

# The human *BTG2/TIS21/PC3* gene: genomic structure, transcriptional regulation and evaluation as a candidate tumor suppressor gene

Cyril Duriez<sup>a</sup>, Nicole Falette<sup>a</sup>, Carole Audouinaud<sup>a</sup>, Caroline Moyret-Lalle<sup>a</sup>, Karim Bensaad<sup>b</sup>,  
Stéphanie Courtois<sup>a</sup>, Qing Wang<sup>a</sup>, Thierry Soussi<sup>b</sup>, Alain Puisieux<sup>a,c,\*</sup>

<sup>a</sup>Unité INSERM U453, Unité d'Oncologie Moléculaire, Centre Léon Bérard, 28 rue Laënnec, F-69373 Lyon Cedex 08, France

<sup>b</sup>Institut Curie, Génotoxicologie des Tumeurs, 26 rue d'Ulm, 75248 Paris Cedex 05, France

<sup>c</sup>Faculté de Pharmacie, 8 avenue Rockefeller, F-69373 Lyon Cedex 08, France

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## Abstract

*BTG2/TIS21/PC3* protein is involved in the regulation of G1/S transition of the cell cycle by inhibiting pRb function, suggesting that *BTG2/TIS21/PC3* regulation is critical for normal cell growth and proliferation. To understand the regulatory mechanisms for the expression of *BTG2/TIS21/PC3* we cloned the human gene. Potential binding sites for several transcription factors were identified in the 5'-flanking region of the gene. Transient expression assays with *BTG2/TIS21/PC3* promoter deletions and electrophoretic mobility shift analysis identified a major wild-type p53 response element located -74 to -122 relative to the start codon. This genomic fragment was sufficient to constitute a promoter element in the presence of p53. The *BTG2/TIS21/PC3* gene is an antiproliferative gene which maps within a chromosomal segment (1q32) frequently altered in breast adenocarcinomas. However, no mutations of *BTG2/TIS21/PC3* were detected in breast cancer cells, suggesting that the inactivation of this gene is not a frequent genetic event during breast carcinogenesis. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** BTG; Promoter region; p53 binding site; Breast cancer; Mutation

## 1. Introduction

The p53 tumor suppressor is a nuclear phosphoprotein involved in the control of cell growth. It is a critical component of the cellular response to certain stresses such as hypoxia, DNA damage, ribonucleotide depletion or onco-gene activation (Kastan et al., 1991; Hansen and Oren, 1997). After exposure to DNA-damaging agents, p53 protein nuclear levels increase by post-translational stabilization. This induction leads either to apoptosis or cell cycle arrest, thereby preventing replication of cells sustaining DNA damage (Diller et al., 1990; Mercer et al., 1990; Yonish-Rouach et al., 1991). p53 acts as a transcription factor (Levine, 1997) that binds in a sequence-specific manner to well defined DNA elements and induces the transcription of genes residing in the vicinity of such p53

response elements (El-Deiry et al., 1992). p53 consensus sequences have been found in many p53 downstream target genes including the *p21Waf1* gene, *14-3-3σ*, *GADD45*, *BAX*, *Killer DR5* and *MDM2* (El-Deiry, 1998). Mainly based on the observation of an endogenous overexpression of *BTG2/TIS21/PC3* in response to DNA damage or after experimental induction of p53, we previously proposed the *BTG2/TIS21/PC3* gene as a novel p53 target gene (Rouault et al., 1996). *BTG2/TIS21/PC3* belongs to a family of structurally related proteins including BTG1, BTG3/ANA, PC3K/PC3B, TOB and TOB2 (Rimokh et al., 1991; Matsuda et al., 1996; Guehenneux et al., 1997; Yoshida et al., 1998; Ikematsu et al., 1999; Buanne et al., 2000). Although precise functions of the BTG members remain to be elucidated, they appear to act as antiproliferative proteins (Rimokh et al., 1991; Rouault et al., 1992; Matsuda et al., 1996; Guehenneux et al., 1997; Ikematsu et al., 1999). In NIH3T3 cells, forced expression of the *BTG2/TIS21/PC3* gene provokes a marked inhibition of cell proliferation (Rouault et al., 1992, 1996; Matsuda et al., 1996; Montagnoli et al., 1996; Yoshida et al., 1998). In various *in vitro* models, such as 12-*O*-tetradecanoylphorbol-13-acetate-

Abbreviations: BS, binding site; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay

\* Corresponding author. Unité INSERM U453, Unité d'Oncologie Moléculaire, Centre Léon Bérard, 28 rue Laënnec, F-69373 Lyon Cedex 08, France. Tel.: +33-4-78-78-26-67; fax: +33-4-78-78-28-21.

E-mail address: puisieux@lyon.fnclcc.fr (A. Puisieux).

treated mouse fibroblasts and nerve growth factor-stimulated PC12 cells, and *in vivo* during neuron cell birthday in mouse embryo, *BTG2/TIS21/PC3* mRNA expression increases when cells cease to proliferate, or when they differentiate (Fletcher et al., 1991; Iacopetti et al., 1999). The inhibition of cell growth is correlated with the appearance of a pRb protein hypophosphorylated isoform and the inhibition of the transition G1 to S (Guardavaccaro et al., 2000). To better characterize the role of p53 and other factors in the regulation of *BTG2/TIS21/PC3* expression, we identified and characterized the human *BTG2/TIS21/PC3* promoter.

## 2. Materials and methods

### 2.1. Cell lines and treatments

Parental cell lines (Saos-2, HCT116, MCF-7, and SW480) employed in these studies were obtained from the American Culture Collection (Rockville, MD). Breast adenocarcinoma MCF-7, osteosarcoma Saos-2 and colon carcinoma HCT116 and SW480 cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies) containing 10% fetal calf serum. MCF-7 and HCT116 cell lines express an endogenous wild-type *p53* gene. SW480 cells express a mutant *p53*, whereas Saos-2 cells exhibit an homozygous deletion of the *p53* gene.

The CX5-11 cell line was derived from the human osteosarcoma cell line Saos-2 by stably integrating a murine temperature-sensitive mutant *p53* (val135). This cell line was maintained in Dulbecco's modified Eagle's medium (Life Technologies) containing 10% fetal calf serum at a non-permissive temperature (37°C) under 0.25 mg/ml G418 (Gibco BRL) selection. Cells were plated at 10<sup>6</sup> cells per 100 mm dish 24 h prior to shifting to a *p53*-permissive temperature (32°C).

### 2.2. Human DNA library screening and sequencing

The human *BTG2/TIS21/PC3* genomic clone was isolated by screening a λEMBL3 human genomic library using a 629 pb *KpnI/SnaBI* fragment from the 5'-untruncated region of the gene (Rouault et al., 1996). Among the different clones obtained, two clones (clones 16 and 19) hybridized to the *BTG2/TIS21/PC3* cDNA. After subcloning, 3 kb of the promoter region as well as the intron of the *BTG2/TIS21/PC3* gene were sequenced using an ABI PRISM 377 DNA sequencer (Perkin-Elmer) as described by the manufacturer.

### 2.3. Luciferase reporter gene assay

The 5'-flanking sequences of the *BTG2/TIS21/PC3* gene were inserted into the pGL3 basic (Promega) to generate promoter/firefly luciferase constructs. The *BTG2/TIS21/PC3* promoter fragments were generated using the native restriction enzyme sites present in the promoter. All the

constructs were confirmed by restriction analysis and sequencing. To generate deletion mutants of the *p53* binding site (BS4), the Erase a base<sup>®</sup> system (Promega) was used according to the manufacturer's instructions.

Before transfections, cells were seeded at 1.5 × 10<sup>5</sup> cells/well in 12-well microtiter plates. Transient transfection was performed using Lipofectin reagent (Life technologies) containing 325 ng of reporter construct and 125 ng of the *p53* expression plasmid (either the wild-type form or the mutant forms). To evaluate transfection efficiencies, cells were co-transfected with 50 ng of the *renilla* luciferase plasmid (pRL-SV40; Promega). Twenty-four to 72 h after transfection, cells were washed with PBS, exposed to 100 μl of 1 × passive lysis buffer (Promega) for 15 min, and harvested. Cytosolic cell extracts were cleared of debris by centrifugation. A total of 20 μl of the supernatant was assayed for luciferase activity on a luminometer (TD 20/20; Turner Designs) using the dual luciferase reporter assay system (Promega).

When indicated, adriamycin (0.25 μg/ml) was added 24 h after transfection, and the following day cells were harvested with the method described above. All transfection experiments were repeated at least three times.

### 2.4. RNA isolation and Northern blot analysis

Total RNA was extracted by the guanidium isothiocyanate method, as previously described (Guillot et al., 1996). RNA samples (10 μg) were separated by electrophoresis through denaturing formaldehyde agarose gel and transferred on nylon membranes (Hybond-N; Amersham). Membranes were hybridized with labeled *BTG2/TIS21/PC3* cDNA, or *p21<sup>waf1/cip1</sup>* cDNA.

### 2.5. Oligonucleotides and electrophoretic mobility shift assay (EMSA)

Complementary oligonucleotides, which correspond to nucleotides -74 to -122 of the *BTG2/TIS21/PC3* gene, were hybridized and end-labeled using the Ready to Go kinase kit (Pharmacia) and [ $\gamma$ -<sup>32</sup>P]ATP. Human recombinant *p53* was expressed in insect cells using recombinant baculovirus. Nuclear extracts containing 50% pure *p53* were used for the DNA binding assay (Hardy-Bessard et al., 1998). The EMSA method was previously described (Bensaad et al., 2001). For the gel shift assay, 0.2 ng of probe was mixed with purified *p53* protein. For the super-shift assay, anti-*p53* (carboxy-terminus monoclonal antibody HR231) was included in the reaction mixture. The competition experiment was performed by adding 50 times excess of unlabeled oligonucleotide in the reaction mixture.

### 2.6. Studies with microsatellite markers

Microsatellite markers used for the PCR analyses are

Table 1  
Microsatellite markers used for loss of heterozygosity assay<sup>a</sup>

Marker	Annealing (°C)	Sequence
AFMa 123 yf1	60	
AFMa 109 zh9	61	
AFMa 272 xd9	61	
AFM 238 xd10	64	
AFMb 032 yf1	65	
AFM 291 vf1	65	
AFM 289 ye9	66	
AFMa 132 yc9	66	
AFM 234 wf6	66	
C4BP	61	5'-TTA CCT CTT ATT GGG CTG AAA-3' 5'-CAT TTG GCC TTT TCA CAT TCT-3'
CAi	60	5'-GGG TGT GCC AAA AGC AAT-3' 5'-GGT GGA TTC CTC TGA GTT CT-3'

<sup>a</sup> Sequences indicated for C4BP and CAi markers correspond to the oligonucleotides used for PCR reaction.

given in Table 1. PCR and electrophoresis were performed as previously described (Lalle et al., 1995).

### 2.7. Heteroduplex analysis

DNA was extracted from 18 primary breast carcinomas and 12 breast cancer cell lines by proteinase K digestion and phenol purification. Both exons of the *BTG2/TIS21/PC3* gene were amplified with primers and conditions described in Table 2. For heteroduplex analysis, each PCR was performed with the addition of 0.05 l [<sup>33</sup>P]dATP. Five microliters of the PCR product were then electrophoresed through a 0.6 × MDE Matrix (FMC BioProducts, Rockland, ME) and visualized by autoradiography.

## 3. Results and discussion

### 3.1. Characterization of the human *BTG2/TIS21/PC3* promoter region

We previously identified *BTG2/TIS21/PC3* as a p53-transcriptional target (Rouault et al., 1996; Cortes et al., 2000). To better characterize the mechanisms of regulation of *BTG2/TIS21/PC3* transcription, we cloned the human gene, including the promoter region. For this purpose, the λEMBL3 human placenta genomic DNA library was screened with the *BTG2/TIS21/PC3* cDNA as a probe. A positive genomic clone of 5 kb was isolated and sequenced (Fig. 1). This fragment contained exons 1 and 2 of *BTG2/TIS21/PC3* and 3074 bp of the 5'-flanking region upstream

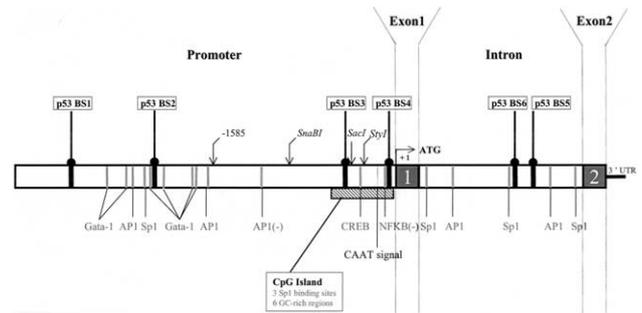


Fig. 1. Diagrammatic representation of the human *BTG2/TIS21/PC3* gene. The first nucleotide of the translation initiation site ATG is indicated by an arrow. The position of a CpG island is shown as a strip rectangle. The main transcription factor binding sites and the CAAT box are indicated. The potential p53 binding sites (p53 BS1–6) are indicated in boxes. Restriction enzyme sites are indicated by arrows. The complete nucleotide sequence is deposited with GenBank (Accession number: AF361937).

from the ATG start codon. The *BTG2/TIS21/PC3* gene contains one intron of 1384 bp. No discernible TATA box was identified in the 5'-flanking region. However, a CCATT box was located –158 upstream from the initiation site ATG. Furthermore, we identified several GC-rich regions containing three Sp1 binding sites (Fig. 1). These features are commonly found in the promoter regions of genes without a TATA box (Ji et al., 1996). Promoter DNA sequence analysis by computer search predicted a CpG island located from nucleotide positions –554 to –80. This CpG island presented an average GC content of 62% and a CpG score of 0.79.

Sequence analysis of the uncoding region identified several putative binding sites for transcription factors including AP-1, GATA-1, NFkappaB or CREB. Furthermore, sequencing analysis of the *BTG2/TIS21/PC3* gene (including the 5'-flanking region and the intron) revealed the presence of six sites (p53 BS1–6) highly related to the consensus binding site for p53. The consensus binding site consists of four pentameric repeats of RRRCW in which R is a purine and W represents either an A or T residue. Two palindromic pentamers (half-site) are juxtaposed to a second set of two palindromic pentamers, the two half-sites being separated by no insert or insertions from 1 to 13 bp (El-Deiry et al., 1992). Among the four elements identified in the promoter region, three (p53 BS1–3) exhibited 85–90% homology with this consensus binding sequence (Fig. 2). p53 BS4, which we previously reported (Rouault et al., 1996), showed a perfect palindromic 20 bp sequence, sharing 14 of 20 nucleotides with one of the two p53 binding

Table 2  
Characteristics of the oligonucleotides used for heteroduplex analysis of the *BTG2/TIS21/PC3* gene coding region

Exon	Oligonucleotide	Sequence	Annealing (°C)	Size (bp)
1	F8	5'-CGC GAC ATG AGC CAC GGG AAG-3'	63	255
	R9	5'-GCT GGC CAC CCT GCT GAT GAT-3'		
2	F9	5'-TAC CGC TGC ATT CGC ATC AAC-3'	65	290
	R8	5'-AGG GCC TAG CTG GAG ACT GC-3'		

Site Designation	5' Position Relative to ATG	Sequence
P53 BS1	-2633 (promoter)	$\begin{array}{c} \text{9/10} \quad \quad \quad \text{9/10} \\ \text{AGACA T} \underline{\text{A}} \text{CCC AG GAAGA TGTTC} \end{array}$
P53 BS2	-1960 (promoter)	$\begin{array}{c} \text{9/10} \quad \quad \quad \text{9/10} \\ \text{A} \underline{\text{C}} \text{GCA TGTTC AGGCA TGC} \underline{\text{A}} \text{T} \end{array}$
P53 BS3	-430 (promoter)	$\begin{array}{c} \text{8/10} \quad \quad \quad \text{9/10} \\ \text{AGGC} \underline{\text{G}} \text{AG} \underline{\text{G}} \text{CC G GGG} \underline{\text{C}} \text{C TGCC} \end{array}$
<b>P53 BS4</b>	-119 (promoter)	$\begin{array}{c} \text{5/10} \quad \quad \quad \text{9/10} \quad \quad \quad \text{10/10} \quad \quad \quad \text{10/10} \\ \text{CTGAA AAACG C TGCCC GGGGA A AGTCC GGGCA G AGCCC GAGCA} \end{array}$
P53 BS5	+924 (intron)	$\begin{array}{c} \text{8/10} \quad \quad \quad \text{9/10} \\ \text{GGC} \underline{\text{C}} \text{ TGCC} \underline{\text{T}} \text{ GGACA TGCC} \underline{\text{A}} \end{array}$
P53 BS6	+1060 (intron)	$\begin{array}{c} \text{8/10} \quad \quad \quad \text{8/10} \quad \quad \quad \text{8/10} \quad \quad \quad \text{8/10} \\ \text{AGTTT TCACT C CCCTT AGGCA C TGCTA AGGAA GG TCTC} \underline{\text{C}} \text{GACT} \end{array}$
P53 ConsensusBS		$\begin{array}{c} \text{5'-PuPuPuC(A/T) (A/T)GPyPyPy-N(0-13)- PuPuPuC(A/T) (A/T)GpyPyPy-3'} \end{array}$

Fig. 2. Sequences of elements in the *BTG2/TIS21/PC3* promoter and intron (p53 BS1–6) sharing homologies with the p53 consensus binding site (shown below). Mismatches to the consensus sequence are underlined. Nucleotide numbers refer to the residues relative to the first nucleotide of the translational start codon.

sites of the *p21Waf1* gene (El-Deiry et al., 1995). Interestingly, the tandem p53 BS4 was directly preceded by an additional decamer (see Fig. 5) exhibiting 70% homology with the p53 binding motif. Additional motifs were showed to enhance the p53 interaction (Bourdon et al., 1997).

### 3.2. Transcriptional activation of the *BTG2/TIS21/PC3* promoter by wild-type p53

We previously demonstrated that a sequence containing the first 760 bp upstream of the *BTG2/TIS21/PC3* translation initiation site was able to confer inducibility by p53 when located *cis* to a heterologous promoter or upstream of a promoterless reporter gene (Rouault et al., 1996). To precisely identify p53 binding sites in the non-coding region of the *BTG2/TIS21/PC3* gene, we tested the entire promoter region and intron of *BTG2/TIS21/PC3*. As a first experiment, 2658 bp of the 5'-flanking region of the *BTG2/TIS21/PC3* promoter (−1/−2658) was inserted upstream of a luciferase reporter gene in the pGL3 basic vector and designated pGL3-BTG2/2658. We showed previously that genotoxic treatment induces *BTG2/TIS21/PC3* mRNA upregulation through both p53-dependent and p53-independent mechanisms. To confirm the promoter activity of the 5'-flanking region of the *BTG2/TIS21/PC3* gene, we first tested whether the induction of DNA damage by adriamycin treatment could induce the reporter activity in the wild-type p53-expressing HCT116 cell line. A 24 h exposure of HCT116 to 0.2 μg/ml adriamycin triggered a significant increase of basal luciferase activity strongly suggesting that the 2658 bp fragment contained the minimal *BTG2/TIS21/PC3* promoter (data not shown). To directly address whether p53 was able to activate *BTG2/TIS21/PC3* transcription, the p53 negative Saos-2 cell line was transiently co-trans-

ected with the pGL3-BTG2/p2658 construct together with an expression vector encoding wild-type p53 (pCMV-p53) or with control plasmid (pCMV-neo). The *BTG2/TIS21/PC3* promoter had low activity in the absence of p53 but the luciferase activity was greatly enhanced in the presence of wild-type p53 expression (Fig. 3A). Similar results were obtained in the colon carcinoma SW480 and HCT116 cell lines (data not shown). CX5-11 cells are derived from the Saos-2 cell line and express a murine *p53val135* thermo-sensitive mutant (ts-p53). As previously described, the ts-p53 protein adopts a wild-type conformation at 32°C and a mutant conformation at 37°C. As shown in Fig. 3B, following transfection with the pGL3-BTG2/p2658 construct, the shift from 37 to 32°C caused a significant increase of transactivation of the *BTG2/TIS21/PC3* promoter.

p53 binding motifs of several p53 target genes are located in intronic sequences. As shown in Fig. 1A, sequencing analysis of the *BTG2/TIS21/PC3* gene revealed two putative p53 binding sites within the *BTG2/TIS21/PC3* intron (BS5 and BS6). A plasmid containing the 1.5 kb *BTG2/TIS21/PC3* intron and a minimal promoter inserted upstream of the luciferase reporter gene was constructed. This construct was transiently co-transfected with either pCMV-p53 or pCMV-neo in Saos-2, SW480 and HCT116 cells. Luciferase activity was not affected by p53, suggesting that intronic sequences are not significantly involved in the p53-dependent regulation of *BTG2/TIS21/PC3* (data not shown).

p53 has been previously shown to interact with the Sp1 transcription factor (Borellini and Glazer, 1993). To evaluate the implication of the Sp1 binding sites in the transactivation of the *BTG2/TIS21/PC3* promoter, Saos-2 cells were co-transfected with pGL3-BTG2/p2658 and pCMV-Sp1 with or without pCMV-p53. No induction of luciferase expression was observed following Sp1 overexpression

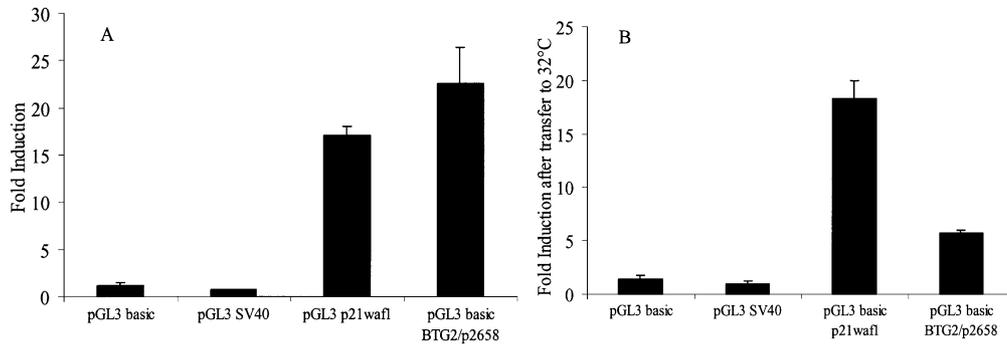


Fig. 3. Effect of wild-type p53 on transcriptional activity of the *BTG2/TIS21/PC3* promoter. (A) Saos-2 cells were co-transfected with either pCMV-wtp53 (expression plasmid encoding wild-type p53) or pCMV-neo (control plasmid) together with pGL3 *firefly* luciferase reporter gene plasmids containing a 2658 bp fragment of the *BTG2/TIS21/PC3* promoter (pGL3-BTG2/p2658). Three vectors were used as controls: pGL3 basic, which represented the low background of the reporter vector; pGL3-SV40, in which luciferase expression was driven by the SV40 promoter; and pGL3-p21Waf1, which contained the p53-responsive *p21Waf1* promoter region. Total luciferase activity was measured after 48 h. The efficiency of transfection was normalized by co-transfecting the *renilla* luciferase vector pRL-SV40. The fold induction indicates the relative induction observed in the presence of wtp53 (pCMV-wtp53) compared to the induction in the absence of p53 (pCMV-neo). Standard deviations are shown. (B) pGL3-BTG2/p2658 construct was transfected into CX5-11, a cell line derived from the human osteosarcoma cell line Saos-2 by integrating a murine temperature-sensitive mutant p53 (val135). At 32°C the cell line expressed a p53 protein with a wild-type conformation and at 37°C a p53 protein with a mutant conformation. pGL3 basic, pGL3-SV40 and pGL3-p21Waf1, described in (A), were used as controls. The fold induction indicates the relative induction at 32°C compared to that observed at 37°C. Standard deviations are shown.

and no co-operation between Sp1 and p53 could be detected (data not shown).

In order to localize the control elements involved in the p53 response, 5'-deletion analysis of the human *BTG2/TIS21/PC3* promoter was undertaken using the existing

restriction sites. Fig. 4 represents luciferase assays done after transfection of Saos-2 cells with six deletion derivatives of the promoter in the presence or absence of the wild-type p53 expression plasmid. pGL3-BTG2/p373 was the most responsive promoter construct to p53. However, dele-

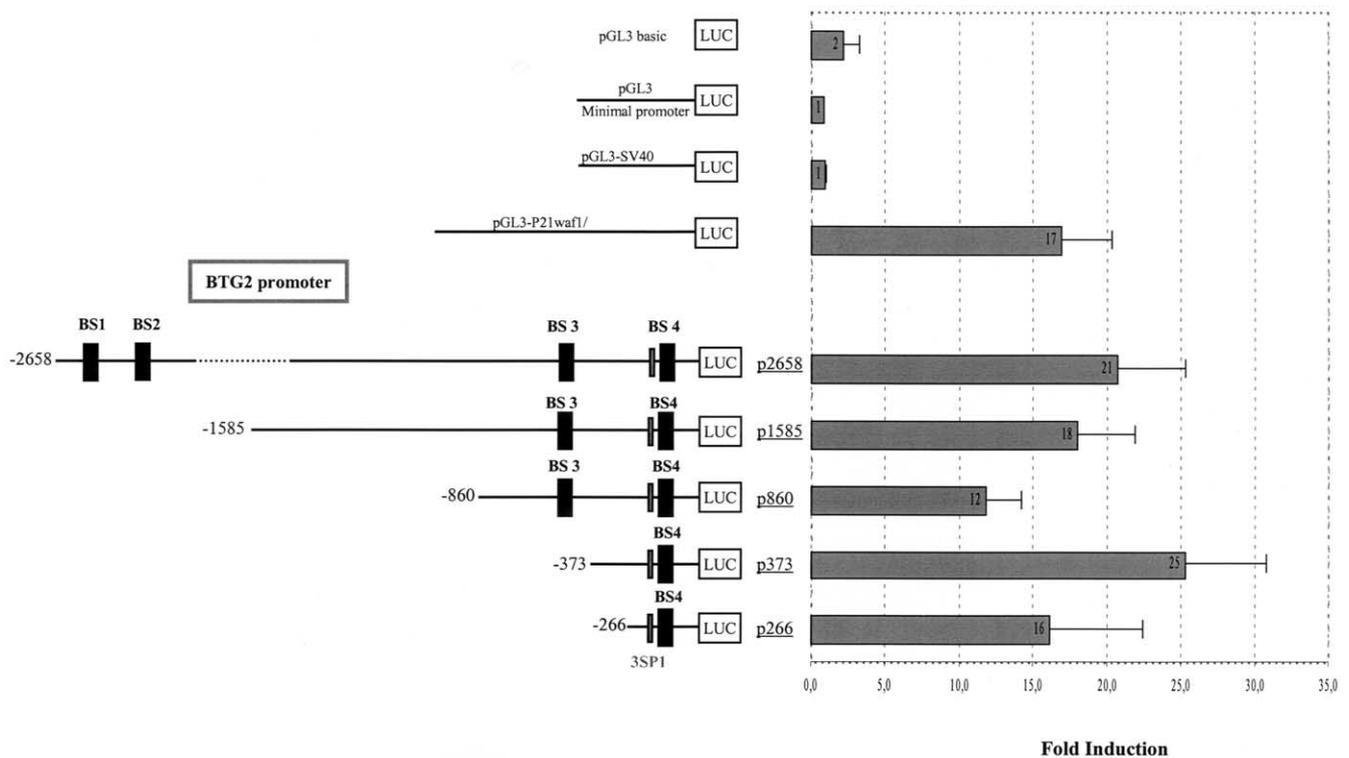


Fig. 4. Deletion analysis of the 5'-flanking region of the *BTG2/TIS21/PC3* gene. Reporter constructs (pGL3-derived plasmids) contain indicated regions of the *BTG2/TIS21/PC3* promoter linked to the luciferase gene. The position of the deletion upstream of the *BTG2/TIS21/PC3* open reading frame was indicated for each construct. Saos-2 cells were co-transfected with either pCMV-p53 or pCMV-neo together with a pGL3-derived construct. pGL3 basic, pGL3-SV40 and pGL3-p21Waf1, described in Fig. 3A, are used as controls. The values represent the relative expression in the presence of p53 compared to the induction in the absence of p53. Standard deviations are shown.

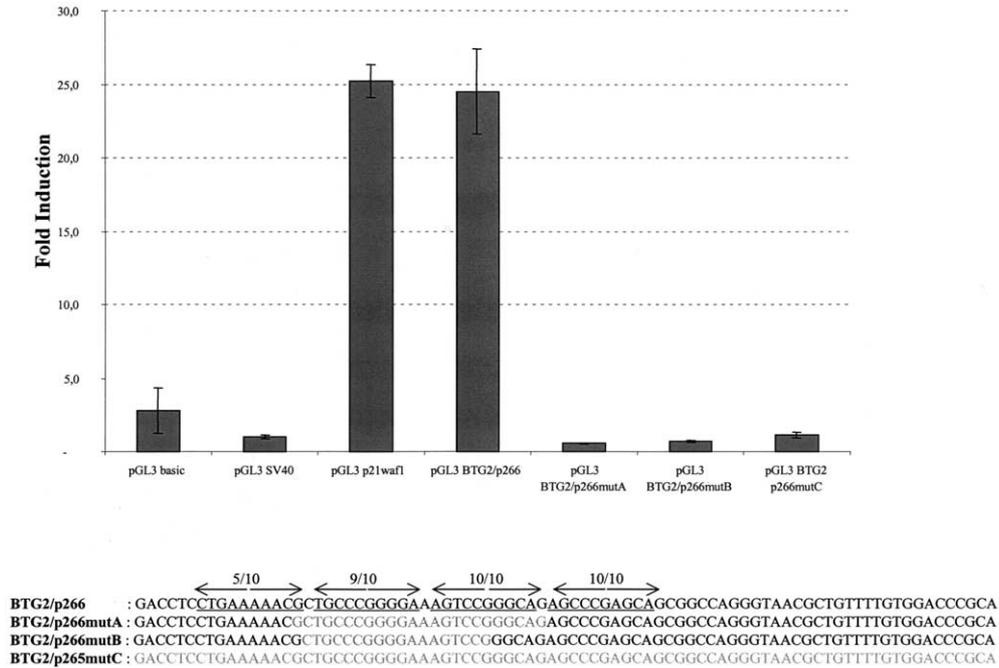


Fig. 5. Role of the p53 binding site 4 in the *BTG2/TIS21/PC3* promoter activity. Deletions within the BS4 site abolish p53 responsiveness. Constructs derived from the PGL3-BTG2/p266 plasmid were generated by deletions encompassing the BS4 sequence (pGL3-BTG2/p266mutA-C). The diagram below the graphic indicates the deleted bases (written in gray). Transcriptional activity has been studied by co-transfecting Saos-2 with pGL3-derived constructs together with either pCMV-P53 or pCMV-neo. The values represent the relative expression in the presence of p53 compared to the induction in the absence of p53. Standard deviations are shown.

tion up to -266 preserved the p53 response. This suggested that the major p53 response element was located within the 266 bp sequence 5' of the *BTG2/TIS21/PC3* translational initiation site. In order to demonstrate that the p53-dependent *BTG2/TIS21/PC3* transactivation was driven from the BS4 site (see Fig. 1), we generated constructs with deletions encompassing the BS4 sequence in the PGL3-BTG2/p266 construct. Then, transcriptional activity was studied in the presence or absence of the wild-type p53 expression plasmid in the Saos-2 cellular context. As shown in Fig. 5, alteration of the BS4 site destroyed the capacity of the promoter to be activated by p53.

We next used the gel retardation assay to assess whether p53 protein (produced in baculovirus-infected insect cells) was able to bind to the BS4 site. A *BTG2/TIS21/PC3* fragment from -74 to -122 was sufficient to complex with p53 (Fig. 6). As controls, the addition of a 50-fold excess of unlabeled oligonucleotide reduced labeling of the protein/DNA complex and addition of a monoclonal antibody recognizing the C-terminus of p53 activated the DNA binding and supershifted the complex indicating that the labeled protein/DNA complex indeed contained p53 (Fig. 6).

All together our results strongly suggested that the BS4 site was responsible for the p53-dependent transactivation of the *BTG2/TIS21/PC3* gene. This observation is in concordance with our former hypothesis based on the preliminary study of the region from -760 to +20 bp (Rouault et al., 1996). Since the *BTG2/TIS21/PC3* promoter is TATA-less and had little activity in Saos-2 cells in the absence of the

wild-type p53 expression plasmid, we wanted to test whether a synthetic p53 binding site alone could constitute

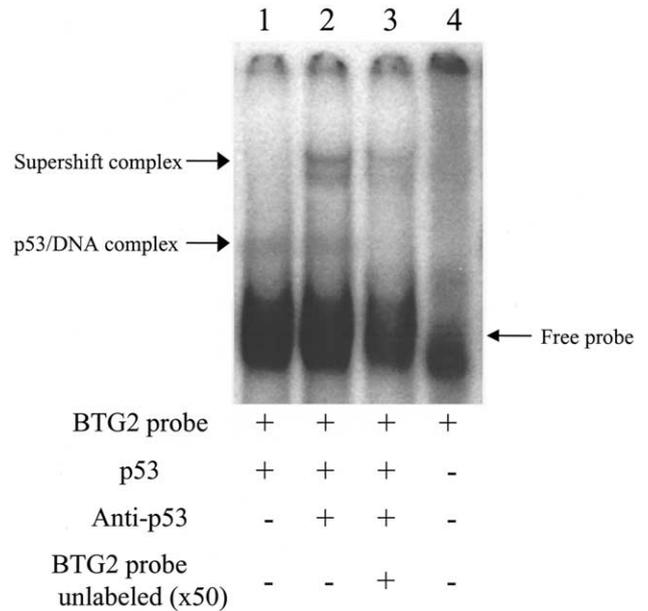


Fig. 6. EMSA. The gel shift mobility assay was performed by using a 48 bp DNA fragment containing the putative p53 binding site, BS4, of the *BTG2/TIS21/PC3* gene. The reaction contains DNA alone (lane 4), DNA plus purified p53 protein (lane 1), DNA, p53 protein and mAb to p53 (lane 2). Lane 3 represents the reaction with competitor DNA by adding 50 × excess of specific unlabeled DNA fragment in the reaction mixture containing 48 bp DNA probe, p53 protein and mAb to p53.

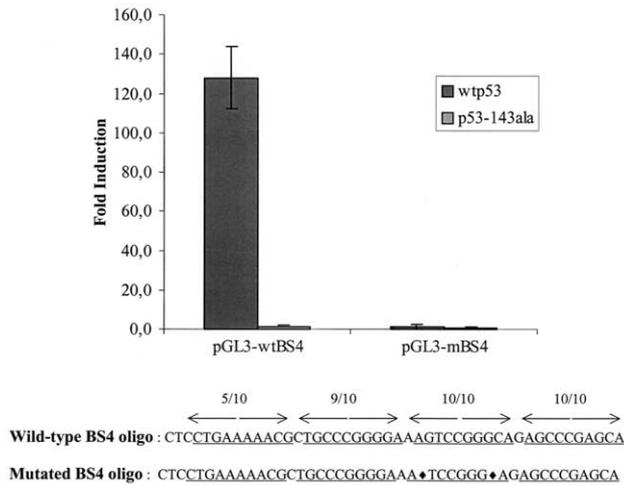


Fig. 7. Mutation of BS4 abolishes wild-type p53-mediated transactivation. The BS4 element acts as a promoter in the presence of p53. An oligonucleotide containing the BS4 site was cloned upstream of the luciferase gene (pGL3-wtBS4). Saos-2 cells were co-transfected with pGL3-wtBS4 together with plasmid expressing either wild-type p53 or mutant p53 (p53-143ala). pGL3-mBS4 presents a deletion of two nucleotides within the BS4 site, as indicated by black lozenges.

a promoter element in the presence of p53. For this purpose, an oligonucleotide containing the BS4 site was cloned upstream of the luciferase gene (pGL3-wtBS4). Co-transfection experiments showed that wild-type p53, but not mutant p53 (ala143), was able to induce the reporter expression through the BS4 site (Fig. 7). As a control, a mutated version of the BS4 site was included in these experiments. Two nucleotide deletions completely eliminated wild-type p53-mediated transactivation (Fig. 7). These observations confirm previous studies demonstrating that p53 binding sites may constitute promoters that can become active in the presence of p53 (Deb et al., 1994).

### 3.3. Mutation analysis of the *BTG2/TIS21/PC3* gene

We previously located the human *BTG2/TIS21/PC3* gene on chromosome 1q32. Chromosome 1q rearrangements appear to be one of the primary lesions associated with the development of breast cancer (Pathak et al., 1991). Homozygous deletions or loss of heterozygosity (LOH) are thought to unmask recessive mutations that inactivate or remove suppressor genes that regulate normal cell growth. Because of (i) its chromosomal localization, (ii) its regulation by the tumor suppressor p53 and (iii) its anti-proliferative properties, we hypothesized that *BTG2/TIS21/PC3* inactivation might be involved in breast tumor formation or progression. In a first approach, LOH on 1q32 was assayed by PCR amplification of genomic DNA from 18 matched breast adenocarcinomas and peripheral blood leukocyte pairs. Eleven polymorphic short tandem repeats from region 1q32 were amplified using primers from GeneThon (Evry, France). We also amplified a CA repeat that we

identified within the *BTG2/TIS21/PC3* intron. Four of 14 informative cases showed LOH for at least one locus. On the four cases, one LOH appeared to involve the entire region and three were restricted to a relatively small chromosomal region including the CA repeat within the *BTG2/TIS21/PC3* intron. To directly evaluate the involvement of *BTG2/TIS21/PC3* in breast carcinogenesis we examined 18 primary breast carcinomas and 12 breast cancer cell lines for mutations of *BTG2/TIS21/PC3* using heteroduplex analysis and sequencing. We designed primers to amplify both exons and splice sites. No mutations nor nucleotide polymorphisms were detected, strongly suggesting that *BTG2/TIS21/PC3* is not a frequent mutational target in breast cancer. Nevertheless, this does not definitively discard the hypothesis that *BTG2/TIS21/PC3* may act as a tumor suppressor gene. Recently, Ficazzola et al. (2001) demonstrated that *BTG2/TIS21/PC3* protein expression was significantly reduced in prostate cancer, as a potential consequence of increased proteosomal degradation. This suggests that *BTG2/TIS21/PC3* may be inactivated in cancer cells in the absence of genetic mutations. Further experiments need to be conducted at the mRNA and protein levels to test this hypothesis in several types of human cancer.

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