induced cell cycle delay is attributed to the activation of p53 (45), which induces cyclin-dependent kinase-inhibitory protein p21, which is, in turn, required for specific cell cycle checkpoints (30, 46). However, p21 showed little response to anticancer agent-induced stress in some tumor cells. Loss of p21 results in an induction of apoptosis, but no difference was found in the clonogenic survival (47, 48), in IR-induced S-phase checkpoint (41), or in DNA repair responses to UV radiation (21). We report here that, although p53 and p21 were activated in IR-derived MCF+FIR cells, no difference was detected in p21 protein and nuclear translocation in control and MCF+FIR cells after IR. In addition, our published results have shown that blocking p21 by antisense transfection did not enhance the radiosensitivity of IR-derived MCF+FIR cells (9). These results argue that although p21 transcription can be up-regulated because of p53 activation, p21 protein may not function as a rate-limiting element in chronic IR-induced stress response.

The reported (49) and present data (Table 3; Fig. 5) demonstrate that MDM2 transcripts are increased by IR. To determine MDM2 response in IR-derived cell, however, we found that MDM2 was inhibited in MCF+FIR cells after the stress with a single dose of IR. In contrast, MDM2 was induced (~7-fold) by IR (Fig. 5A) and MDM2 was detected in the p53/DNA binding complex of sham-FIR control but not IR-derived MCF+FIR cells (Fig. 5, B and C). On the basis of the interaction of MDM2 with p53, our results suggest that MDM2 functions to inhibit p53 in cells with a normal status of p53, and this interaction is inhibited in chronic IR-derived cells that showed a transient radioresistance. Interaction of MDM2 with p53 NH2-terminal region accelerates p53 degradation and inhibits the ability of p53-mediated gene transcription (50). In addition, the sequence required for MDM2-mediated p53 nuclear export has also been identified (34, 50). Therefore, MDM2 accumulated in the control sham-FIR cells after IR appears to be required for a feed-back control of p53 activation because p53/DNA binding was increased with a relative low enhancement of p53-controlled luciferase activity. Further evidence is provided by the fact that MDM2 was directly detected in p53/DNA complex in control MCF-7 but not in IR-derived MCF+FIR cells (Figs. 5, B and C).

Down-regulation of the p53 inhibitory element may be a critical adjustment to preserve p53 proteins in cells adapted to chronic IR. Mechanisms underlying MDM2 inhibition in IR-derived cells need to be identified.

An important finding in the present microarray and protein expression studies is the activation of p14ARF. In mammalian cells, the INK4a-p14ARF locus has been identified to encode two different cell cycle inhibitors (p16INK4a and p14ARF) by alternative splicing (51, 52). It has been suggested that ARF family proteins regulate p53 activity (36, 53) via a negative regulation by MDM2 (53, 54). p14ARF is also required for MDM2 degradation by small ubiquitin-like protein (SUMO; Ref. 42). In the present study, we found that p14ARF was activated in MCF+FIR cells that showed down-regulation of MDM2 and up-regulation of p14ARF. As a result, p53 luciferase transcription was much higher in MCF+FIR cells than control cells after IR. Correspondingly, expression of p14ARF and MDM2 was found to be oppositely regulated in sham-FIR control cells after IR. This difference between control and chronic IR-derived cells suggests an adjustment of p14ARF and MDM2 proteins, which appears to be required for maintaining a relatively high level of p53 responsiveness in IR-derived cells. This adjustment may contribute to a quick transactivation of p53 without the recruitment of p53 into the nucleus.

Inhibition of p53 has radiosensitized human diploid fibroblasts (55) and expression of p53 is associated with local failure of neck and head tumors in radiotherapy (56). Although a direct linking between p53 activation and radioresistance has not been identified, transcription factor NF-κB, evidently related to radioresistance (10, 57, 58), is associated with p53 activation. The inhibition of NF-κB abrogates p53-inhibited apoptosis (59), and expression of p65, the key subunit of NF-κB, activates p53 gene promoter activity (60). We have reported that both p53 and NF-κB are activated by IR in MCF-7 cells (9), and NF-κB activation is causally related to IR-derived radioresistance (10, 15). The coordinated modulation of p53 and NF-κB pathways has been indicated in IR-induced response in human malignant melanoma cells (61).

When we follow this line of reasoning, p53 appears to participate the radioresistance-signaling network leading to the activation of NF-κB. In addition, p53 and NF-κB likely regulate the function of Ku86 a subunit of DNA-dependent protein kinase (DNA-PK) and a key protein in DNA repair (62). Our microarray data showed that, compared with sham-FIR control cells, Ku86 transcripts were up-regulated in IR-derived MCF+FIR cells (Table 3). Because Ku86 and its isoform KARP-1 can be regulated by p53 (63) and, importantly, DNA-dependent protein kinase is shown to phosphorylate IkBα for NF-κB activation (64), NF-κB can be activated in IR-derived cells by Ku86 as a result of p53 activation. Such a Ku86-mediated connection...
between p53 and NF-κB networks may function as a potential molecular junction in IR-derived radiosensitivity.

In conclusion, MCF-7 cells exposed to chronic IR showed increased basal and IR-induced p53 activity. MDM2 was down-regulated and p14ARF, an inhibitor of MDM2, was up-regulated in IR-derived MCF-7/FIR cells, and these two proteins were oppositely regulated in the sham-FIR control cells. These results suggest that adjustment in p14ARF and MDM2 expression is required to maintain a high responsiveness of p53 in chronic IR-derived radiosensitive cells.

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