p73γ transactivates the p21 promoter through preferential interaction with the p300/CBP-associated factor in human prostate cancer cells

YASUTOMO MOMII[1], HIROTO IZUMI[1], MASAKI SHIOTA[1], TAKAMITSU ONITSUKA[1], TATSUYA ABE[2], HIDENORI KOBAYASHI[2], NAOYA MIYAMOTO[1], TAKESHI UCHIUMI[1] and KIMITOSHI KOHNO[1]

[1]Department of Molecular Biology, School of Medicine, University of Occupational and Environmental Health Japan, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555; [2]Department of Neurosurgery, Oita University Faculty of Medicine, 1-1 Idaigaoka Hasama-machi, Oita 879-5593, Japan

Received February 25, 2007; Accepted April 10, 2007

Abstract. Several p73 variants have been reported with different carboxy-terminal structures and transcriptional activities. We showed that p73γ had stronger transactivation activity than the other splicing variants such as α, ß and δ by analysing p21 promoter activity in human prostate cancer PC3 cells. The transactivation activity of p73γ was similar to that of p53 and was enhanced by co-transfection with p300/CBP-associated factor (PCAF). *In vitro* pull-down assay, p73 variants were able to bind to PCAF with a similar extent. However, *in vivo* co-immunoprecipitation assays showed that p73γ interacted preferentially with PCAF. Neither *in vitro*-translated nor *in vivo*-immunoprecipitated p73γ were able to bind to oligonucleotides containing the p53 consensus binding site. However, p73γ acetylated by PCAF restored DNA binding activity. Differential functions of p73 variants are supposed to be regulated by the structural differences of carboxy-terminal region. Our results revealed that p21 promoter activity was affected by differential interactions of p73 variants with PCAF and its acetylation.

Introduction

The tumour suppressor p53 functions as the master regulator of the genome (1) and is inactivated in human cancers (2). p53 regulates the response of cells to various stresses, including DNA damage, and its transcriptional activity controls gene expression related to cell-cycle progression and apoptosis (3,4). Such p53-mediated transcription can be enhanced through molecular interactions with the non-histone chromosomal protein high-mobility group B1 (HMGB1) (5). p53 has been reported to accumulate in cisplatin-resistant cells (6) and we previously demonstrated that it augments the binding of cisplatin-modified DNA induced by HMGB1 (7), therefore playing an important role in DNA damage signalling and recognition.

p73 has both functional and structural similarities to p53. Its overexpression has been reported to transactivate DNA repair genes and to induce cisplatin resistance (8). In addition, it has been suggested that p73 overexpression enhances cisplatin-induced apoptosis by reducing cyclin D1 and cyclin B1 expression in HeLa cells (9). Y-box binding protein 1 (YB-1) is overexpressed in cisplatin-resistant cells (10) where it binds to cisplatin-modified DNA and is thought to be involved in DNA repair (11). YB-1 gene expression is induced by p73 (12), which also interacts with HMGB1 (13).

To date, six p73 isoforms have been identified, with varying expression levels among different cell lines. The carboxy (C)-terminal region of the p73 variants produced by alternative splicing might modulate their functions, including transcriptional activities and protein-protein interactions (14). The transactivation abilities of these isoforms have been compared previously, and p73ß was shown to have the strongest activity in p53-null cell lines such as Saos-2 human osteosarcoma cells and Jurkat cells (15,16). p53 acetylation induces conformational changes that give the protein a higher affinity to target DNA (17). Both p300/CBP-associated factor (PCAF) and p300 are coactivators of p53-mediated transcription (18). PCAF has also been shown to coactivate p73-mediated transcription (19). Here we describe the functional activities of p73 isoforms in human cancer cells lacking p53 expression. Contrary to previous reports, our results demonstrate that p73γ shows the greatest transactivation activity and interacts with PCAF in human prostate cancer (PC3) cells.

Materials and methods

Cells and culture conditions. Saos-2 and H1299 human lung adenocarcinoma cells were grown in RPMI-1640 medium

Correspondence to: Dr Hiroto Izumi, Department of Molecular Biology, School of Medicine, University of Occupational and Environmental Health Japan, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan
E-mail: h-izumi@med.uoeh-u.ac.jp

Key words: acetylation, gene expression, p73, p300/CBP-associated factor

(Nissui Seiyaku, Tokyo, Japan). PC3 cells were grown in Eagle's minimal essential medium supplemented with 10% foetal bovine serum. All cell lines were maintained in a 5% CO_2 atmosphere at 37˚C.

Antibodies. The anti-haemagglutinin (anti-HA; clone F-7) monoclonal antibody and anti-HA-peroxidase (clone 3F10) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Roche Molecular Biochemicals (Mannheim, Germany), respectively. The anti-FLAG (clone M2) monoclonal antibody and anti-Flag M2-agarose affinity gel were purchased from Sigma Chemical Co. (St. Louis, MO).

Plasmid construction. The p21-Luc plasmid that was utilised for the luciferase assay has been described previously (20). The amino (N)-terminal haemagglutinin-tagged plasmids (pcDNA3-HA-p73α, pcDNA3-HA-p73ß, pcDNA3-HA-p73δ and pcDNA3-HA-p73γ) expressed in mammalian cells were kindly provided by Dr G. Melino (University of Rome, Italy) (15). To obtain Flag-PCAF, a Flag-tagged PCAF complementary DNA (cDNA) fragment was cloned into the pcDNA3 vector (Invitrogen, San Diego, CA). PCAF dn1 and PCAF dn2, which are mutants lacking histone acetyltransferase activity (Δ579-608 and Δ609-624, respectively), were constructed using the method described previously (21). To obtain the plasmids expressing GST-p73α, ß, γ and δ, cDNA fragments of p73α, ß, γ and δ were ligated in the pGEX4T vector (Amersham Biosciences, Piscataway, NJ), respectively. The plasmid expressing HA-PCAF in *E. coli* was obtained to ligate the HA-tagged PCAF fragment to the pThioHis vector (Invitrogen) in which thioredoxin had been deleted.

Luciferase assays. The transient transfection and luciferase assays were performed as described previously (22,23). Briefly, PC3, Saos-2 and H1299 cells were seeded in 12-well plates at a density of $5x10^4$ cells/well. The following day, the cells were transiently transfected with the p21-Luc plasmid and the expression plasmids described above. The total amount of DNA per well was adjusted to 1.5 μg by adding a mock DNA plasmid. After 72 h, the luciferase activity was measured according to the manufacturer's instructions (Promega, Madison, WI). The results were normalised according to the protein concentration and were representative of at least three independent experiments.

Immunoprecipitation and Western blot analysis. *In vivo* binding assays were performed as described previously (23). Briefly, PC3 cells were co-transfected with HA-fusion and Flag-fusion plasmids (1 μg of each) along with SuperFect according to the manufacturer's instructions (Qiagen, Hilden, Germany). After 48 h, the cells were lysed in buffer X [50 mM Tris/HCl (pH 8.0), 1 mM ethylenediaminetetraacetic acid (EDTA), 120 mM NaCl, 0.5% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulphonylfluoride (PMSF)]. The lysates were centrifuged at 21000 x g for 10 min at 4˚C, and the supernatants (300 μg) were incubated with anti-Flag (M2)-agarose affinity gel for 60 min at 4˚C. The immunoprecipitated and pre-immunoprecipitated samples (50 μg) were separated on a 10% (w/v) SDS gel and transferred to a polyvinylidene fluoride (PVDF) microporous membrane (Millipore, Bedford, MA). Western blot analysis was performed with anti-Flag (M2) and anti-HA antibodies.

In vitro-translation reactions and electrophoresis mobility-shift assay (EMSA). The *in vitro*-translated proteins were prepared as described previously (23). Oligonucleotides containing the p53 binding site were used as probes: 5'-GGC AGAACATGTCTAAGCATGCTGG-3' and 5'-GGCCAGCA TGCTTAGACATGTTCTG-3'. Preparation of the end-labelled oligonucleotides with [α-^{32}P]-dCTP (Amersham Biosciences) using the Klenow fragment (Fermentas, Vilnius, Lithuania) and purification were performed as described previously (24). EMSA of the *in vitro*-translated protein was performed as reported previously (23). For the competition experiments and supershift assay, preincubation was performed in the presence of 20-, 60- or 180-fold concentrations of unlabelled oligonucleotides for 5 min at room temperature (25˚C), or with 0.5 μg anti-HA antibody for 60 min at 4˚C.

In vitro GST pull-down assay. Preparation of GST fusion proteins and pull-down assay were described previously (12,23). Briefly, GST-p73 variants and HA-PCAF expressed in *E. coli* were induced by 1 mM isopropyl-1-thio-ß-D-galacto-pyranoside for 1 h at 30˚C. The cells were sonicated for 10 sec in binding buffer [50 mM Tris (pH 8.0), 1 mM EDTA, 120 mM NaCl, 10% glycerol, 0.5% Nonidet P-40, 1 mM dithiothreitol and 0.5 mM PMSF], and the soluble fraction was obtained by centrifugation at 21000 g for 10 min at 4˚C. For pull-down assay, GST fusion proteins were immobilized on glutathione-sepharose 4B beads, and mixed with the soluble fraction including HA-PCAF. The mixtures were incubated for 2 h at 4˚C with gentle inversion, and washed three times with binding buffer. Pull-down samples and 10% of the starting material were subjected to Western blot analysis with anti-HA antibody as described above.

In vitro acetylation and binding analysis. The *in vitro*-acetylation reactions were performed as described previously, with slight modifications (25,26). Briefly, samples immunoprecipitated with anti-Flag (M2)-agarose affinity gel (see above) were incubated with 2 μM acetyl-CoA in acetylation buffer [50 mM Tris (pH 8.0), 10% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM PMSF, 10 mM sodium butyrate] for 15 min at 30˚C. The beads were washed twice with buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 mM NaCl, 10% glycerol, 0.05% Nonidet P-40, 1 mM dithiothreitol and 0.1 mg/ml bovine serum albumin, and were mixed with ^{32}P-labelled oligonucleotides containing the p53 binding site. After incubation for 15 min at room temperature, the beads were washed three times, and then mixed with lysis buffer and phenol (10 μl of each). After vortexing and brief centrifugation, 10 μl supernatant was applied on a 20% polyacrylamide gel with 1 x TBE buffer. The gel was dried and evaluated using a bio-imaging analyser (FLA2000, Fuji Photo Film, Tokyo, Japan).

Results

p73 splicing variants transactivate the p21 promoter. The transactivation abilities of the p73 variants were examined using the

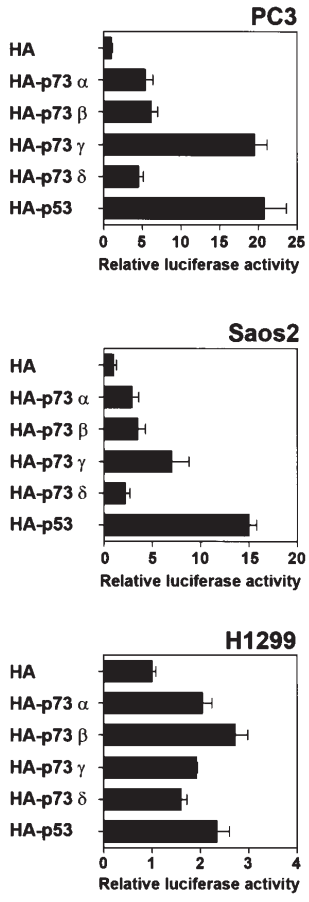

Figure 1. Induction of p21 gene-reporter activity by p53 and p73 variants. PC3, Saos-2 and H1299 cells were transiently co-transfected with the p21-Luc plasmid and the HA, HA-p73α, HA-p73ß, HA-p73δ, HA-p73γ or HA-p53 expression plasmids at a molecular ratio of 1:1. The maximum amount of plasmid per well was 1.5 μg. After 72 h, the luciferase activity was measured. All values represent the mean promoter activity ± the standard deviation (SD) of triplicate samples. The luciferase activity of the cell lysates transfected with the p21 promoter-luciferase construct and the HA expression plasmid was set to 1.

p21 promoter in three cancer cell lines: Saos-2, H1299 and PC3 (Fig. 1). Saos-2 cells are often used to analyse p53 target genes because they do not express functional p53. The H1299 and PC3 cell lines also showed no p53 expression. p53 transactivated the p21 promoter in the PC3 and Saos-2 cells, but not in the H1299 cells. Among the p73 variants, p73γ transactivated the p21 promoter activity in PC3 cells as efficiently as p53.

In vitro-translated p73γ showed no DNA binding activity. We next examined whether the transactivation abilities of the p73 variants were correlated with DNA binding activity. To this end, the *in vitro*-translated p73 variants were subjected to gel mobility-shift assays (Fig. 2). Unexpectedly, p73γ did not bind to the p53 binding consensus sequences. The DNA binding activity of p73δ was the strongest among the variants. The shifted band was confirmed by supershift assay with anti-HA antibody and competition experiment with cold competitor.

PCAF-acetylated p73γ binds to DNA containing the p53 binding site. The DNA binding activity of p53 is enhanced by acetylation (17,26). Furthermore, PCAF is known to be a coactivator of p73-mediated transactivation (19). To examine whether the interaction between PCAF and p73 differed among the variants, we carried out a glutathione *S*-transferase (GST) pull-down assay. The results did not reveal differential interactions between PCAF and the p73 variants *in vitro* (Fig. 3A). To confirm these findings, co-immunoprecipitation assays were performed. Unexpectedly, PCAF interacted preferentially with p73γ *in vivo* in PC3 cells (Fig. 3B). PCAF also interacted weakly with p73α, but not with p73ß or p73δ. In addition, PCAF functioned as a coactivator when both p53 and p73γ were co-transfected (Fig. 4A). The p73-mediated transactivation was repressed by co-transfection with the Δ579-608

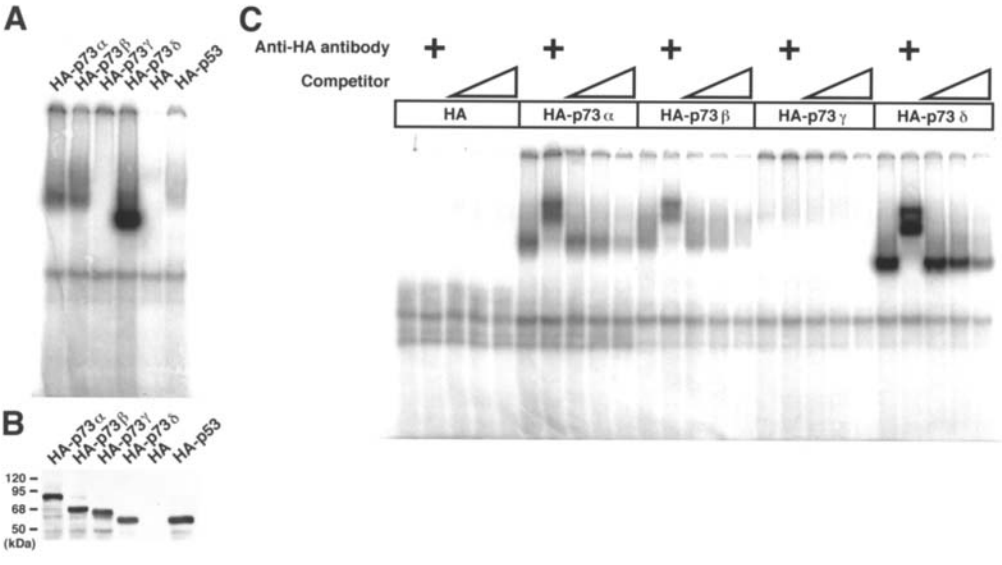

Figure 2. (A) Binding activity of p53 and p73 variants to oligonucleotides containing the p53 binding site. HA, HA-p73α, HA-p73ß, HA-p73δ, HA-p73γ and HA-p53 expression plasmids were translated *in vitro* using a coupled transcription and translation (TNT) system (Promega). A 2-μl sample of each protein was incubated with 4 ng ^{32}P-labelled oligonucleotides containing the p53 binding site for 15 min at room temperature. (B) Western blot analysis of the p73 variants used in (A). A 2-μl sample of each HA-fusion protein was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The transferred membrane was immunoblotted with anti-HA antibody. (C) The DNA binding activity of the p73 variants. The reaction mixtures were analysed as described in (A).

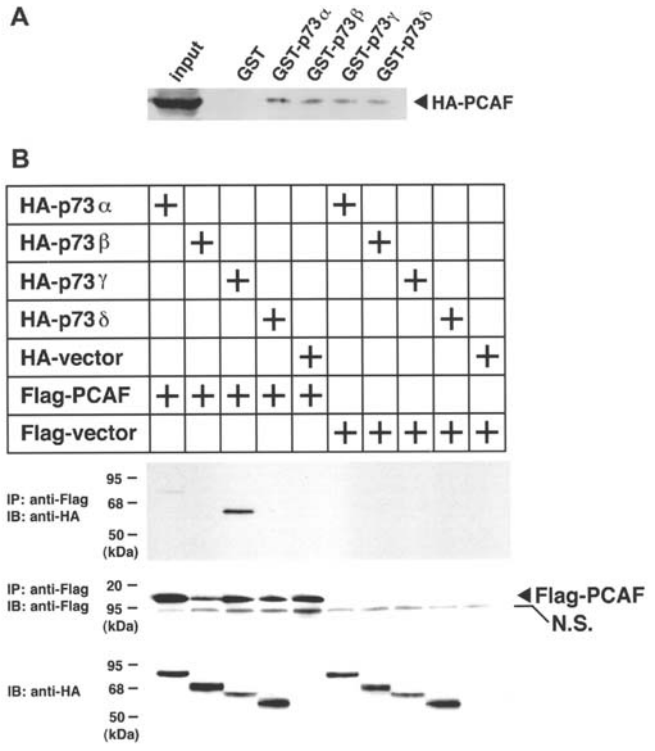

Figure 3. (A) *In vitro* interaction between PCAF and p73 variants. GST, GST-p73α, GST-p73ß, GST-p73γ and GST-p73δ immobilized on glutathione-sepharose 4B beads were incubated with HA-PCAF expressed in bacteria. Pull-down samples and 10% of the starting material were subjected to Western blot analysis with anti-HA antibody. (B) *In vivo* interaction between PCAF and p73 variants. PC3 cells were co-transfected with 1 μg HA, HA-p73α, HA-p73ß, HA-p73δ, HA-p73γ and HA-p53 expression plasmids with 1 μg Flag or Flag-PCAF expression plasmid. After immunoprecipitation with anti-Flag M2-agarose affinity gel, the samples were subjected to SDS-PAGE. The transferred membrane was immunoblotted with anti-HA antibody (upper panel). The same membrane was re-immunoblotted with anti-Flag antibody after stripping anti-HA antibody (middle panel). The cell lysates pre-immunoprecipitation were also subjected to SDS-PAGE. The transferred membrane was immunoblotted with anti-HA (lower panel).

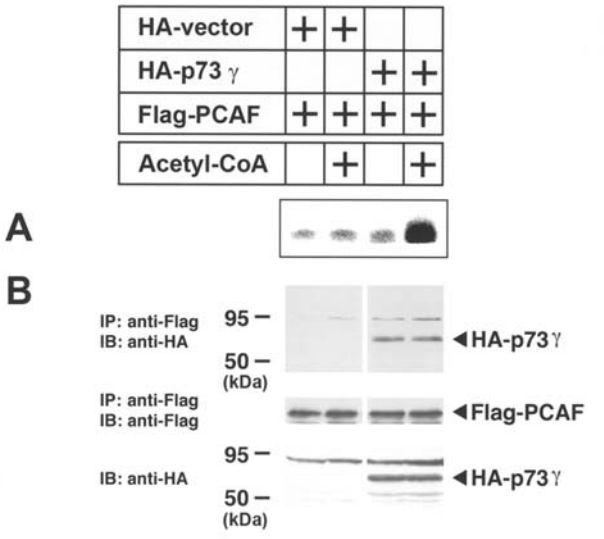

Figure 5. (A) p73γ acetylated by PCAF bound to oligonucleotides containing the p53 binding site. PC3 cells were co-transfected with Flag-PCAF and HA-p73γ or HA-p53 expression plasmids (Fig. 3). (B) Efficiency of expression and immunoprecipitation. The cell lysates used in (A) were analysed by Western blotting as described in Fig. 3. The middle and upper panels show the efficiency of the immunoprecipitated Flag-PCAF and co-immunoprecipitated HA-p73γ by Flag-PCAF, respectively. The lower panel shows the expression of HA-p73γ with pre-immunoprecipitated cell lysate.

and Δ609-624 PCAF mutants (Fig. 4C). Based on these results, we investigated whether acetylated p73γ could bind to DNA. DNA binding was observed when an immune complex containing PCAF and p73γ was incubated with acetyl-CoA (Fig. 5A). This finding implied that the acetylation of p73γ by PCAF was critical for its DNA binding activity.

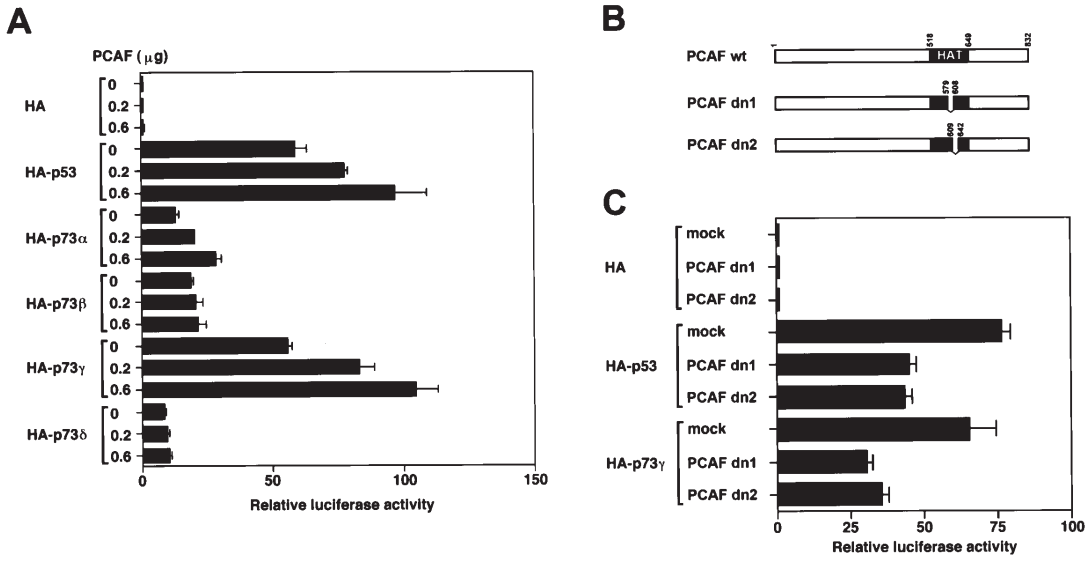

Figure 4. (A) Enhancement of p21 gene-reporter activity by PCAF. PC3 cells were transiently transfected with 0.6 μg p21-Luc plasmid, and 0.2 or 0.6 μg of HA, HA-p73α, HA-p73ß, HA-p73δ, HA-p73γ or HA-p53 expression plasmids. After 72 h the luciferase activity was measured. All values represent the mean promoter activity ± SD of triplicate samples. The luciferase activity of the cell lysates transfected with the p21 promoter-luciferase construct and HA expression plasmid was set to 1. (B) Schematic presentation of wild-type and PCAF mutants lacking histone acetyltransferase activity (Δ579-608 and Δ609-624). (C) The p21 gene-reporter activity induced by mutant PCAF lacking histone acetyltransferase activity. PC3 cells were transiently transfected with 0.5 μg p21-Luc plasmid and 0.5 μg HA-p73γ or HA-p53, along with 0.5 μg PCAF dn1 or PCAF dn2 expression plasmid. After 72 h the luciferase activity was measured as described above.

Discussion

p73 variants possess distinct C-terminal structures as a result of alternative splicing (14,15). Similar to p53, p73 is known to activate the transcription of p21, which plays a role in cell growth arrest. Indeed, the p73-mediated transactivation of the p21 promoter is correlated with cell growth arrest. Thus, the physiological significance of p73 variants is related to their transactivation abilities. However, until now, this activity has not been investigated in cancer cells.

In previous studies, p73ß appeared to show greater transactivation activity than the other variants (15,27). Both p73ß and p73δ are deletion forms of p73α, suggesting that most of the C-terminal region of p73α between amino acids 495 and 636 functions as an inhibitory domain for transactivation (28,29). In addition, p73δ (deletion form of p73ß) suggests that the region between amino acids 400 and 495 functions as a stimulatory domain for TAD (19). It has been proposed that structural differences in the C-terminal region might contribute to either the oligomerisation of, or the interaction with, other molecules that are involved in transcription.

In the present study, we investigated the functional differences between p73 variants in human cancer cell lines that lack p53 expression. Contrary to previous reports, we found that p73γ induces greater transactivation of the p21 promoter than the other variants in PC3 cells (Fig. 1). The 75 amino acids of the C-terminal region of p73γ are unique, due to a frame shift caused by alternative splicing, and contain many basic residues. We thus speculated that the C-terminal region of p73γ might support its DNA binding activity. To evaluate the DNA binding activity of the p73 variants, *in vitro*-translated forms were subjected to an EMSA. However, the results showed that p73γ did not bind to the p53 consensus sequence (Fig. 2). Among the variants, p73δ showed the strongest DNA binding activity, although its activation of the p21 promoter was the weakest observed. Thus, the transactivation abilities of the p73 variants were not correlated with their DNA binding activity.

To understand this discrepancy, we investigated the role of PCAF, which is a coactivator of p73-mediated transactivation. PCAF reportedly binds to p73α and stimulates transcription from the p21 promoter (19). We found that PCAF interacted equally well with the GST-fused p73 variants *in vitro* (Fig. 3A). We subsequently performed a co-immunoprecipitation assay to evaluate the interactions between PCAF and the p73 variants *in vivo*. The results showed that PCAF interacted preferentially with p73γ (Fig. 3B) and efficiently transactivated the p21 promoter (Fig. 4A). The acetylation of p53 can dramatically increase its DNA binding activity (17,26). In the current study, we observed enhanced DNA binding when an immunocomplex containing p73γ and PCAF was incubated with acetyl-CoA (Fig. 5). A latent DNA binding form of p53 revealed the intermolecular interactions between the DNA binding domain and the C-terminal tail domain. Acetylation resulted in neutralisation of the positive charges and disruption of the intermolecular interactions, thereby facilitating DNA binding (26). The C-terminal region of p53 is rich in basic amino acids, which is similar to p73γ but not the other variants. Thus, although the exact mechanism by which the acetylation of p73γ enhanced DNA binding was not clear, it might alter the intermolecular interactions between the unique C-terminal region and the other regions of the p73γ variant. PCAF is expressed in all tissues, but its precise levels might have a critical impact on transactivation by p73. In this study, the levels of PCAF expression were similar among the PC3, H1299 and Saos-2 cells (data not shown).

Our findings clearly revealed functional differences among p53 and p73 variants in the cell lines tested. These discrepancies might have occurred because the promoter with a p53 binding site was regulated by other molecules involved in p53/p73-mediated transcription, the expression of which varied among the cell lines.

In summary, our study indicated that p73γ is structurally and functionally similar to p53. Further investigations will be required to detail the functional regulation of p73 variants *in vivo*.

Acknowledgements

This study was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Mext), Kakenhi (17590257, 13218132 and 18590307) and a Grant-in-Aid for Cancer Research from the Fukuoka Cancer Society, Japan.

References

1. Royds JA and Iacopetta B: p53 and disease: when the guardian angel fails. Cell Death Differ 13: 1017-1026, 2006.
2. Gomez-Lazaro M, Fernandez-Gomez FJ and Jordan J: p53: twenty five years understanding the mechanism of genome protection. J Physiol Biochem 60: 287-307, 2004.
3. Harris SL and Levine AJ: The p53 pathway: positive and negative feedback loops. Oncogene 24: 2899-2908, 2005.
4. Schuler M and Green DR: Transcription, apoptosis and p53: catch-22. Trends Genet 21: 182-187, 2005.
5. Jayaraman L, Moorthy NC, Murthy KG, Manley JL, Bustin M and Prives C: High mobility group protein-1 (HMG-1) is a unique activator of p53. Genes Dev 12: 462-472, 1998.
6. Torigoe T, Izumi H, Ishiguchi H, Yoshida Y, Tanabe M, Yoshida T, Igarashi T, Niina I, Wakasugi T, Imaizumi T, Momii Y, Kuwano M and Kohno K: Cisplatin resistance and transcription factors. Curr Med Chem Anticancer Agents 5: 15-27, 2005.
7. Imamura T, Izumi H, Nagatani G, Ise T, Nomoto M, Iwamoto Y and Kohno K: Interaction with p53 enhances binding of cisplatin-modified DNA by high mobility group 1 protein. J Biol Chem 276: 7534-7540, 2001.
8. Vikhanskaya F, Marchini S, Marabese M, Galliera E and Broggini M: p73α overexpression is associated with resistance to treatment with DNA-damaging agents in a human ovarian cancer cell line. Cancer Res 61: 935-938, 2001.
9. Kim KC, Jung CS and Choi KH: Overexpression of p73 enhances cisplatin-induced apoptosis in HeLa cells. Arch Pharm Res 29: 152-158, 2006.
10. Ohga T, Koike K, Ono M, Makino Y, Itagaki Y, Tanimoto M, Kuwano M and Kohno K: Role of the human Y box-binding protein YB-1 in cellular sensitivity to the DNA-damaging agents cisplatin, mitomycin C, and ultraviolet light. Cancer Res 56: 4224-4228, 1996.
11. Ise T, Nagatani G, Imamura T, Kato K, Takano H, Nomoto M, Izumi H, Ohmori H, Okamoto T, Ohga T, Uchiumi T, Kuwano M and Kohno K: Transcription factor Y-box binding protein 1 binds preferentially to cisplatin-modified DNA and interacts with proliferating cell nuclear antigen. Cancer Res 59: 342-346, 1999.
12. Uramoto H, Izumi H, Ise T, Tada M, Uchiumi T, Kuwano M, Yasumoto K, Funa K and Kohno K: p73 Interacts with c-Myc to regulate Y-box-binding protein-1 expression. J Biol Chem 277: 31694-31702, 2002.

13. Stros M, Ozaki T, Bacikova A, Kageyama H and Nakagawara A: HMGB1 and HMGB2 cell-specifically down-regulate the p53- and p73-dependent sequence-specific transactivation from the human Bax gene promoter. J Biol Chem 277: 7157-7164, 2002.
14. Levrero M, De Laurenzi V, Costanzo A, Gong J, Wang JY and Melino G: The p53/p63/p73 family of transcription factors: overlapping and distinct functions. J Cell Sci 113: 1661-1670, 2000.
15. De Laurenzi V, Costanzo A, Barcaroli D, Terrinoni A, Falco M, Annicchiarico-Petruzzelli M, Levrero M and Melino G: Two new p73 splice variants, gamma and delta, with different transcriptional activity. J Exp Med 188: 1763-1768, 1998.
16. Ueda Y, Hijikata M, Takagi S, Chiba T and Shimotohno K: New p73 variants with altered C-terminal structures have varied transcriptional activities. Oncogene 18: 4993-4998, 1999.
17. Dornan D, Shimizu H, Perkins ND and Hupp TR: DNA-dependent acetylation of p53 by the transcription coactivator p300. J Biol Chem 278: 13431-13441, 2003.
18. Iyer NG, Ozdag H and Caldas C: p300/CBP and cancer. Oncogene 23: 4225-4231, 2004.
19. Zhao LY, Liu Y, Bertos NR, Yang XJ and Liao D: PCAF is a coactivator for p73-mediated transactivation. Oncogene 22: 8316-8329, 2003.
20. Okamoto T, Izumi H, Imamura T, Takano H, Ise T, Uchiumi T, Kuwano M and Kohno K: Direct interaction of p53 with the Y-box binding protein, YB-1: a mechanism for regulation of human gene expression. Oncogene 19: 6194-6202, 2000.
21. Yang XJ, Ogryzko VV, Nishikawa J, Howard BH and Nakatani Y: A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature 382: 319-324, 1996.
22. Torigoe T, Izumi H, Wakasugi T, Niina I, Igarashi T, Yoshida T, Shibuya I, Chijiiwa K, Matsuo K, Itoh H and Kohno K: DNA topoisomerase II poison TAS-103 transactivates GC-box-dependent transcription via acetylation of Sp1. J Biol Chem 280: 1179-1185, 2005.
23. Izumi H, Molander C, Penn LZ, Ishisaki A, Kohno K and Funa K: Mechanism for the transcriptional repression by c-Myc on PDGFß-receptor. J Cell Sci 114: 1533-1544, 2001.
24. Yoshida Y, Izumi H, Torigoe T, Ishiguchi H, Yoshida T, Itoh H and Kohno K: Binding of RNA to p53 regulates its oligomerization and DNA-binding activity. Oncogene 23: 4371-4379, 2004.
25. Izumi H, Ohta R, Nagatani G, Ise T, Nakayama Y, Nomoto M and Kohno K: p300/CBP-associated factor (P/CAF) interacts with nuclear respiratory factor-1 to regulate the UDP-N-acetyl-α-d-galactosamine: polypeptide N-acetylgalactosaminyltransferase-3 gene. Biochem J: 373: 713-722, 2003.
26. Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD and Berger SL: p53 sites acetylated *in vitro* by PCAF and p300 are acetylated *in vivo* in response to DNA damage. Mol Cell Biol 19: 1202-1209, 1999.
27. Takagi S, Ueda Y, Hijikata M and Shimotohno K: Overproduced p73α activates a minimal promoter through a mechanism independent of its transcriptional activity. FEBS Lett 509: 47-52, 2001.
28. Liu G and Chen X: The C-terminal sterile alpha motif and the extreme C terminus regulate the transcriptional activity of the α isoform of p73. J Biol Chem 280: 20111-20119, 2005.
29. Racek T, Mise N, Li Z, Stoll A and Putzer BM: C-terminal p73 isoforms repress transcriptional activity of the human telomerase reverse transcriptase (hTERT) promoter. J Biol Chem 280: 40402-40405, 2005.