p53R2 Inhibits the Proliferation of Human Cancer Cells in Association with Cell-Cycle Arrest

Keqiang Zhang¹, Jun Wu¹, Xiwei Wu², Xiaochen Wang¹, Yan Wang¹, Ning Zhou², Mei-ling Kuo¹, Xiyong Liu¹, Bingsen Zhou¹, Lufen Chang¹, David Ann¹, and Yun Yen¹

Abstract
Deregulation of the expression of p53R2, a p53-inducible homologue of the R2 subunit of ribonucleotide reductase, has been found in various human cancer tissues; however, the roles p53R2 plays in cancer progression and malignancy remain controversial. In the present study, we examined changes in gene expression profiles associated with p53R2 in cancer cells, using the analysis of cDNA microarray. Gene set enrichment analysis identified that the gene set regulating cell-cycle progression was significantly enriched in p53R2-silencing human oropharyngeal carcinoma KB cells. Attenuation of p53R2 expression significantly reduced p21 expression and moderately increased cyclin D1 expression in both wild-type p53 cancer cells (KB and MCF-7) and mutant p53 cancer cells (PC3 and MDA-MB-231). Conversely, overexpression of p53R2-GFP resulted in an increase in the expression of p21 and decrease in the expression of cyclin D1, which correlated with reduced cell population in S-phase in vitro and suppressed growth in vivo. Furthermore, the MAP/ERK kinase inhibitor PD98059 partially abolished modulation of p21 and cyclin D1 expression by p53R2. Moreover, under the conditions of nonstress and adriamycin-induced genotoxic stress, attenuation of p53R2 in KB cells significantly increased phosphorylated H2AX, which indicates that attenuation of p53R2 may enhance DNA damage induced by adriamycin. Overall, our study shows that p53R2 may suppress cancer cell proliferation partially by upregulation of p21 and downregulation of cyclin D1; p53R2 plays critical roles not only in DNA damage repair but also in proliferation of cancer cells. Mol Cancer Ther; 10(2); 269–78. ©2011 AACR.

Introduction
Ribonucleotide reductase plays an essential role in catalyzing conversion of ribonucleoside diphosphates to the corresponding 2'-deoxyribonucleoside diphosphates, a rate-limiting step in the production of 2'-deoxyribonucleoside-5'-triphosphates (dNTP) required for DNA synthesis and repair (1, 2). Human ribonucleotide reductase consists of 2 subunits, R1 and R2 subunits of ribonucleotide reductase (RRM1 and RRM2), both of which are required for enzymatic activity (3). p53R2, the p53-inducible homologue of the RRM2, encodes a peptide that showed striking similarity to RRM2 (4). Expression of the p53R2 can be induced by signals activating p53 such as DNA-damaging agents, ionizing radiation, and UV irradiation in a wild-type p53-dependent manner to synthesize the dNTP for DNA repair after DNA damage (5).
Elevated ribonucleotide reductase activity and overexpression of RRM2 have been found to significantly increase the drug-resistant properties and the angiogenic and invasive potential of human cancer cells (3, 6–8). Therefore, RRM2 is well accepted as an important therapeutic target for DNA replication–dependent diseases such as cancer. However, the roles p53R2 plays in carcinogenesis and malignancy of human cancer remain largely controversial. Some studies support the idea that p53R2 is also a potential target for cancer gene therapy such as RRM2. The idea is mainly based on the crucial role p53R2 plays in deoxyribonucleotide synthesis and DNA damage repair. Inhibition of p53R2 enhances 5-fluorouracil sensitivity of cancer cells in vitro (9). Several studies have also observed that elevated p53R2 expression was positively correlated with anticancer agent resistance of human malignancies, including oral cavity (10) and esophageal cancers (11). Recently, a study by Devlin and colleagues showed that p53R2 was overexpressed in prostate tumor cell lines whereas silencing p53R2 enhances the apoptotic effects of ionizing radiation and doxorubicin (12). On the other hand, another study identified that disruption of the p53R2-mediated DNA repair in ulcerative colitis initiated carcinogenesis of colon (13).
Consistently, an early study also showed that p53R2−/− embryonic fibroblasts (MEF) became immortal much earlier and were more susceptible to apoptosis induced by oxidative stress than p53R2+/− MEFs. They also showed an increased rate of proliferation after 7 passages (14). Some studies reported that positive p53R2 expression was significantly correlated with depth of invasion, lymph node metastasis, stage, and poor prognosis in patients with esophageal squamous cell carcinoma and lung cancer (15, 16). However, another study showed that the expression of p53R2 did not correlate with stage, grade and histologic types, of gastric tumors (17). Controversially, Yoshida and colleagues addressed that p53R2 expression significantly decreased with progression from ulcerative colitis–associated carcinogenesis dysphasia to carcinoma, indicating an inverse relationship between p53R2 and cancer development (13). Our previous studies identified that p53R2 was negatively correlated with the metastasis of colon adenocarcinoma samples (18, 19).

In the present study, we reported the changes in gene expression profile associated with knockdown of p53R2 in human KB cancer cells and the impact of p53R2 on cancer cells proliferation. We identified that the gene set regulating cell-cycle progression was significantly enriched in p53R2-attenuated KB cells, using gene set enrichment analysis (GSEA) with cDNA microarray (20). Overexpression of p53R2 significantly suppressed cancer cells proliferation regardless of p53 status of cells. Our results indicated that overexpression of p53R2 may suppress cancer cells proliferation through alterations in the expression of cell-cycle–regulating genes.

Materials and Methods

Cell culture and plasmid transfection

Human oropharyngeal carcinoma KB (wild-type p53) and breast adenocarcinoma MCF-7 cells (wild-type p53) were cultured in DMEM with 10% FBS. Human breast carcinoma MDA-231 (mutated p53) and prostate adenocarcinoma cells PC3 (truncated p53) were cultured in RPMI with same supplements. Cell lines were purchased from American Type Culture Collection previously; they were tested and authenticated for genotypes by DNA fingerprinting (Supplementary Fig. S1). Adriamycin and PD98059 were purchased from Sigma-Aldrich. The human p53R2 gene–specific siRNA (sc-36338) and scramble siRNA were purchased from Santa Cruz Biotechnology; anti-phospho-
oblate-ATM, ERK, and phosphorylated ERK1/2 were obtained from Santa Cruz Biotechnology; anti-phospho-

gamma-histone H2AX (Ser139, clone JBW301) was purchased from Millipore. Signals were densitometrically assessed and normalized to the signals of GAPDH.

Microarray analysis of p53R2-associated genes

siRNA transfection and total RNA from p53R2-silencing KB and control cells were carried out as reported previously (8). Duplicated RNA samples were subjected to analysis of cDNA microarray. The Affymetrix GeneChip Human Gene 1.0-ST array (Affymetrix) was used to define gene expression profiles from the samples. Hybridization, data generation, and analysis were done at Microarray Core at City of Hope according to the user manual of the kits. Genes that were differentially expressed between p53R2-attenuated KB cells and the control KB cells were selected with a cutoff of adjusted P < 0.05 and log2 ratio of 1.5. GSEA (v2.0; ref. 20) was used to determine whether an a priori defined set of genes in C2 of GSEA shows statistically significant, concordant differences between control siRNA and p53R2 siRNA–transferred KB cells.

Quantitative reverse transcriptase PCR and Western blot analysis

The primers used in the study are listed in Supplementary Table S1. Quantitative real-time PCR (qRT-PCR) was carried out in the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). The reaction mixture of 20 µL consisted of 1× ABI SYBR Green PCR Master Mix, 0.25 µL cDNA, and 0.2 µmol/L of each primer. Relative gene expression quantification method as reported previously was used to calculate the fold change of mRNA expression according to the comparative Ct method using β-actin as an endogenous control (8). Data were represented as ratio or fold change to control sample. The antibodies against GAPDH (glyceraldehyde 3-phosphate dehydrogenase), p53R2, p53, cyclin D1, phosphorylated-ATM, ERK, and phosphorylated ERK1/2 were obtained from Santa Cruz Biotechnology; anti-phospho-

gamma-histone H2AX (Ser139, clone JBW301) was purchased from Millipore. Signals were densitometrically assessed and normalized to the signals of GAPDH.

Dual luciferase reporter assay

KB cells were seeded at a concentration of 5.0 × 10^4 cells per well of a 24-well plate. Cells were transfected with 125 ng of p21-Luc or PG13 plus 10 ng Renilla luciferase reporters with 250 ng of GFP (green fluorescent protein) or p53R2-GFP plasmid per well by Lipofectamin-2000. Forty-eight hours after reporter plasmid transfection, cells were washed once with PBS and lysed with reporter lysis buffer from Promega. Firefly and 10 ng Renilla luciferase activities were determined according to the manufacturer’s instruction of dual luciferase assay (Promega). Relative luciferase activity of each reporter was normalized to the value of Renilla luminescence. The experiment was repeated 3 times. All experiments were done in triplicates. Data are reported as average ± SD.

Cell-cycle analysis

One million cells were washed in cold PBS, fixed in 70% ethanol in PBS for at least 1 hour on ice, washed, resuspended in PBS containing 25 µg/mL propidium
iodide and 100 μg/mL ribonuclease A, and incubated for 30 minutes at 37°C. Fluorescence was measured on a Becton Dickinson FACScalibur flow cytometer (excitation 488 nm, measurement 564–607 nm) within 1 hour. Data were analyzed using the MODFIT 2.0 program (Verity Software). The mean and standard errors for the percentage of cells in each phase of the cell cycle were derived from at least 3 independent experiments, each in duplicate.

In vitro proliferation and in vivo tumor growth
A total of 2.5 × 10⁶ KB/PC3-GFP and KB/PC3-p53R2-GFP cells were seeded into wells of 16-well devices compatible with a W200 real-time cell electronic sensing (RT-CES) analyzer and 16× station (Acea Biosciences). Cell growth was monitored periodically (typically, every 0.5 or 1 hour) for indicated durations via calculation of a cell index (normalized impedance) for each well. Data were analyzed using the MODFIT 2.0 program (Verity Software). The mean and standard errors for the percentage of cells in each phase of the cell cycle were derived from at least 3 independent experiments, each in duplicate.

Statistics
Data were collected using an MS-Excel spreadsheet. Data were analyzed using the JMP Statistical Discovery Software version 6.0 (SAS Institute, Inc.). Group comparisons for continuous data were done with Student’s t test for independent means or 2-way ANOVA. Statistical significance was set at P < 0.05.

Results
GSEA with cDNA microarray data revealed that gene set regulating cell-cycle progression was enriched in p53R2-attenuated KB cells
GSEA with cDNA microarray identified that 17 of 429 gene sets from the C2 inventory created by Molecular Signatures Database (MSigDB; ref. 20) were significantly enriched and 24 were significantly reversibly enriched in p53R2-attenuated KB cells compared with control cells (Nom P < 0.05). These top enriched gene sets (Nom P < 0.05) included the gene sets of cell cycle, ABC transporter-general, and inhibition of matrix metalloproteinase, which are shown in Supplementary Table S2.

As shown by the enrichment plot in Figure 1A, the gene set regulating cell-cycle progression was significantly enriched in p53R2-attenuated KB cells. The heat map (Fig. 1B) displayed names, positions, and expression levels of the enriched genes in the set. The qRT-PCR analysis was carried out to validate the differential expression of the 10 selected genes in the set (Fig. 1C). The analysis validated that attenuation of p53R2 expression significantly upregulated the mRNA expression of several cell-cycle regulatory proteins such as cyclin D1 and CDK1.
and CDK1, while decreasing mRNA expression of several cell-cycle inhibitors such as CDKN1A (p21WAF1/CIP1, p21) and CDKN1C (KIP2; Fig. 1C). In addition, we used an alternative method of ingenuity pathways analysis to validate the GSEA pathway analysis result that cell-cycle pathway was enriched by p53R2 knockdown. We selected the genes that are differentially expressed between p53R2 and control with \( P < 0.01 \) (equivalent to false discovery rate < 0.1), which resulted in 1,997 probe sets. These genes were analyzed using ingenuity pathways analysis, and the significant functional categories (\( P < 0.01 \)) were identified and shown in Supplementary Figure S2. The original microarray data were submitted to Gene Expression Omnibus of National Center for Biotechnology Information, which can be obtained from the Gene Expression Omnibus website of National Center for Biotechnology with accession number of GSE25238.

**Attenuation of p53R2 decreased p21 expression and increased cyclin D1 in cancer cells**

At gene level, cDNA microarray analysis identified that attenuation of p53R2 caused about 100 genes differentially expressed in KB cells (\( P < 0.05 \)). These genes including p21 and cyclin D1 are listed in Table S3 of Supplementary Data. p21 and cyclin D1 are essential cell-cycle regulators; we further examined the regulation of p53R2 on these two genes in various cancer cell lines. In addition, because p21 is a well-known target of p53, we also questioned whether the regulation of p21 by p53R2 was wild-type p53 dependent or not. Attenuation of p53R2 expression by siRNA (Fig. 2A) significantly decreased p21 and increased cyclin D1 expression in both wild-type p53 (KB and MCF-7) and p53-mutated (PC3 and MDA-MB-231) cancer cells (Fig. 2B and C), which indicates that the regulation was wild-type p53 independent. Quantitative analysis of the triplicate data showed that the differential expression of RNA and protein of p21 and cyclin D1 was statistically significant (Fig. 2B and D).

**Overexpression of p53R2 increased p21 and decreased cyclin D1 and phosphorylation of ERK1/2 in both KB and PC3 cells**

To further elucidate the p53R2-regulated expression of cell-cycle regulatory genes, we constructed 2 cancer cell lines stably overexpressing p53R2-GFP. FACScan technique was applied to enrich cancer cells highly expressing GFP/p53R2-GFP. As shown in Figure 3A, KB-p53R2-GFP and PC3-p53R2-GFP cells highly expressed p53R2-GFP,
which was clearly observed by the fluorescent microscope (Fig. 3A). Western blot assay further showed that the p53R2-GFP fused protein (around 76 kDa) was highly expressed in both KB-p53R2-GFP and PC3-p53R2-GFP cells (Fig. 3B). Compared with KB-GFP and PC3-GFP, the p21 protein expression was significantly increased and cyclin D1 expression was moderately decreased in KB-p53R2-GFP and PC3-p53R2-GFP as shown in Figure 3C (P < 0.05). A recent study showed that p53R2 negatively regulates MAP/ERK kinase (MEK) extracellular signal regulated kinase (ERK) signal pathway through direct interaction with MEK2 (22). Using Western blot analysis, we also found that the overexpression of p53R2-GFP significantly decreased phosphorylated ERK1/2 in KB and PC3 cancer cells (Fig. 3B). Knockdown of p53R2 moderately increased the level of phosphorylated ERK1/2 in these cancer cells (Fig. 3D). Furthermore, the MEK inhibitor PD98059 partially abolished the impact of p53R2 on the expression of cell-cycle regulatory genes, p21 and cyclin D1, in KB cells (Fig. 3D). This observation indicates that p53R2 may regulate the expression of cell-cycle regulatory genes partially through inhibition of ERK1/2 phosphorylation.

p53R2-GFP reduced the percentage of cells in S-phase of both KB and PC3 cells

To test the impact of p53R2 on cell-cycle progression, we first measured the impact of attenuation of p53R2

Figure 3. Overexpression of p53R2 increased p21 and decreased cyclin D1 and phosphorylated ERK1/2 in both KB and PC3 cells. A, photographs of fluorescence microscopy (×100) showed a high expression of p53R2-GFP fused protein in GFP and p53R2-GFP cancer cells. B, Western blot analysis showed that p21 protein was significantly upregulated; cyclin D1 and phosphorylated ERK1/2 were moderately downregulated in KB-p53R2-GFP/PC3-p53R2-GFP cells compared with KB-GFP/PC3-GFP. C, relative quantitative level of indicated proteins in cells was determined by measuring the density of the band of target protein and normalized to that of GAPDH. Data are presented as the ratio to control and are the mean ± SD of 3 experiments. *, P < 0.05; **, P < 0.01, compared with control cells. D, knockdown of p53R2 moderately upregulated serum-induced phosphorylated ERK1/2. The MEK inhibitor PD98059 partially abolished the impact of p53R2 on expression of p21 and cyclin D1 in KB cells, which were transfected with p53R2 siRNA and serum-starved overnight after 24 hours, followed by stimulation with 20% serum in the absence or presence of the MEK inhibitor PD98059 (25 μmol/L).
on cell-cycle progression of KB and PC3 cancer cells. We found that transient attenuation of p53R2 by siRNA moderately increased the percentage of cells in S phase in both wild-type KB (about 6%) and PC3 (p53 truncated) cells (about 4%). We further compared cell-cycle profiles of KB-p53R2-GFP and PC3-p53R2-GFP with their controls. The representative cell-cycle profiles of KB-GFP/p53R2-GFP and PC3-GFP/p53R2-GFP cells are shown in Figure 4A and B, respectively. In comparison with KB-GFP cells (28.36% ± 3.12%), the percentage of KB-p53R2-GFP cells in S-phase (14.67% ± 4.49%) was significantly decreased (P < 0.01). Simultaneously, compared with KB-GFP (58.72% ± 5.43%), the percentage of KB-p53R2-GFP cells in G1-phase (74.52% ± 4.22%) was markedly increased (P < 0.01). Compared with PC3-GFP (S-phase: 23.43% ± 5.66%, G1-phase: 53.52% ± 3.28%), the percentage of PC3-p53R2-GFP cells in S-phase (13.57% ± 3.56%) was significantly decreased (P < 0.01) whereas that in G1-phase (65.57% ± 4.98%) was significantly increased (P < 0.01). The mean and standard errors for the percentage of cells in each phase of the cell cycle were derived from at least 3 independent experiments, each in duplicate.

Overexpression of p53R2-GFP inhibited proliferation of human cancer cells in vitro and in vivo

These data strongly suggest that p53R2 may regulate cancer cell proliferation. To further elucidate this question, we compared the growth of p53R2-GFP cancer cells with their control cells. RT-CES traces displayed that the proliferation of KB-p53R2-GFP and PC3-p53R2-GFP was significantly slower than their control cells over a period of 96 hours (Fig. 5A and B). We also observed that transient attenuation of p53R2 by siRNA slightly increased the proliferation of KB and PC3 (data not shown). We further assessed the effect of p53R2-GFP on the growth of tumor xenografts in vivo. We found that the growth of KB-p53R2-GFP and PC3-p53R2-GFP xenografts was significantly slowed down compared with that of KB-GFP and PC3-GFP xenografts (Fig. 5C and D; P < 0.05). Mean weight of xenografts was 0.90 ± 0.13/0.29 ± 0.09 g for KB-GFP/PC-GFP cells (n = 8) and 0.56 ± 0.12/0.15 ± 0.07 g for KB-p53R2-GFP/PC3-p53R2-GFP cells (n = 8), respectively, and the decrease was
statistically significant ($P < 0.05$). Western blot analysis also displayed that KB-p53R2-GFP and PC3-p53R2-GFP xenografts expressed more p21 and less cyclin D1 (small photographs in Fig. 5C and D).

**Impact of attenuation of p53R2 on DNA damage in KB cells**

We further investigated whether p53R2 has a feedback impact on p53 and how p53R2 impacts p21 expression after DNA damage. As shown in Figure 6A, Western blot analysis identified neither the attenuation nor overexpression of p53R2 changed p53 protein level in KB cells. Luciferase report assays also showed that overexpression of p53R2 significantly increased luciferase activity of p21-Luc by 1.6-fold ($P < 0.05$) but not the luciferase activity of p53 reporter plasmid PG13 (Fig. 6B), which consistently indicates that the p53R2-regulated p21 expression is wild-type p53 independent. We examined the impact p53R2 have on p53 and p21 expression under genotoxic stress caused by adriamycin. qRT-PCR analysis and Western blot assays (Fig. 6C and D) showed that attenuation of p53R2 significantly decreased the p21 mRNA and protein expression but did not change p53 protein level in KB cells under the genotoxic stress of adriamycin. DNA damage induced by adriamycin significantly increased the phosphorylation of histone H2AX in KB cells. The level of phosphorylated H2AX was comparable with that of phosphorylated ATM. p53R2-attenuated KB cells had a substantial increase in the phosphorylation of H2AX not only under the stress of adriamycin but also under normal condition. Consistently, we found that overexpression of p53R2 only marginally decreased the phosphorylation of H2AX and ATM caused by genotoxic stress of adriamycin in KB cells, which indicates that overexpression of p53R2 may slightly increase the resistance of cancer cells against DNA-damaging reagent (Supplementary Fig. S3).

**Discussion**

The roles of p53R2 in the biological characteristics of cancer cells and the underlying mechanisms remain largely unclear and even controversial. Using GSEA, a more reproducible and more interpretable method that focuses on pathways and processes rather than on high scoring individual gene (20, 23), we surprisingly found that the gene set regulating cell-cycle progression was significantly enriched in the p53R2-attenuated KB cells.
The finding promoted us to examine the impact of p53R2 on cell-cycle progression. Coincidentally, transient attenuation of p53R2 by siRNA moderately increased the percentage of cells in S-phase in both wild-type KB and PC3 cells. To further examine the impact, we constructed 2 cancer cell lines highly overexpressing p53R2-GFP. Unlike GFP-expressing cells, we observed that the percentage of cells with high level of p53R2-GFP expression progressively decreased among G418-resistant cells during the process of selection and following culture with G418. The phenotype of p53R2-GFP–expressing cells during G418 selection was very similar to cells overexpressing cell-cycle inhibitor as reported previously by others (21, 24). Overexpression of GFP may be cytotoxic to cells; however, the phenomena may not be caused by GFP, as the intensity and viability of GFP-expressing cells were stable during G418 selection and culture. We found that overexpression of p53R2-GFP significantly suppressed cancer cells growth regardless of the status of p53. Consistently, early study by Kimura and colleagues revealed that p53R2−/− and p53R2+/− embryonic fibroblasts (MEFs) showed an increase in the rate of proliferation after 7 passages, indicating an inhibitory role of p53R2 in cell proliferation (14). Study by Yamaguchi and colleagues suggested that the suppression of cancer growth by inactivation of p53R2-dependent pathway owes to the activation of p53-dependent apoptotic pathway due to the shortage of dNTP for DNA synthesis (25). However, we previously reported that RRM2 might complement p53R2 in response to UV-induced DNA repair in cancer cells with mutant p53 (26). A different study also showed that disruption of the p53R2-mediated DNA

Figure 6. Impact of attenuation of p53R2 on DNA damage in KB cells. A, neither attenuation nor overexpression of p53R2 modulated p53 protein level (p53 protein was developed by immunoprecipitation–Western blot; mouse and rabbit anti-p53R2 antibodies were used for pull-down and blot, respectively). B, overexpression of p53R2 significantly increased the luciferase activity of p21 promoter reporter p21-Luc (*, $P < 0.05$) but not the luciferase activity of p53 reporter plasmid PG-13. C, qRT-PCR. D, Western blot analysis showed that the attenuation of p53R2 did not change p53 accumulation induced by adramycin but significantly decreased p21 expression in KB cells. Under both normal and stressed conditions, attenuation of p53R2 substantially enhanced the DNA damage in KB cells, which was indicated by increased phosphorylation of γ-H2AX and phosphorylated ATM proteins.
repair in ulcerative colitis could initiate carcinogenesis in colon (13).

At the transcriptional level, we found that attenuation of p53R2 in KB cells significantly altered p21 and cyclin D1 levels. Because p21 and cyclin D1 play well-known roles in the regulation of cell-cycle progression (27, 28), therefore, we further examined the impact of p53R2 on the expression of p21 and cyclin D1 in wild-type p53 cancer cells (KB and MCF-7) and p53-mutated cancer cells (PC3 and MDA-MB-231). A most recent study showed that p53R2 negatively regulates MEK-ERK signal pathway through binding to MEK2 (22). We found that overexpression of p53R2 moderately decreased phosphorylated ERK1/2 in KB cells. Because MEK-ERK signaling pathway regulates diverse cellular functions including cell proliferation, cell-cycle progression, and cell survival, inhibition of this pathway is a logical therapeutic target for malignancies (29, 30). We further found that the MEK inhibitor PD98059 partially abolished the effect of p53R2 on the expression of cell-cycle-regulating genes. Therefore, we considered that impact of p53R2 on cancer cells growth might mediate partially through its inhibition on MEK-ERK signaling pathway.

Both p53R2 and p21 are targets and executors of ATM-p53 pathway after DNA damage that provides dNTP for DNA repair and causes cell-cycle arrest (21, 31). We further investigated whether p53R2 has a feedback impact on p5, and whether p53R2 still regulates p21 expression after DNA damage. Our data showed that neither attenuation nor overexpression of p53R2 changed wild-type p53 protein in KB cancer cells. Consistent with the observation of study by Devlin and colleagues (12), we also found that p21 protein level was significantly decreased in p53R2-attenuated KB cells under the condition of DNA damage induced by adriamycin and attenuation of p53R2 impaired DNA damage repair in KB cancer cells as indicated by enhanced phosphorylated γ-H2AX (12, 32). We were surprised to find that attenuation of p53R2 in KB cells without genomic stress increased phosphorylated γ-H2AX. We consider that knockdown of p53R2 may increase reactive oxygen species from endogenous sources such as oxidative phosphorylation, cytochrome P450 metabolism, and peroxisomes (33). In addition, reactive oxygen species may cause DNA damage and induce phosphorylated γ-H2AX. Indeed, the previous study by Kimura and colleagues also found that p53R2−/− MEFs were much more sensitive to oxidative stress damage than p53R2 wild-type MEFs (14). Coincidently, a recent study further showed that knockdown of p53 decreased mitochondrial and cellular superoxide levels and increased cellular hydrogen peroxide in human primary fibroblast cells under normal culture conditions, which was accompanied by a reduction in the p53R2 and mitochondrial DNA depletion (34).

Several studies found that silencing p53R2 enhanced the apoptotic effects of ionizing radiation, UV, and genotoxic agent in a wild-type p53R2-dependent manner. p53R2 is a potential therapeutic target for cancer (11, 12, 28). However, both downregulation and upregulation of p53R2 in human cancer tissues were reported. Studies of ours, and others, indicated that basal transcription of p53R2 is expressed in almost all of normal human tissues and regulated through the p53-independent mechanism (17, 26). Furthermore, the low constitutive level of p53R2 in mammalian cells is essential for the supply of dNTPs for basal levels of DNA repair and mitochondrial DNA synthesis in G0/G1 cells (35). Studies indicated that p53R2 plays an essential role in maintenance of genomic stability (13, 14). It is well known that genomic instability also contributes to tumor development, progression, and resistance to therapy. Because p53 mutation is frequently observed in human cancers, genomic instability is often seen in cancer tissues without wild-type p53 protein, which may reflect the dysfunction of ribonucleotide reductase due to the failure of p53R2 induction (36). Therefore, the novel finding of our study that p53R2 may suppress cancer cells proliferation regardless of p53 status of cells may be a careful consideration for p53R2-targeted cancer therapy, especially for cancers with mutant p53, as studies indicate that attenuation of p53R2 enhances only the apoptotic effects of ionizing radiation, UV, and genotoxic agent in wild-type p53 cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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