# Human p53-p51 (p53-Related) Fusion Protein: A Potent *BAX* Transactivator

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**We recently discovered human *p51*, a new gene structurally and functionally related to human *p53*. This gene encodes two major splicing variants, p51A and p51B, which differ in their carboxyl-terminal structure. However, p51A shows strong transactivation potential, while p51B has only weak potential. To clarify the reason for this difference, we made chimeric gene constructs expressing fusion proteins of p53-p51A and p53-p51B, having an N-terminus of p53 and a C-terminus of p51A or p51B, respectively. In a *BAX* promoter-luciferase assay using p53-deficient SAOS-2 cells, they exhibited up to 30-fold stronger transactivation potential than p53 and p51A themselves, suggesting that the C-terminus of p51B does not simply serve as a repressor. We obtained similar results with *p21^WAF1* promoter-reporter plasmids. These chimeras will be valuable tools for gene therapy.**

Key words:    p53-p51 — Chimeric construct — Transactivator — Gene therapy

Tumor suppressor gene *p53* is activated by DNA damage, and causes cells to undergo G1-arrest or apoptotic cell death, thereby playing a critical role in human carcinogenesis.[1–3]

We recently reported the discovery of human *p51* (also known as *p40*, *p63*, *p73L*), a new gene related to *p53*.[4–7] Splicing variants of *p51*, termed *p51A* and *p51B*, were shown to be capable of exerting apoptotic function similar to that of *p53* and *p73*, another *p53*-related gene[8] in a p53-deficient human osteogenic sarcoma cell line, SAOS-2. However, the transactivation potential and apoptotic potential of these two forms were strikingly different, p51A being strong, and p51B, weak.[4, 6] These two splicing variants differ only in their carboxyl-terminal sequence, sharing an identical amino-terminal 408-residue polypeptide with an additional 40-residue polypeptide for p51A and a 233-residue polypeptide for p51B at the carboxyl terminus.[4] Moreover, this carboxyl-terminal sequence of p51B contains a protein sequence motif called PY-motif or SAM domain, which is speculated to function as a protein-protein interaction module of a type characteristic of transactivators involved in differentiation processes.[9, 10] This notion led us to investigate the nature of the difference of the carboxyl-terminal polypeptides of p51A and p51B which results in the major differences in transactivation potential and apoptotic potential. To this end, we prepared chimeric gene constructs capable of expressing p53-p51 fusion proteins and analyzed the transactivation potentials of the products. We encountered a striking and unexpected result: these fusion proteins exhibited extraordinarily strong transactivation potentials for a sample p53 target gene. They may turn out to be invaluable tools for gene therapeutic applications and for functional analyses of p53 family proteins.

We made the chimeric genes using PCR (polymerase chain reaction) technology basically as described[11] with some modifications (Fig. 1). We constructed the p53, p51A, and p51B genes in pRc/CMV backbone vector (Invitrogen, Carlsbad, CA), which was expected to allow easy construction of the chimeric genes, and evaluation of the transcription potential. We designed one hybrid primer 5′-GCTGCCCCCAGGGAGCACTAAGCGCCCGTTTC-GTC-3′, so as to juxtapose the 293rd amino acid of the human p53 and the 336th amino acid of the human p51A and p51B (Fig. 2A), and anti-sense primer 5′-AGTGCTC-CCTGGGGGCAGC-3′ corresponding to the p53 portion of the hybrid primer. PCR amplification (30 s at 94°C, 30 s at 60°C, 1 min at 72°C for 20 cycles using EX Taq (Takara Shuzo, Kyoto) as suggested by the supplier) was performed using the antisense primer and the T7 primer (positioned 5′ to the inserted genes in the pRc/CMV vector) and the hybrid primer and SP6 primer (positioned 3′ to the inserted genes in the pRc/CMV vector) using p53 and p51A expression construct as templates, respectively (Fig. 1A). Following gel purification, the amplified fragments were mixed and PCR-amplified using T7 and SP6 primers as above except for a 2-min extension at 72°C (Fig. 1A). The resulting amplified fragment was recloned back into the pRc/CMV vector and subjected to sequence
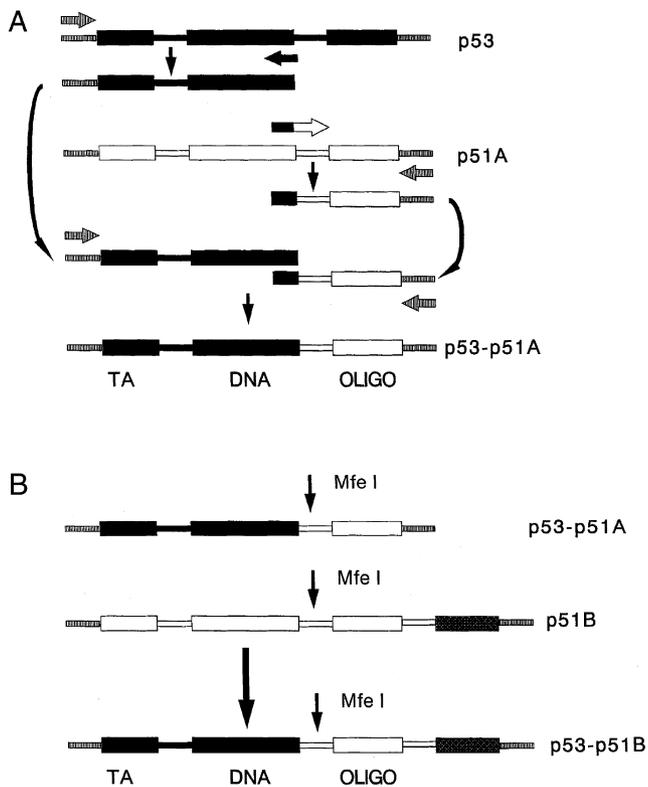
Fig. 1. Preparation of chimeric gene constructs expressing p53-p51A (A) and p53-p51B (B). For construction of p53-p51A plasmid (A), PCR amplification was performed using the antisense primer and the T7 primer with p53 expression construct as a template and the hybrid primer and SP6 primer with p51A as a template. The amplified fragments were mixed and PCR-amplified using T7 and SP6 primers. Construction of the p53-p51B plasmid (B) was easily done by combining fragments derived from p53-p51A and p51B plasmid, utilizing the restriction enzyme *Mfe*I recognition site unique to the p51 coding sequence and pRc/CMV vector. Solid box, portion derived from p53; open box, portion derived from p51; striped box, portion derived from pRc/CMV; stippled box, SAM domain of p51B; TA, transactivation domain; DNA, DNA binding domain; OLIGO, oligomerization domain.
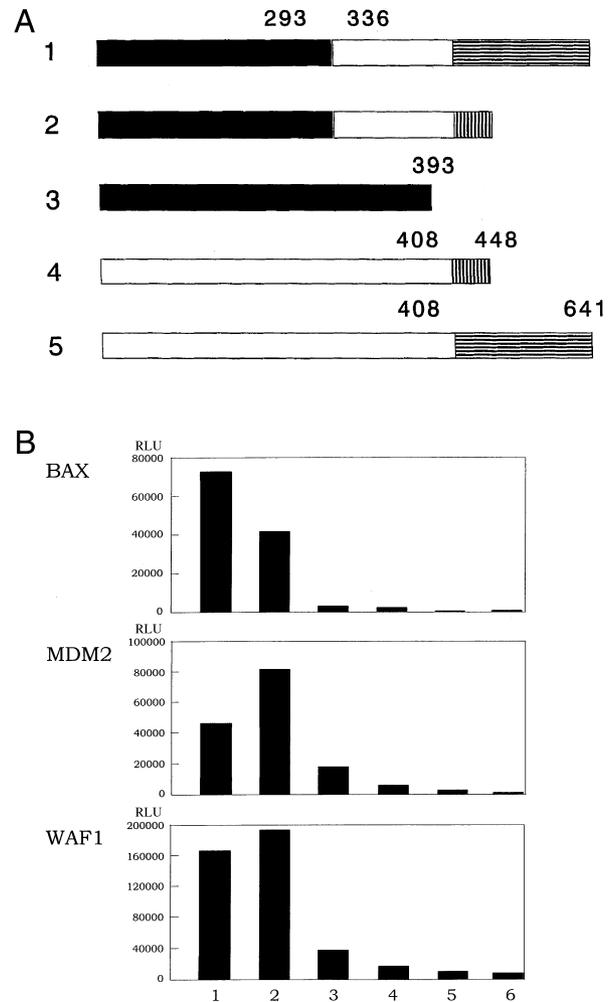


Fig. 2. Transactivation potentials of p51, p53 and their derivatives. Schematic representation of the constructs used in the luciferase assay (A). Numbers above the bars indicate amino acid residue number of the junctions and termination of the proteins. 1, p53-p51B plasmid; 2, p53-p51A plasmid; 3, p53 plasmid; 4, p51A plasmid; 5, p51B plasmid. Solid box, portion derived from p53; open box, portion derived from p51 common region; box with horizontal stripes, portion derived from p51B specific region; box with vertical stripes, portion derived from p51A specific region. Result of luciferase assay shown in relative light unit (RLU) (B). BAX, *BAX* promoter plasmid; MDM2, *MDM2*-P2 promoter plasmid; WAF1, *p21*^WAF1 promoter plasmid. Columns 1–5 correspond to constructs shown in (A). Column 1, p53-p51B plasmid; column 2, p53-p51A plasmid; column 3, p53 plasmid; column 4, p51A plasmid; column 5, p51B plasmid; column 6, pRc/CMV.

analysis of the entire coding region to confirm that the construct was devoid of mutations (p53-p51A plasmid, construct 2 of Fig. 2A).

Construction of the p53-p51B plasmid (construct 1 of Fig. 2A) was easily done by combining p53-p51A plasmid and p51B plasmid, utilizing the restriction enzyme *Mfe*I recognition site unique to the p51 coding sequence and pRc/CMV vector (Fig. 1B).

Transcription potentials of these chimeras were determined by luciferase reporter assay using *BAX*, *MDM2*-P2 and *p21*^WAF1 promoters as target promoters. Four hundred nanograms of the p53-p51B, p53-p51A, p53,

p51A, p51B, and pRc/CMV plasmids was co-transfected with 100 ng of reporter plasmid (*BAX* or *MDM2*-P2 or *p21*^WAF1 promoter-luciferase plasmid) and 0.5 ng of the internal control plasmid, pRL-CMV (Promega, Madison,

WI) into the SAOS-2 cell line using a 24-well plate as described.[4] For the BAX promoter-luciferase plasmid, we amplified the whole *BAX* promoter region by PCR,[12] inserted it into pGL3basic (Promega), and sequenced the clone to confirm its correctness. *MDM2*-P2 promoter-luciferase plasmid (pGL2hmdm2-HX-luc) was a gift from Dr. M. Oren,[13] and *p21^WAF1* promoter-luciferase plasmid (pWAF1luc) was described previously.[4] Luciferase activity was measured using a Dual Luciferase assay kit (Promega) as suggested by the supplier and the results were collected from triplicate experiments and standardized by reference to the internal control of *Renilla* luciferase activity of the pRL-CMV (Fig. 2). Unexpectedly, using *BAX* promoter, the p53-p51B chimera exhibited up to 30-fold (72,300 RLU) and the p53-p51A chimera exhibited up to 30-fold (41,800 RLU) stronger transactivation potential than those of p53 and p51A themselves (2,520 and 2,250 RLU, respectively). Accordingly, the carboxyl-terminal domain of p51B, which was originally thought to be a trans-repressor domain, does not simply serve a repressor function in that it can be turned into a potent transactivater in the context of p53-p51B. Note that, although it is not clearly apparent in the figure, p51B had a significant activity of 320 RLU compared to 65 RLU for pRc/CMV. Enhanced transactivation potential was also observed when using *MDM2*-P2 and *p21^WAF1* reporter plasmid, although the potentiation was not as strong as was observed in the case of *BAX* promoter (2- to 5-fold potentiation, depending on the effector and reporter plasmids). We do not know at present the exact meaning of this potentiation by the p51 carboxyl-terminal sequence, since the transactivation process which we measured is the result of many biological processes. Possible causes include stabilization of the proteins resulting from lowered susceptibility to MDM2-dependent destruction, increased oligomerization potential of the oligomerization domain of p51, resulting in a stable oligomer capable of stronger transactivation, or simply increased capacity for transacti-

vation and so forth. It is unlikely, however, that this potentiation can simply be ascribed to deletion of a putative regulatory domain residing in the carboxyl-terminal 30 amino acids, deletion of which region was shown to enhance DNA binding activity *in vitro* by means of electrophoretic mobility shift assay (EMSA).[14, 15] To our knowledge, the deletion of the domain was never shown to potentiate transactivation, apoptosis or any other biological function of p53. For further substantiation of our results, we created mutant p53 expression constructs by introducing a termination codon at amino acids 351 and 360 of the wild type p53. Transactivation potential of these mutants were measured by luciferase assay using the RGC (ribosomal gene cluster) promoter as a reporter, and they were shown to retain only 46% and 3.3% of the transactivation potential of the wild-type p53, respectively (unpublished observation).

Nevertheless, irrespective of the mechanism of potentiation, these chimeras may be valuable tools for gene therapeutic applications. It may also be possible to supply tailor-made gene therapeutic agents by combining other domains of p51 and p53 as well as p73, and these may be effective in treating tumors resulting from malfunction of the p53 pathway, such as p14^ARF mutations, MDM2 amplification and p53 mutation. They may also prove to be useful for functional analyses of p53 family proteins. Along this line, we are currently applying the technology described here for constructing various chimeric genes composed of *p53*, *p51* and *p73*, and the results will be presented elsewhere in the near future.

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