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Transcriptional suppression of breast cancer resistance protein (BCRP) by wild-type p53 through the NF-κB pathway in MCF-7 cells

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ABSTRACT

Breast cancer resistance protein (BCRP) has been shown to confer multidrug resistance, but the mechanisms of its regulation are poorly understood. Here, we investigate the effects of wild-type and mutant p53, and nuclear factor kappa-B (NF-κB) (p50) on BCRP promoter activity in MCF-7 cells. Our results demonstrated that wild-type p53 markedly suppressed BCRP activity and enhanced the chemosensitivity of cells to mitoxantrone, whereas mutant p53 had little inhibitory effect. After inhibition of NF-κB, similar results were obtained. Following knockdown of endogenous p53, BCRP and p50 expressions were increased, and the chemosensitivity of the cells to mitoxantrone was decreased. We conclude that wild-type p53 acts as a negative regulator of *BCRP* gene transcription.

1. Introduction

The development of multidrug resistance (MDR) by tumor cells is a major obstacle to successful chemotherapy for cancer. One pivotal mechanism by which tumor cells can become resistant to cytotoxic drugs used in chemotherapy is the increased expression of certain ATP-binding cassette (ABC) transporters which including P-glycoprotein (P-gp, MDR1), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP) [1]. These proteins are thought to function as energy-dependent efflux pumps of a variety of structurally diverse chemotherapeutic agents, thereby decreasing intracellular drug accumulation. Comparison of BCRP protein sequences with that of P-gp and MRP revealed that, unlike P-gp and MRP, which are arranged in two repeated halves, BCRP is a half-transporter consisting of only one nucleotide binding domain followed by one membrane-spanning domain. This cell line (MCF-7/AdrVp) was selected by continuous exposure to doxorubicin in combination with verapamil to avoid development of resistance due to expression of P-gp. Interestingly, MCF-7/AdrVp cells also did not express the multidrug resistance-associated protein MRP [2]. BCRP was also discovered by investigating cell lines selected for resistance to mitoxantrone – a poor substrate for P-gp and MRP, which is a high-affinity substrate for BCRP [3]. BCRP expression status is a significant determinant of sensitivity of cancer cells to its substrate anticancer agents.

P53 is a critical tumor suppressor in the human cells that mediates growth arrest, senescence and apoptosis in response to several cellular stresses and often referred to as "the guardian of the genome". Mutations of the p53 tumor suppressor gene have been estimated to occur in close to 50% of human tumors and p53 mutation is not a random process [4]. According to the p53 mutation database (www.iarc.fr/p53), the majority of DNA mutations are located in the core domain responsible for DNA binding, such as codons 175, 245, 248, 249, 273, and 282. The hot spot mutations at codons 175 and 248 occur with highest frequency in human cancers. P53 functions primarily as a transcription factor which exerts its downstream functions by activating or repressing a large number of its downstream genes [5,6]. The majority of the promoter studies seem to confirm a downregulation of the MDR1 or MRP promoter by wild-type p53 (wt-p53) and an upregulation or activation by certain mutants of p53 [7,8].

Up to now, whether *p53* gene can regulate *BCRP* gene expression has not been reported. The mechanisms of BCRP overexpression in drug-resistant cells are not clearly understood. In this study, we investigated the role of *p53* gene in the regulation of human *BCRP* gene expression. Disclosing the relationship between

Abbreviations: MDR, multidrug resistance; ABC, ATP-binding cassette; BCRP, Breast cancer resistance protein; MRP, multidrug resistance-associated protein; P-gp, P-glycoprotein; NF-κB, nuclear factor kappa-B; RNAi, RNA interference; wt-p53, wild-type p53; PBS, phosphate balanced solution; EMSA, electrophoretic mobility shift assay

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p53 gene and *BCRP* gene could give rise to new evidence for an explanation of the mechanisms of drug resistance.

2. Materials and methods

2.1. Materials

The human breast cancer cell line MCF-7 and JAR (a human choriocarcinoma cell line overexpressing endogeneous BCRP) were purchased from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The wild type (wt) p53 expression plasmid, pC53-SN3, and the empty vector, the mutants of pC53–175 and pC53–248 were kindly provided by Dr. Liang Cao at the University of Hong Kong of China and their constructions have been described elsewhere [9]. Plasmid pAVU6 + 27 (a gift of Dr. ML He, University of Hong Kong, China) was used to generate a plasmid that expressing hairpin RNAs. The nuclear factor kappa-B (NF-κB) promoter/luciferase reporter plasmid (pNF-κB) was a gift of Dr. Cao L (University of Hong Kong, China) [10]. pBabe-IκBα was constructed by IκBα mutant gene into EcoRI sites of retroviral plasmid pBabe.

2.2. Constrution of BCRP promoter-luciferase plasmids

Genomic DNA was extracted from the human JAR cells using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The promoter region of the human BCRP gene, a 358 bp fragment was amplified by PCR using the following primers: 5′-CCGCTCGAGGAGGTACT GATCAGCCCAATGAG-3′ and 5′-CCCAAGCTTAGCGCTGACACGAACTTCCTAAG-3′. The promoter fragment was cloned into the Hind III and Xbal I sites in the pGL2-Basic (Promega, Madison, WI). All of the constructed DNAs were purified through Qiagen columns (Qiagen) and confirmed by restruction analysis and sequencing.

2.3. Generation and characterization of p53-overexpression cell lines MCF-7/wtp53

The MCF-7 cells were plated in the wells of a 6-well plates at a density of 1.0×10^5 cells per well and incubated overnight to $90 \sim 95\%$ confluent and transfected with 5.0 μg of wt-p53 expression plasmid DNA or the empty vector DNA for pCMV-Neo-Bam by using Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, G418 (800 μg/ml) was added to the medium and was supplemented in the media throughout the cloning process. After growing the cells in the presence of G418 for 3 weeks, a few colonies formed in plates transfected with p53 plasmids, compared with about 100 times more colonies with empty vector transfection. Individual colonies were isolated with a cloning ring from the transfection plates and expanded as established cell lines (MCF-7/wtp53, and MCF-7/pCMV). Other cell lines established similar to the above method.

2.4. Transient transfection and luciferase reporter gene assays

MCF-7 cells were plated in the wells of a 24-well plates at a density of 1.0×10^5 cells per well and incubated overnight to $90 \sim 95\%$ confluent. Using Lipofectamine 2000 kit (Invitrogen) as instructed by the manufacturer, plasmid DNA (2.0 μg) and DNA for cotransfection were transfected into the cells. Luciferase plasmids were cotransfected with 1.0 μg β-galactosidase or Renilla control vector (Promega) to monitor transfection efficiency. For luciferase assays, cells were washed three times with 2.0 ml of ice-cold phosphate balanced solution (PBS) and were lysed in 150 μl of lysis buffer. Samples were assayed in a Lumi-Scint Luminometer (Bioscan, Washington, USA) using 20 μl of cell lysate and 100 μl of Luciferase assay reagent (Promega). Luciferase activity was measured a 10 s delay and 30 s integration time and was normalized to β-galactosidase or Renilla luciferase activity to determine transfection efficiency.

2.5. Transfection of RNA interference plasmid

The short-hairpin-RNA-encoding complementary single-stranded oligonucleotides corresponding to p53 were designed according to the literatures [11]. These oligonucleotides 5′-tcgacGACTCCAGTGGTAATCTACttcaagagaGTAGATTACCACTGGAGTCtttttt-3′ and 5′-ctagaaaaaGACTCCAGTGGTAATCTACtctcttgaaGTAGATTACCACTGGAGTCG-3′; 5′-tcgacAGCTTCATAAGGCGCATGCttcaagagaGCATGCGCCTTATGAAGCTttttt-3′ and 5′-ctagaaaaaAGCTTCATAAGGCGCATGCtctcttgaaGCATGCGCCTTATGAAGCTG-3′, were annealed and cloned into pAVU6+27 vector to generate p53 (pAVU6 + 27/sip53) and control construction (pAVU6+27/sicontrol), respectively. Then, MCF-7 cells were transfected with the above constructions using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol.

2.6. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) assay

Total cellular RNA was isolated from cells using TRIzol reagent (Invitrogen) and quantified by UV absorbance spectroscopy. BCRP, NF-κB (p50), β-actin mRNA transcripts were detected using RT-PCR assays. A 206 bp BCRP cDNA fragment was amplified with the primers 5′-CACCTTATTGGCCTCAGGAA-3′ (sense) and 5′-CCTGCTTGGAAGGCTCTATG-3′ (antisense); a 176 bp NF-κB (p50) cDNA fragment with the primers: 5′-CTGGAAGCACGAATGACAGA-3′ (sense) and 5′-TGAGGTCCATCTCCTTGGTC-3′ (antisense); a 194 bp p53 cDNA fragment with the primers: 5′-CCAGCCAAAGAAGAAACCAC-3′ (sense) and 5′-TATGGCGGGAGGTAGACTGA-3′ (antisense). As an internal control, amplification of β-actin mRNA (309 bp) was carried out with the primers 5′-ACCGTGGAGAAGAGCTACGA-3′ (sense) and 5′-GTACTTGCGCTCAGAAGGAG-3′ (antisense).

2.7. Western blot and electrophoretic mobility shift assays (EMSA)

Western blotting and EMSA analyses were carried out as previously described [12,13]. Biotin-labeled double-strand NF-κB oligonucleotides (5′-CCCGACCTGGGGAAACCCGGGCGCTGGGG-3′) containing the NF-κB (p50) binding site in the BCRP promoter and mutant NF-κB oligonucleotides (5′-CCCGACCTTTTTTAATTTGGGGCGCTGGGG-3′) were obtained from Zhaorui Biotech Co., Ltd. (Shanghai, China) and used as probes (underlining indicates NF-κB binding site).

2.8. Chromatin immunoprecipitation assay (ChIP)

Immunoprecipitation experiments with an anti- NF-κB (p50) antibody (Santa Cruz Biotech., CA) to examine protein-DNA interactions were done using the chromatin immunoprecipitation assay kit (Upstate, Charlottesville, VA) following manufacturer's instructions. To analyze the target regions (NF-κB-binding site at −27/−18 regions in the BCRP promoter), the DNA samples were amplified by PCR, forward; 5′-CGGGAGTGTTTGGCTTGTC-3′; reverse: 5′-CAGAGCTGAACGCAGTGGC-3′ that generated a 233-bp PCR product.

2.9. Cytotoxicity assay

Cytotoxicity of mitoxantrone on cells was assessed by MTT as described previously [14]. Cells were counted and then cultured in 96-wells plate, and treated with those anticancer drugs of

different concentration for 48 h. For comparison of cell lines, the absorption of untreated cells was normalized to 1. The anti-proliferative effects of the drugs were evaluated by survival rate. Fifty percent cell growth inhibitory concentrations (IC_{50}) were calculated from linear regression analysis of the linear portion of the growth curves.

2.10. Statistical analysis

For all statistical tests, the SPSS-11.0 version software package (SPSS Inc., Cary, USA) was used. All data are expressed as the mean ± S.D. from three or more experiments. Statistical analyses of the data were carried out using the Student's *Z*-test with $P < 0.05$ considered as statistically significant.

3. Results

3.1. Wt-p53 but not mutant p53 inhibits BCRP expression in MCF-7 cells

As shown in Fig. 1A and B, MCF-7/wt-p53 cell line overexpressed p53 mRNA, as determined by RT-PCR, compared with MCF-7 cell line and empty vector-transfected cell line, MCF-7/pCMV. Expression of the p53 protein in the MCF-7/wt-p53 cell line was confirmed by Western blot analysis. Of importance, in the MCF-7/wt-p53, there was a decrease in BCRP transcripts compared with MCF-7 cell line and empty vector-transfected cell line, MCF-7/pCMV, suggesting that exogenous expression of wt-p53 inhibited endogenous BCRP transcription in MCF-7 cells. Contrary to the MCF-7/wt-p53 cells, there were no markedly changes in BCRP mRNA and protein levels in mutant p53 overexpression cell lines.

To further define the effects of endogenous p53 on BCRP expression, MCF-7 cells were transfected with p53 knockdown constructs (pAVU6/sip53) or control constructs (pAVU6/sicontrol). As shown in Fig. 1A and B, the endogenous p53 expression in MCF-7 was disrupted after transfected with pAVU6/sip53. Whereas, there were increases in BCRP mRNA and protein levels in MCF-7/sip53.

To know whether expression of wild-type p53 can enhance chemosensitivity, we detected cells survival rate on various kinds of cells after the different concentrations of drugs treated.

As shown in Fig. 1C, the chemosensitivity of MCF-7/wt-p53 to mitoxantrone was enhanced, which might predict that the level of BCRP was decreased. However, there were no significant changes on the mutant p53 overexpression cell lines chemosensitivity to mitoxantrone. The endogenous p53 expression in MCF-7 was disrupted by RNA interference (RNAi). The survival rate of endogenous p53 silencing cells treated with mitoxantrone increased significantly.

IC_{50} values were calculated in accordance with the survival curve (Fig. 1C). The IC_{50} in MCF-7/pCMV, MCF-7/wt-p53, MCF-7/$p53^{R175H}$ and MCF-7/$p53^{R248W}$ cells under mitoxantrone condition was 0.654 ± 0.043 μmol/L, 0.174 ± 0.012 μmol/L, 0.652 ± 0.038 μmol/L

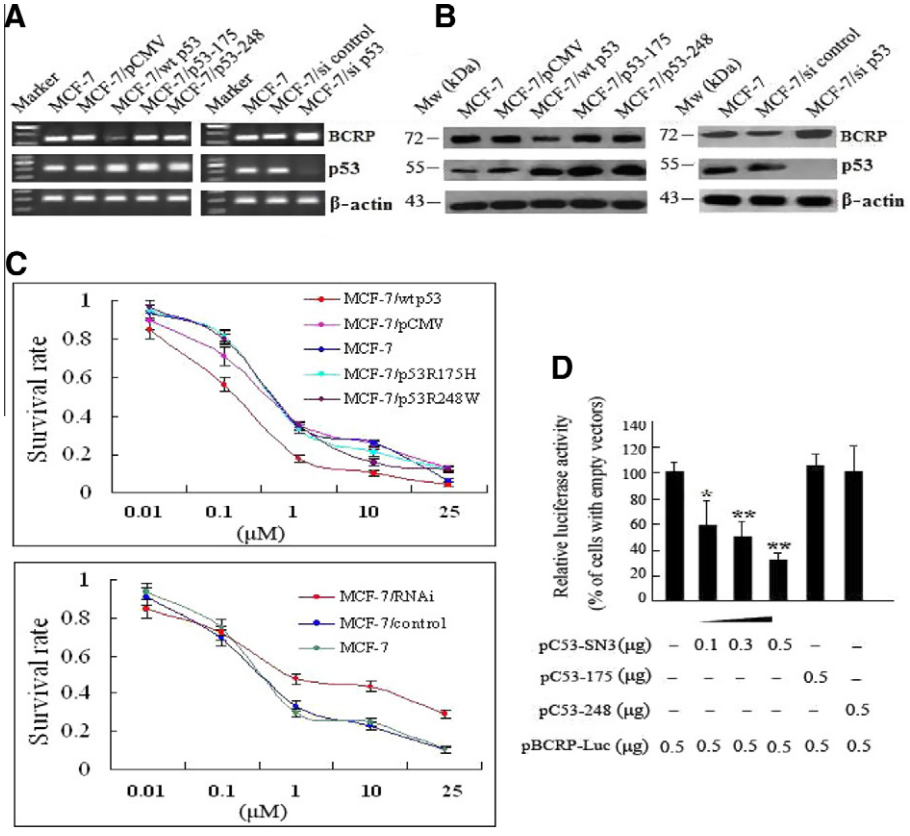

Fig. 1. Effects of p53 on BCRP expression and promoter activity. (A) By RT-PCR assay, there was an increase in p53 expression on the MCF-7/wt-p53 compared with the MCF-7/pCMV. Between MCF-7/wt-p53 and mutant p53 cells, there were no markedly changes on p53 in mRNA levels. After transfected with MCF-7/si p53, BCRP expression was increased and p53 was decreased. β-Actin was used as an internal control. MCF-7 cells were used as control. The data shown are representative of three to four independent experiments. (B) By Western blot assay, similar results were obtained. (C) Survival curves of various kinds of cells after the different concentrations of drugs treated. The cells were seeded at a density of 10^4 cells per well in 96-well plates and then incubated for 48 h in the presence or absence of increasing mitoxantrone concentrations. Cell survival was assessed by the MTT assay. Data points represent means from various kinds of cells in three independent experiments; bars, S.D. (D) MCF-7 cells were cotransfected with different dose (0.1 μg, 0.3 μg, 0.5 μg, respectively) of p53 expression plasmids (pC53-SN3) and BCRP luciferase promoter reporter constructs. Luciferase activity was measured after 48 h of transfection and normalized by β-galactosidase activity. Values are expressed as the percentage of the control activity (100%) in cell extract from MCF-7 cells that were not transfected with p53 expression plasmid. Values are mean ± S.D. ($n = 3$). Statistical significance compared with control: $^{*}P < 0.05$, $^{**}P < 0.01$.

and 0.651 ± 0.047 μmol/L, respectively. The sensitivity of MCF-7/wt-p53 cells to mitoxantrone increased 3.76 times (compared with MCF-7/pCMV), while the sensitivity of mutant p53 to mitoxantrone had no significant change. After knocking down endogenous wt-p53 through RNAi, the IC_{50} in MCF-7/control and MCF-7/RNAi cells was 0.515 ± 0.138 μmol/L and 1.378 ± 0.512 μmol/L, respectively. The resistance of MCF-7/RNAi cells to mitoxantrone increased 2.68 times (compared with MCF-7/control).

To test the effect of exogenous p53 on BCRP transcription in MCF-7 cells, the experiments were conducted in MCF-7 cells by co-transfecting the wt-p53 expression plasmids with the BCRP luciferase promoter reporter constructs. As shown in Fig. 1D, the wt-p53 expression plasmid caused marked inhibition of the BCRP promoter activity in a dose-dependent manner, with 67.7% inhibition at a dose of 0.5 μg. In contrast to wt-p53, mutant p53 with either mutation in protein-binding region ($p53^{R175H}$) or mutation in DNA-binding region ($p53^{R248W}$) of p53 protein lost most of the inhibitory effect of wt-p53. The inhibition of BCRP promoter activity is specific because expression of p53 had no inhibitory effect on the empty luciferase vectors.

3.2. Wt-p53 but not mutant p53 inhibits NF-κB subunit p50 expression in MCF-7 cells

To define the effects of p53 on NF-κB expression, we co-transfected the p53 expression plasmids with the NF-κB luciferase promoter reporter constructs. As shown in Fig. 2A, wt-p53 inhibited NF-κB promoter activity in a concentration-dependent manner, with 79.6% inhibition at a dose of 0.5 μg. Contrary to wt-p53, mutant p53 with either mutation in protein-binding region ($p53^{R175H}$) or mutation in DNA-binding region ($p53^{R248W}$) of p53 protein lost the effect.

To further determine whether NF-κB is involved in the transcriptional suppression of the BCRP gene by p53 in MCF-7 cells, we performed RT-PCR and Western blot to assess the levels of NF-κB in p53 overexpression cells. Results showed that NF-κB was downregulated in MCF-7/wt-p53 cells. In contrast to the MCF-7/wt-p53 cells, there were no markedly changes in the levels of NF-κB (p50) mRNA and protein in mutant p53 overexpression cell lines (Fig. 2B and C). When endogenous p53 expression in MCF-7 was disrupted by RNA interfere, NF-κB expression was upregulated.

3.3. Suppression of BCRP by wt-p53 in MCF-7 cells may be dependent of NF-κB

To assess the hypothesis that NF-κB plays a role in p53-mediated suppression of BCRP, we transfected MCF-7 cells with different dose of a dominant-negative IκBα mutant expression plasmid to obtain cells with different NF-κB activity. Meanwhile, wt-p53 expression plasmid and BCRP luciferase promoter reporter construct were co-transfected. The results showed a lower inhibitory effect of wt-p53 on BCRP promoter activity after inhibition of NF-κB activity (Fig. 3A). These data demonstrated that NF-κB might play a role in p53-mediated repression of BCRP.

To investigate the effects of IκBα on the regulation of BCRP expression, we established IκBα overexpression cell lines, MCF-7/IκBα and JAR/IκBα cells. RT-PCR and Western blot asssays were enformed to confirm the levels of BCRP mRNA and protein. As shown in Fig. 3B and C, there was a decrease in BCRP expression in MCF-7/IκBα and JAR/IκBα cells lines, compared with MCF-7 cells (mock) and empty vector-transfected cell line, MCF-7/pBabe or JAR/pBabe (vector).

3.4. NF-κB activates BCRP expression by directly binding to BCRP promoter

To investigate the effects of NF-κB on BCRP expression, we then constructed three plasmids, in which BCRP/promoter was inserted into the pGL3 and NF-κB-p50 as well as NF-κB-p65 was inserted into the pcDNA3.1, respectively. As shown in Fig. 4A. Co-transfecting with pcDNA3.1-p50 and pcDNA3.1-p65 respectively, the activities of pGL3-BCRP/promoter are approximately 1.5-fold and 1.1-fold higher than basal activity of the pGL3-BCRP/promoter, indicating that NF-κB plays a regulatory role for the activities of the BCRP promoter.

To further determine the possibility of NF-κB binding to BCRP promoter binding site, EMSA was done with specific oligonucleotides and mutant oligonucleotides. Fig. 4B shows efficient binding to the radiolabeled specific oligonucleotides. NF-κB (p50) can be combined with specific oligonucleotide probes but not with the mutant oligonucleotide probes. This combination can be competitively inhibited by 100-fold unlabeled specific oligonucleotides but not be inhibited by 100-fold unlabeled non-specific oligonucleotides. These results suggest that BCRP promoter has specific

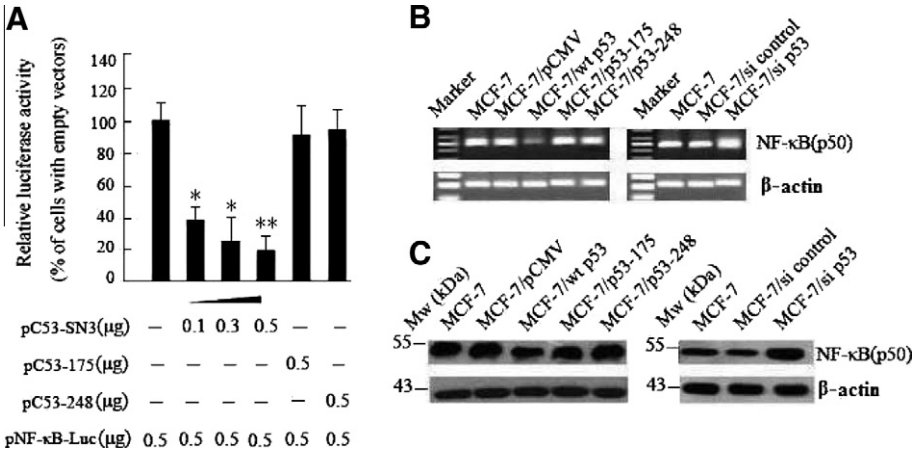

Fig. 2. Effects of p53 on NF-κB expression and promoter activity. (A) MCF-7 cells were cotransfected with different dose (0.1 μg, 0.3 μg, 0.5 μg, respectively) of p53 expression plasmids (pC53-SN3) and NF-κB luciferase promoter reporter constructs. Luciferase activity was measured after 48 hours of transfection and normalized by β-galactosidase activity. Values are expressed as the percentage of the control activity (100%) in cell extract from MCF-7 cells that were not transfected with p53 expression plasmid. Values are mean ± S.D. ($n = 3$). Statistical significance compared with control: $^{*}P < 0.05$, $^{**}P < 0.01$. (B) By RT-PCR assay, there was a decrease in NF-κBon the MCF-7/wt-p53 compared with the MCF-7/pCMV. Between MCF-7/wt-p53 and mutant p53 cells, there were markedly changes on NF-κB in mRNA levels. After transfected with p53siRNA, NF-κB expression was increased. β-actin was used as an internal control. MCF-7 cells were used as control. The results shown are representative of three independent experiments. (C) By Western blot assay, similar results were obtained.

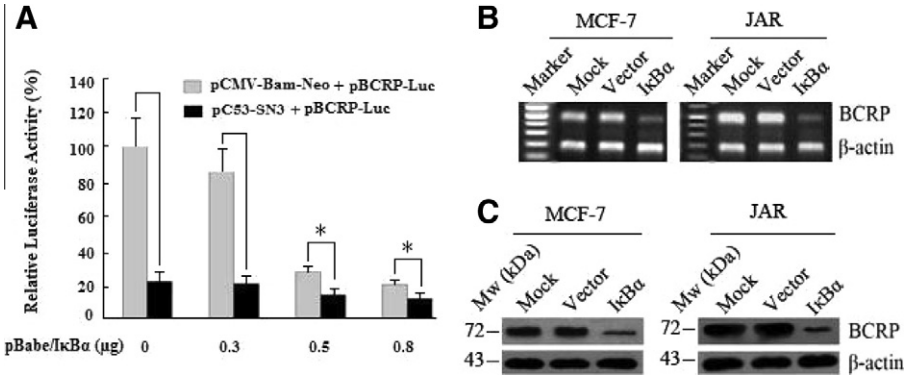

Fig. 3. Effects of IκBα on BCRP expression and promoter activity. (A) MCF-7 cells were transfected with various amounts of a dominant-negative IκBα mutant expression plasmid (pBabe-IκB) to obtain cells with different NF-κB activity. These cells were cotransfected with wt-p53 expression plasmids (or empty vectors) and BCRP luciferase promoter reporter constructs. Values are the means of the relative luciferase activity, normalized to β-galactosidase activity, from three separate experiments; bars, S.D. Statistical significance compared with control: $^{*}P < 0.05$, $^{**}P < 0.01$. (B) MCF-7 cells and JAR cells were transfected with pBabe/IκBα. By RT-PCR assay, there was a decrease in BCRP expression on the MCF-7/IκBa or JAR/IκBα cells compared with the MCF-7/vector or JAR/vector cells. β-actin was used as an internal control. MCF-7 cells were used as control or mock. Quantitative data from three independent experiments were shown in right panel. (C) By Western blot assay, similar results were obtained.

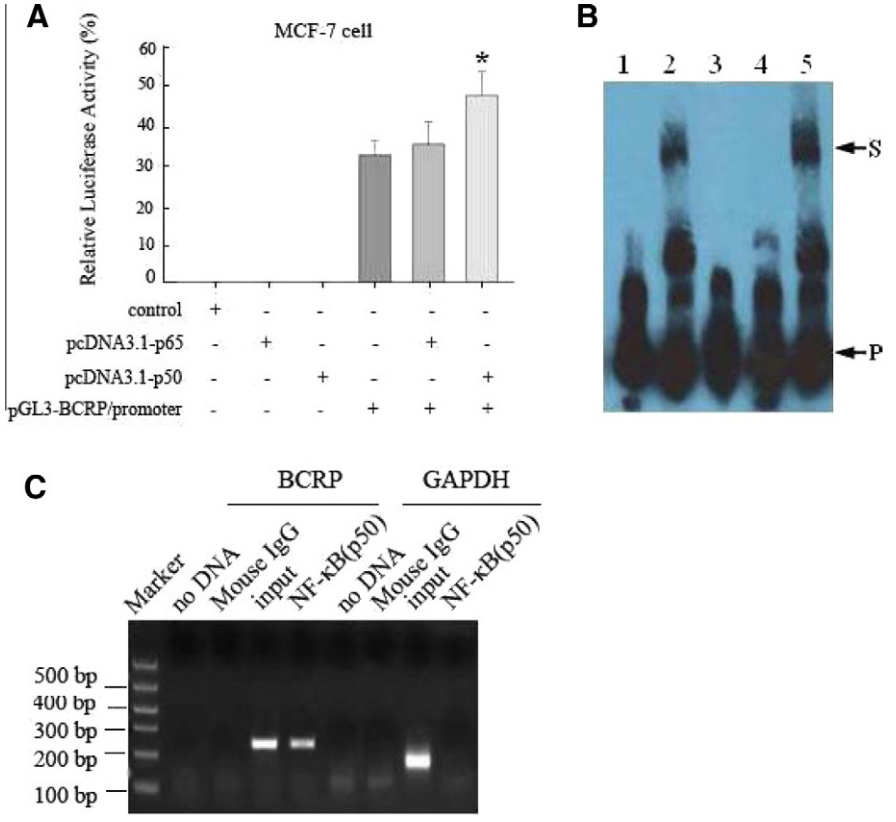

Fig. 4. Effects of NF-κB on BCRP promoter activity and p50 binding site assay. (A) MCF-7 cells were transiently transfected with pcDNA3.1-p65 (100 ng), pcDNA3.1-p50 (100 ng), pGL3-BCRP/promoter (100 ng), pcDNA3.1-p65 + pGL3-BCRP/promoter (100 ng) and pcDNA3.1-p50 (100 ng) + pGL3-BCRP/promoter (100 ng), respectively. Cells were also transfected with empty pGL3 (100 ng) as negative control. All luciferase assay results expressed as relative luciferase activity (i.e., firefly luciferase light units/Renilla luciferase light units). The results were quantified based on three experiments and are presented as mean ± S.D. Statistical significance compared with pGL3-BCRP/promoter group: $^{*}P < 0.01$. (B) Electrophoretic mobility shift assay of NF-κB (p50) binding site within the BCRP promoter. Lane 1: negative control, the reaction mixture of the biotin-labeled specific oligonucleotides and mutant oligonucleotides; lane 2: labeled specific oligonucleotides and nuclear extracts; lane 3: labeled mutant oligonucleotides and nuclear extracts; lane 4: labeled specific oligonucleotides and 100-fold excess amounts of unlabeled specific oligonucleotides added to nuclear extracts; lane 5: labeled specific oligonucleotides and 100-fold excess amounts of unlabeled mutant oligonucleotides added to nuclear extracts. "S" indicates the specifically shift band, while "P" indicates free probe. The representative experiment out of the three experiments is shown. (C) Chromatin immunoprecipitation assay was performed using anti-NF-κB (p50) or Normal Mouse IgG. PCR primers covering the NF-κB (p50) binding sites of BCRP promoter region were used to detect promoter fragment resulting from the −215 to +18 promoter regions in immunoprecipitates. Input is DNA that had been processed as samples but without any immunoprecipitation and was used as a PCR positive control. The negative control is Normal Mouse IgG, which controls for the non-specific immunoselection of chromatin by immunoglobulins. Control Primers are included for detection of a 166 base pair region of the human GAPDH promoter. The experiment shown is representative of three.

binding site of the NF-κB (p50). To examine whether NF-κB interacts with the endogenous BCRP promoter in MCF-7cells, we performed chromatin immunoprecipitation using anti-NF-κB (p50). We detected a specific band after PCR on the MCF-7

immunoprecipitate with oligonucleotides corresponding to the −215 to +18 BCRP promoter regions (Fig. 4C).

4. Discussion

We have demonstrated in this study that the human *BCRP* gene is negatively regulated at the transcriptional level by wt-p53. This inhibitory effect on *BCRP* gene expression by wt-p53 is due at least, in part, to attenuating the effect of a powerful transcription activator, NF-κB. Our data presented here strongly support the conclusion that BCRP expression is transcriptionally downregulated by wt-p53 but not mutant p53 in MCF-7 cells. First, the overexpression of wt-p53 decreased BCRP expression at the mRNA and protein levels, but the mutant p53 with either mutation in protein-binding region ($p53^{R175H}$) or mutation in DNA-binding region ($p53^{R248W}$) lost this inhibitory effect. Second, after the endogenous wt-p53 was disrupted by RNAi, the BCRP expression was upregulated. Third, cotransfection of the p53 expression plasmids and BCRP promoter into MCF-7 cells also demonstrated the ability of p53 to regulate the BCRP promoter. Furthermore, to investigate the effect of *p53* gene on *BCRP* gene expression, we established several stable cell lines with overexpression of exogenous wild-type, MCF-7/wt-p53 and mutant type p53, MCF-7/$p53^{R175H}$ and MCF-7/$p53^{R248W}$ and found the chemosensitivity of MCF-7/wt-p53 to mitoxantrone, which is a specific substrate of BCRP, was enhanced.

Using the MatInspector program [15] with a core similarity of 1.0 and a matrix similarity of at least 0.85, we did not find any potential p53 consensus binding sites within the BCRP promoter, but a potential NF-κB (p50) binding site. Moreover, we were unable to detect p53 direct interaction with BCRP promoter using EMSA assay (data not shown). It seems likely that p53 may mediate the repression through an indirect mechanism rather than direct DNA binding. We found that NF-κB activated BCRP expression through binding to the specific NF-κB (p50) site (−27/−18) within BCRP promoter. Our results indicate that p53 represses BCRP through an indirect mechanism involving the binding of NF-κB to BCRP promoter in MCF-7 cells, at least in part. This interpretation is supported by the following evidences: first, p53 does not direct interact with BCRP promoter; second, NF-κB can bind to BCRP promoter and increase BCRP expression; third, in MCF-7 cells, both overexpression of wt-p53 and inhibition of NF-κB decrease BCRP promoter activity, and after inhibition of NF-κB by IκBα mutant or MG132, the inhibitory effect of wt-p53 on BCRP promoter activity is lower; forth, in Saos-2 cells, p53 induces NF-κB activity and p53 also increases BCRP promoter activity (data not shown).

The interactions between p53 and its downstream genes are mainly through binding to a specific DNA responsive element. Several human genes whose expression is positively regulated by wild-type p53 have been identified [16,17]. Additionally, results obtained from experiments performed on NF-κB in virally transformed or p53-null cells might differ if performed in cell lines capable of mounting a wild-type p53 response. Furthermore, it is possible that novel cancer therapies based on reactivation of wild-type p53 function might benefit from coordinate suppression of NF-κB in order to promote a beneficial outcome. We have also shown that mutant p53 do not inhibit NF-κB gene expression and it is worth noting that many previous experiments on NF-κB transcriptional regulation have been performed in cell lines with mutated p53 or in which it has been functionally inactivated due to the expression of viral proteins. Previous studies have suggested that wt-p53 inhibits *MDR1* and *MRP* gene expression and the inactivation of wt-p53 led to increased expression of the *MDR1* and *MRP* and drug resistance [18]. However, little is known about the molecular mechanisms regulating BCRP expression. Here, for the first time, we have demonstrated that *BCRP* gene expression is also under the negative control of wt-p53. In the present study, our results show that wt-p53 can inhibit *BCRP* gene expression by antagonizing the transactivating effect of NF-κB subunits p50 on BCRP promoter. We suggest that the breakdown of this pathway may result in BCRP overexpression and development of BCRP-associated MDR in tumors.

In conclusion, we present that BCRP expression is activated by NF-κB through direct DNA binding and downregulated by wt-p53 through a decrease of NF-κB activity in MCF-7 cells. Given that BCRP account for the atypical multidrug resistance and p53 plays important role in tumor development, our data should contribute to a better understand of not only regulation of BCRP, but also cancer multidrug resistance (MDR) especially the relationship between MDR and the development of tumor.

Acknowledgments

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