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TAp63 γ enhances nucleotide excision repair through transcriptional regulation of DNA repair genes

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Abstract

p63 and p73, two p53 family members, play crucial roles in development and tumor suppression. p63 and p73 have multiple isoforms, which have similar or distinct biological functions. Transactivation (TA) isoforms of p63 and p73 have high similarity with p53 and often have biological functions similar to p53. p53 plays an important role in nucleotide excision repair (NER) through transcriptional regulation of target genes involved in NER, including DDB2, XPC and GADD45. To investigate whether TAp63 and TAp73 play a similar role in NER, Saos2 cells with inducible expression of specific isoforms of TAp63 and TAp73, including TAp63 $\alpha/\beta/\gamma$ and TAp73 $\alpha/\beta/\gamma$ isoforms, were employed. Overexpression of TAp63 γ significantly enhances NER of ultraviolet (UV)-induced DNA damage, including cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts, and enhances cell survival after UV irradiation in Saos2 cells. The enhancement of NER of UV-induced DNA damage by TAp63 γ was also confirmed in H1299 cells with overexpression of TAp63 γ . Consistently, knockdown of endogenous TAp63 decreases NER of UV-induced DNA damage in H1299 cells. TAp63 α/β and TAp73 $\alpha/\beta/\gamma$ isoforms do not have a clear effect on NER in Saos2 or H1299 cells. TAp63 γ overexpression clearly induces the expression of DDB2, XPC and GADD45 at both RNA and protein levels. Furthermore, luciferase reporter assays show that TAp63 γ transcriptionally activates DDB2, XPC and GADD45 genes through the regulation of the p53 binding elements in these genes. These results demonstrate that TAp63 γ enhances NER to remove UV-induced DNA damage and maintain genomic stability through transcriptional induction of a set of NER proteins, which provides an additional important mechanism that contributes to the function of TAp63 in tumor suppression.

Keywords

p63; Isoforms; Nucleotide excision repair; Ultraviolet

1. Introduction

p63 and p73 are two structural and functional homologs of the tumor suppressor p53. These three family members of transcription factor have a similar domain organization, which all contain a transactivation (TA) domain, a proline-rich (PR) domain, a DNA binding domain (DBD) and an oligomerization domain (OD) (Fig. 1A) [1]. p53, p63 and p73 share high similarity in DBD, which binds to similar or identical DNA sequences [2]. p63 and p73 can transcriptionally regulate a group of p53-target genes, which in turn exert some functions similar to p53, including cell cycle arrest, apoptosis, cellular senescence in response to stress.

p63 and p73 have multiple isoforms with diverse properties due to the presence of alternative promoters and alternative splicing at the carboxy-terminal end [3–7]. Each gene has two promoters: a promoter upstream of exon 1 which generates the TA isoforms, and an alternative promoter in intron 3 which leads to the expression of amino-terminally truncated (Δ N) isoforms that lack the TA domain. TAp63 and TAp73 are transcriptionally proficient, and often have biological functions similar to p53. Δ N isoforms are usually transcriptionally inactive, and often function as dominant negative inhibitors of TAp53, TAp63 and TAp73 through repressing transactivation of TA isoforms by competing for the binding elements in their target genes. Alternative splicing at the carboxy-terminal gives rise to three main isoforms of p63 and p73 (α , β , γ). Isoforms that differ at the carboxy-terminal end have different abilities to transactivate gene expression, which in turn have functions in common as well as functions unique to each of isoforms [8].

p63 and p73 have long been suggested to be involved in tumor suppression. However, their precise roles in tumor suppression are not well understood due to their structure complexity and the existence of multiple isoforms. TA isoforms have been suggested to function as tumor suppressors while Δ N isoforms function as oncogenes. It has been shown that p63 and p73 can induce cell cycle arrest, apoptosis and senescence in response to stress. A recent study reported that p63 and p73 also play a role in DNA repair [9]. DNA repair pathways, which include nucleotide excision repair (NER), base excision repair, double strand break (DSB) repair and mismatch repair, play a critical role in maintaining genomic integrity and thus tumor prevention. p63 and p73 have been shown to transcriptionally induce genes involved in homologous recombination repair for DSBs, including BRCA2 and Rad51. Mouse embryonic fibroblasts that are deficient for p63 and p73 have impaired repair capacity for DSBs [9].

NER is the major DNA repair system in mammalian cells, which removes many types of bulky DNA lesions induced by environmental carcinogens, such as UV irradiation, and chemotherapeutic agents. The primary DNA lesions induced by UV are cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4 PPs), both of which are repaired by NER. Mutations or defects in human NER proteins result in xeroderma pigmentosum (XP) syndrome. XP patients are highly sensitive to UV irradiation and have dramatically increased risk for UV-associated skin cancer. It has been known that p53 regulates NER [10]. The p53 deficient cells are deficient in NER of CPD. As a transcriptional factor, p53 regulates NER through transcriptional activation of genes involved in NER, including DDB2, XPC, and GADD45. The role of p63 and p73 in NER is not well understood. Considering the structural and functional similarity of p53 with TA isoforms of p63 and p73, TAp63 and TAp73 may also transcriptionally regulate these NER genes regulated by p53 and thus are involved in NER.

To test this possibility, the impact of TAp63 and TAp73 isoforms upon NER and the transcriptional regulation of p53-regulated DNA repair genes, including DDB2, XPC and GADD45, were determined in Saos2 cells with inducible specific TAp63 and TAp73 isoforms. Saos2 cells are deficient for p53, p63 and p73 [11]. Overexpression of TAp63 γ in cells clearly enhances NER of UV-induced DNA damage, whereas TAp63 α/β and TAp73 $\alpha/\beta/\gamma$ isoforms do not show clear effect on NER. TAp63 γ also enhances cell survival after UV irradiation. Furthermore, TAp63 γ displays a strong transcriptional induction of DDB2, XPC and GADD45 at both mRNA and protein levels, whereas TAp63 α/β and TAp73 $\alpha/\beta/\gamma$ isoforms show limited or no induction of these genes. Luciferase reporter assays demonstrate that TAp63 γ transcriptionally activates DDB2, XPC and GADD45 genes through the regulation of the p53 binding elements in these genes. Together, these results clearly demonstrate that TAp63 γ plays an important role in NER through transcriptional regulation of a group of genes that are involved in NER.

2. Materials and methods

2.1. Cell culture and UV irradiation

Saos2 cells with Tet-on inducible expression of TAp63 α , TAp63 β , TAp63 γ , TAp73 α , TAp73 β , and TAp73 γ (Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ) were generous gifts from Dr. Gerry Melino (University of Rome “Tor Vergata”, Italy). Cells were grown in a 1:1 mixture of Dulbecco’s Minimal Essential Medium/F12 supplemented with 10% fetal bovine serum in a 5% CO₂ humidified incubator. The expression of TAp73 and TAp63 isoforms was induced by doxy-cycline (Dox) (2 μ g/ml). The human lung epithelial H1299 cells (ATCC) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. For UV irradiation, cells grown at 50–70% confluence were irradiated with various doses of UV at a fluency rate of 1 J/m²/s using a GE15118 germicidal lamp (predominate emission, 254 nm).

2.2. Quantitation of the repair of CPD and 6–4 PP by ELISA assays

The removal of CPD and 6–4 PP from genomic DNA was measured by a cellular UV DNA-damage detection kit for CPD (Cyclex) and a OxiSelect cellular UV-induced DNA damage ELISA kit for 6–4 PP (Cell Biolabs), respectively, according to the manufacture’s instructions. In brief, cells cultured with or without Dox (2 μ g/ml) for 24 h were UV irradiated. The repair of CPD and 6–4 PP was determined at different time points after UV irradiation. Genomic DNA was denatured by the fixing/denaturing solution, and incubated with the blocking solution to avoid non-specific antibody binding. The samples were then incubated with anti-CPD and anti-6–4 PP antibody, respectively, followed with HRP-conjugated secondary antibody. The substrate solution was added and absorbance at 450 nm was measured using a microplate reader.

2.3. Host cell reactivation assays

Host cell reactivation assays were performed to determine the repair of UV-induced DNA damage as previously described [12]. pGL3 Firefly luciferase reporter plasmid was irradiated with various doses of UV. Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ cells cultured with or without Dox (2 μ g/ml) for 24 h were transfected with the UV-irradiated plasmid. Non-irradiated pRL-SV40 plasmid expressing Renilla luciferase was co-transfected as an internal control to normalize transfection efficiency. Transfection was performed by using FuGENE 6 transfection reagent (Roche). The UV-irradiated pGL3-luciferase reporter plasmid was also transfected into Saos2 and H1299 cells together with expression plasmids for TAp63 α , TAp63 β or TAp63 γ (pcDNA-TAp63 α , pcDNA-TAp63 β , or pcDNA-TAp63 γ), or siRNA against TAp63 or TAp73 (Ambion). Non-irradiated pRL-SV40 plasmid was co-transfected as an internal control. Luciferase activities were measured at 24 h after transfection by using the Dual-Luciferase Reporter Assay system (Promega). The relative luciferase activity (i.e. reactivation of the damaged plasmids by the host cells) from UV-treated pGL3-luciferase reporter plasmids was expressed as a percentage of luciferase activity from untreated pGL3-luciferase reporter plasmids and was used to represent the capacity of cells to repair UV-induced DNA damage.

2.4. Colony formation ability assays

Logarithmically growing cells were irradiated with different doses of UV (0–6 J/m²). Cells were trypsinized after UV irradiation, seeded (300–40,000 cells/dish) in culture dishes and cultured in medium with or without Dox (2 μ g/ml) for two weeks. Colonies were then fixed with methanol, stained with crystal violet, and counted. Colony formation ability was

calculated by dividing the plating efficiency of irradiated cells by that of non-irradiated control cells.

2.5. Quantitative Taqman real-time PCR

Total RNA was extracted from cells by using RNeasy kit (Qiagen). The cDNA was prepared with random primers by using Taqman reverse transcription kit (Applied Biosystems). Real-time PCR was performed in triplicate with TaqMan PCR Mix (Applied Biosystems) using the ABI Step One System (Applied Biosystems). All primers were purchased from Applied Biosystems. The expression of target genes was normalized to that of β -actin gene.

2.6. Western-blot assays

Standard Western-blot assays were used to analyze protein levels in cells as previously described [13]. Anti-p63 antibody (4A4), anti-p73 antibody (H-79), anti-DDB2 antibody (H-127) and anti-GADD45A antibody (C-4) were purchased from Santa Cruz Biotechnology. Anti-XPC antibody was purchased from Gene Tex, and Anti- β -actin (A5441) was purchased from Sigma-Aldrich.

2.7. Construction of reporter plasmids and dual-luciferase reporter assays

The TOPO II vector (Invitrogen) was used to clone PCR fragments containing the p53 binding elements in human DDB2 and XPC genes, respectively. The sequences of the primers used for DDB2 gene are as follows: 5'-GAAGGGGCGGGTCTCCG-3' and 5'-AGCCATCGCGTCCTCCGTGT-3'. The sequences of the primers for XPC gene are as follows: 5'-CGGGGTACCGGAAAAACACTCACTTAATGCCTACC-3' and 5'-CCGCTCGAGGTTGAACCCGGACTGCCTGACT-3'. The sequences of the primers for GADD45 gene are as follows: 5'-CGGGGTACCGCTGGGTTGCCTGATTGTGGAT-3' and 5'-CCGCTCGAGACGGGAGGCAGTGCAGATGTAGG-3'. The sequence-confirmed clones were subcloned into pGL2-Basic luciferase reporter plasmid (Promega). For luciferase reporter assays, the pGL2 reporter plasmids containing one copy of each p53 binding element were transfected into Saos2 and Saos2-TAp63 γ cells along with the pRL-SV40 plasmid expressing Renilla luciferase as an internal control to normalize transfection efficiency. Transfection was performed by using FuGENE 6 transfection reagent (Roche). After transfection, cells were cultured in medium with or without Dox (2 μ g/ml) for 24 h. Cells were then collected and luciferase activities were measured by using the Dual-Luciferase Reporter Assay system (Promega). The reporter activity was calculated as luciferase activity of reporter plasmids in cells with Dox treatment compared with that in cells without Dox treatment.

2.8. Statistical analysis

The data were expressed as the mean $\pm$ SD. Significance was calculated using the Student's *t*-test. Values of $p < 0.05$ were considered to be significant.

3. Results

3.1. TAp63 γ enhances the repair of UV-induced DNA damage

To investigate whether TAp63 and TAp73 isoforms regulate NER, Saos2-derived cell lines stably transfected with Tet-on inducible TAp63 isoforms α , β , γ or TAp73 isoforms α , β , γ (Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ) were employed. Saos2 cells are deficient for p53, p63 and p73 [11]. As shown in Fig. 1B, the protein levels of TAp63 and TAp73 isoforms were undetectable in cells without Dox treatment, and were clearly induced after Dox treatment (2 μ g/ml). The induction of TAp63 and TAp73 isoforms clearly induced the expression of their known

target genes, Perp (induced by p63) and p21 (induced by p73) (Fig. 1C). Among α , β and γ isoforms of TAp63 and TAp73, TAp63 γ and TAp73 γ showed the strongest transcriptional induction of their target genes, which is consistent with previous reports [1]. Saos2, Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ cells were irradiated with UV (20 J/m²), and the repair of CPD by NER in cells was determined by employing ELISA assays to specifically detect the CPD levels in genomic DNA. In control Saos2 cells, Dox treatment did not change the repair of CPD (data not shown). As shown in Fig. 2A, overexpression of TAp63 γ significantly increased the repair of CPD in Saos2 cells. At 6 h after UV irradiation, ~50% of CPDs were already removed in cells with TAp63 γ overexpression while less than 20% of CPDs were repaired in control Saos2-TAp63 γ cells without Dox treatment. Over the entire 24 h, significantly more CPDs were removed in cells with TAp63 γ overexpression ($p < 0.05$). However, overexpression of other isoforms of TAp63 or TAp73, including TAp63 α , TAp63 β , TAp73 α , TAp73 β , or TAp73 γ , did not clearly increase the removal of CPD in Saos2 cells.

The effect of TAp63 γ on the repair of 6–4 PP was also determined in Saos2 cells by employing ELISA assays to specifically detect the 6–4 PP levels in genomic DNA. As shown in Fig. 2B, overexpression of TAp63 γ significantly increased the repair of 6–4 PP compared with control Saos2-TAp63 γ cells without Dox treatment ($p < 0.05$), whereas overexpression of other isoform of TAp63 (e.g. TAp63 α) did not increase the repair of 6–4 PP in Saos2 cells.

To confirm that TAp63 γ is involved in NER, host cell reactivation assays were performed to determine the impact of TAp63 γ upon the repair of UV-induced DNA damage. Saos2-TAp63 γ cells cultured with or without Dox (2 μ g/ml) were transfected with pGL3-luciferase reporter plasmid irradiated with different doses of UV. The luciferase activity was determined at 24 h after transfection. Since the full-length transcript will not be produced unless UV-induced DNA damage is repaired from the plasmid, the luciferase activity reflects the repair of UV-induced DNA damage in the reporter plasmid. As shown in Fig. 3A, overexpression of TAp63 γ significantly increased the relative luciferase activities of the UV-damaged reporter plasmids ($p < 0.05$). Host cell reactivation assays were also determined in Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ cells. Overexpression of TAp63 α/β (Fig. 3A) or TAp73 $\alpha/\beta/\gamma$ (data not shown) did not increase the relative luciferase activities of the UV-damaged reporter plasmids. Host cell reactivation assays were also performed in Saos2 cells transiently transfected with the UV-irradiated pGL3 plasmid together with expression plasmids for TAp63 α , TAp63 β or TAp63 γ (pcDNA-TAp63 α , pcDNA-TAp63 β , or pcDNA-TAp63 γ). As shown in Fig. 3B, overexpression of TAp63 γ but not TAp63 α or TAp63 β significantly increased the relative luciferase activities of the UV-damaged pGL3 reporter plasmids. The effect of TAp63 γ on the repair of UV-induced DNA damage was also confirmed in H1299 cells. H1299 cells are p53-null cells and express low levels of endogenous p63 and p73. H1299 cells were transfected with UV-irradiated pGL3 reporter plasmids along with expression plasmids for TAp63 α , TAp63 β , or TAp63 γ . Over-expression of TAp63 γ but not TAp63 α or TAp63 β clearly increased the relative luciferase activities of the UV-damaged pGL3 reporter plasmids in H1299 cells ($p < 0.05$) (Fig. 3C). Furthermore, host cell reactivation assays were performed in H1299 cells with knockdown of endogenous TAp63 or TAp73. Due to structural similarity among isoforms of p63 and p73, no siRNA can specifically knockdown one isoform without affecting the expression of other isoforms. Here, siRNAs targeting TA domain of p63 or p73 were employed to knockdown TAp63, including TAp63 γ , or TAp73. As shown in Fig. 3D, knockdown of TAp63 but not TAp73 clearly decreased the relative luciferase activities of the UV-damaged pGL3 reporter plasmids in H1299 cells ($p < 0.05$). Similar results were obtained when two sets of siRNA targeting TA domain of p63 or p73 were used. These results are consistent with the results determining NER of CPD in genomic

DNA. Together, these results clearly demonstrate that TAp63 γ enhances NER of UV-induced DNA damage.

It has been shown that TAp63 γ protein accumulates in response to UV irradiation or actinomycin D treatment in mouse cells, which may be mediated by post-translational modification [14]. However, due to the existence of multiple isoforms of p63 in cells with close molecular weight and high structure similarity, the relative low levels of endogenous TAp63 γ protein, and the lack of antibody specific for TAp63 γ , it is difficult to determine the endogenous levels of TAp63 γ in cells. Therefore, the induction of TAp63 γ protein by UV irradiation or actinomycin D was demonstrated by the induction of the ectopic overexpressed TAp63 γ protein instead of the endogenous protein in that study [14]. Employing the same strategy, the accumulation of TAp63 γ protein in response to UV irradiation was also observed in human Saos2 cells. As shown in Fig. 3E, UV irradiation induced the accumulation of TAp63 γ protein in a time dependent manner in Saos2-TAp63 γ cells pretreated with Dox (50 ng/ml for 8 h); much higher TAp63 γ protein levels were observed in Saos2-TAp63 γ cells treated with Dox and UV together compared with cells treated with Dox only. Furthermore, consistent with the previous report [14], the UV-induced TAp63 γ protein accumulation was not due to the induction of mRNA of TAp63 γ by UV irradiation; similar mRNA levels of TAp63 γ were observed in Saos2-TAp63 γ cells treated with Dox and UV together and cells treated with Dox only (Data not shown). These results strongly suggest that TAp63 γ protein can be accumulated in response to UV, which in turn enhances NER of UV-induced DNA damage.

3.2. TAp63 γ increases cell survival after UV irradiation

In many cell types, the decrease in NER capacity sensitizes cells to cell death induced by DNA-damaging agents, including UV [15–17]. To determine whether the increase in NER capacity by TAp63 γ overexpression protects cells from UV-induced killing, colony formation ability assays were employed to determine cell survival in response to UV irradiation in Saos2 cells. Saos2, Saos2-TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ cells cultured with or without Dox were irradiated with various doses of UV, and their colony formation abilities were determined. As shown in Fig. 4, overexpression of TAp63 γ clearly increased cell survival after UV irradiation ($p < 0.05$). The overexpression of TAp63 α , TAp63 β , TAp73 α , TAp73 β or TAp73 γ did not increase cell survival after UV irradiation. Furthermore, Dox treatment did not increase cell survival after UV irradiation in control Saos2 cells (data not shown). These results strongly suggest that enhancement of NER by TAp63 γ protects cells from DNA damage (UV)-induced cell death.

3.3. Differential induction of genes involved in NER by TAp63 and TAp73

It has been reported that p53 regulates NER through transcriptional regulation of a group of genes that are involved in NER, including DDB2, XPC and GADD45 [10,18,19]. DDB2 is a subunit of the UV-damaged DNA-binding protein complex. XPC is one of the NER factors. Both DDB2 and XPC proteins localize to sites of UV-induced DNA lesions, including CPD, and are involved in the recognition of DNA damage [18,20,21]. GADD45 has been shown to bind to UV-damaged chromatin, which enhances the accessibility of the DNA damage sites for repair proteins [22,23]. It has been reported that TAp63 and TAp73 can bind to similar or identical p53-consensus binding elements and transcriptionally regulate a group of p53 target genes. TAp63 and TAp73 have multiple isoforms due to alternative splicing at the carboxy-terminal end, which could have different transcriptional activities and biological functions. It is therefore possible that TAp63 γ may transcriptionally regulate these p53 target genes, which in turn regulate NER. To test this possibility, the transcriptional regulation of DDB2, XPC and GADD45 by TAp63 and TAp73 was determined in Saos2-

TAp63 α , Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 α , Saos2-TAp73 β , and Saos2-TAp73 γ cells with or without Dox treatment. As shown in Fig. 5A, overexpression of TAp63 γ clearly increased DDB2, XPC and GADD45 protein levels, overexpression of TAp63 β , TAp73 β and TAp73 γ increased GADD45 protein levels, and overexpression of TAp63 α and TAp73 α did not have clear effect on the expression levels of any of these proteins. To test whether the regulation of DDB2, XPC and GADD45 protein levels by TAp63 and TAp73 isoforms is through transcriptional regulation of their mRNA levels, DDB2, XPC and GADD45 mRNA levels were determined in above-mentioned cells by Taqman real-time PCR assays. As shown in Fig. 5B, the mRNA levels of DDB2 and XPC were clearly induced by overexpression of TAp63 γ , whereas the mRNA levels of GADD45 were induced by overexpression of TAp63 β , TAp63 γ , TAp73 β and TAp73 γ . These results are consistent with the expression changes observed at the protein levels. These results demonstrate that the induction of DDB2, XPC and GADD45 by TAp63 and TAp73 isoforms is through transcriptional induction of these genes at mRNA levels.

The induction of DDB2, XPC and GADD45 by TAp63 γ was also observed in H1299 cells. H1299 cells were transiently transfected with TAp63 γ expression plasmids (pcDNA-TAp63 γ), and the mRNA levels of DDB2, XPC and GADD45 were determined by Taqman real-time PCR assays. As shown in Fig. 5C, overexpression of TAp63 γ clearly increased the mRNA levels of DDB2, XPC and GADD45 in H1299 cells.

These results demonstrate that TAp63 and TAp73 isoforms differentially regulate the transcription of DDB2, XPC and GADD45 genes. Among all these isoforms, TAp63 γ isoform shows the clearest induction of all these genes at both RNA and protein levels, which could be an important mechanism by which TAp63 γ regulates NER.

3.4. TAp63 γ transcriptionally activates the p53-consensus DNA binding elements in human DDB2, XPC and GADD45 genes

TAp63 and TAp73 have been shown to be able to recognize and bind to p53-consensus DNA binding elements, which in turn transcriptionally regulate the expression of a group of p53-target genes. It has been shown that TAp63 and TAp73 DNA binding domains bind to the p53 binding element in human GADD45 gene [24]. To test whether TAp63 β and TAp63 γ recognize and transactivate the previously described p53 binding elements in human DDB2, XPC and GADD45 genes, DNA fragments containing one copy of the p53-consensus binding elements in DDB2, XPC and GADD45 genes, respectively, were cloned into the promoter region of a pGL2 fire-fly luciferase reporter plasmid and luciferase reporter assays were performed (Fig. 6A). Saos2 and Saos2-TAp63 γ cells cultured with or without Dox were co-transfected with the luciferase reporter plasmid and a pRL-SV40 plasmid expressing Renilla luciferase as an internal control. As shown in Fig. 6B, the expression of TAp63 γ induced by Dox treatment in Saos2-TAp63 γ cells significantly increased the luciferase activity in the reporter plasmids containing the p53 binding elements of DDB2, XPC and GADD45 genes compared with that in Saos2-TAp63 γ cells without Dox treatment. In control Saos2 cells, which do not contain Tet-on TAp63 γ inducible expression plasmid, Dox treatment did not induce the luciferase activities. These results demonstrate that TAp63 γ transactivates the p53 binding elements in human DDB2, XPC and GADD45 genes, and thus induces the expression of these three genes.

4. Discussion

The p53 family members consist of transcriptional factors p53, p63 and p73 [1]. These proteins have similar structures and some similar biological functions. In addition, they each have distinct biological functions. p53 plays a critical role in tumor suppression; p53 is the most frequently mutated gene in human cancers. Loss of p53 functions commonly leads to

the development of cancers. p63 and p73 play important roles in development. p63 regulates the development of epithelial cell layers and p73 plays an important role in neuronal development and immune response [25,26].

p63 and p73 have also been suggested to play important roles in tumor prevention. However, the precise roles of p63 and p73 in tumor prevention are not well-understood. It has been reported that p63^{+/-} and p73^{+/-} mice are predisposed to spontaneous tumors [27]. p63 and p73 also cooperate with p53 in tumor suppression. p53^{+/-} p63^{+/-} and p53^{+/-} p73^{+/-} mice have higher tumor burden and increased metastasis compared with p53^{+/-} mice [27]. However, due to the existence of the complex p63 and p73 isoforms, some of which have opposite functions, the role of individual isoforms in tumorigenesis has not been clearly addressed. While TAp63 and TAp73 bear structural and functional similarity to p53, the Δ Np63 and Δ Np73 act primarily in dominant-negative fashion against p53, p63 and p73. While the mutations of p63 and p73 genes are rare in tumors, the altered expression levels of different p63 and p73 isoforms are often observed in tumors. Overexpression of Δ Np63/ Δ Np73 and decrease of TAp63/TAp73 have been observed in a number of human tumors [28–30], which are often associated with poor prognosis and chemotherapeutic response in patients [31–34]. The roles of TAp63 and TAp73 iso-forms in tumor prevention have been further demonstrated in TAp63 and TAp73 knockout mice [35,36]. Loss of TAp63 in mice increased genomic instability. TAp63 deficient mice develop primary and metastatic tumors, including carcinomas and sarcomas, and have a shorter life span. TAp73 deficient mice display increased risk of spontaneous and carcinogen-induced tumorigenesis. While these studies with mouse models demonstrate the role of TAp63 and TAp73 in tumor suppression, the precise roles of individual TA isoforms in tumor suppression have not been distinguished yet.

Results presented in this study demonstrate that TAp63, particularly TAp63 γ , plays an important role in NER to maintain genomic stability and prevent tumor formation. The NER pathway is one of the most important repair mechanisms that remove a wide spectrum of different bulky DNA lesions caused by UV and chemical carcinogens. It has been well-documented that in human cells, p53 promotes the NER of CPD. p53 transcriptionally induces DDB2, XPC and GADD45 genes, which all play important roles in NER. In this study, Saos2 cells with inducible expression of specific isoforms of TAp63 and TAp73 were employed to determine their impacts upon NER and the underlying mechanisms. TAp63 γ overexpression greatly increases the removal of CPD and 6–4 PP as determined by both ELISA assays which directly measure the levels of DNA damage in cells and host cell reactivation assays, resulting in the increased cell survival after UV damage. Other isoforms of TAp63 (α , β) and TAp73 (α , β , γ) do not exhibit clear effect on NER. Our finding is consistent with a recent report by Ferguson-Yates et al. showing that knockdown of p63 but not p73 by siRNA impaired the repair of CPD in human keratinocytes lacking p53, which suggests that p63 is involved in NER [37]. However, in that study, siRNA against all p63 isoforms was employed, which cannot distinguish which p63 isoform(s) contributes to the repair of CPD and 6–4 PP. In this study, using the cell lines with expression of specific isoforms of TAp63 and TAp73, TAp63 γ has been identified as the major isoform that regulates the repair of UV-induced DNA damage. Furthermore, knockdown of endogenous TAp63 by siRNA against TAp63 clearly decreased NER of UV-induced DNA damage in H1299 cells, which strongly suggests that TAp63 γ at the natural occurring level enhances NER of UV-induced DNA damage. The decrease of TAp63, including TAp63 γ has been frequently observed in human cancers. Our findings suggest that the decrease of TAp63 γ levels in tumors may impair NER which could contribute to the tumor formation.

It has been reported that among all p63 isoforms, TAp63 γ has the strongest transactivation activity toward a group of p53 target genes [38]. Indeed, overexpression of TAp63 γ highly

induces DDB2, XPC and GADD45 at both mRNA and protein levels. Overexpression of TAp63 β , TAp73 β and TAp73 γ only induce GADD45 but not DDB2 and XPC at protein levels. These results demonstrate the selective transcriptional regulation of target genes by different isoforms of TAp63 and TAp73. While DDB2, XPC and GADD45 proteins are all involved in NER, the transcriptional induction of GADD45 protein alone may not be sufficient to lead to the increased NER as observed in cells with overexpression of TAp63 β , TAp73 β and TAp73 γ . It has been shown that p63 and p73 can regulate many unique target genes in addition to some p53-target genes. It is possible that TAp63 γ transcriptionally regulates additional genes involved in NER and/or other cellular repair pathways. In addition to the transcriptional regulation of genes involved in NER, the p53 protein itself has been implicated in NER through interaction with several proteins involved in NER, including TFIIH, the helicases XPB and XPD [39]. Considering the structural similarity between p53 and TAp63 γ , it will be interesting to test whether TAp63 γ also interact with any of these proteins involved in NER, which may contribute to the role of TAp63 γ in NER. Future studies on the transcriptional program and protein interactions of TAp63 γ will help us to further understand the mechanisms by which TAp63 γ regulates NER and other repair pathways.

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Abbreviations

TA	transactivation
NER	nucleotide excision repair
UV	ultraviolet
CPD	cyclobutane pyrimidine dimer
PR	prolin-rich
DBD	DNA binding domain
OD	oligomerization domain
ΔN	amino-terminal truncated
DBS	double strand break
6–4 PP	6–4 photoproduct
XP	xeroderma pigmentosum
Dox	doxycycline

References

1. Dotsch V, Bernassola F, Coutandin D, Candi E, Melino G. p63 and p73, the ancestors of p53. *Cold Spring Harb Perspect Biol.* 2010; 2:a004887. [PubMed: 20484388]
2. Yang A, Kaghad M, Caput D, McKeon F. On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet.* 2002; 18:90–95. [PubMed: 11818141]

3. De Laurenzi V, Costanzo A, Barcaroli D, Terrinoni A, Falco M, Annicchiarico-Petruzzelli M, Levrero M, Melino G. Two new p73 splice variants, gamma and delta, with different transcriptional activity. *J Exp Med*. 1998; 188:1763–1768. [PubMed: 9802988]
4. De Laurenzi VD, Catani MV, Terrinoni A, Corazzari M, Melino G, Costanzo A, Levrero M, Knight RA. Additional complexity in p73: induction by mitogens in lymphoid cells and identification of two new splicing variants epsilon and zeta. *Cell Death Differ*. 1999; 6:389–390. [PubMed: 10381648]
5. Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP, Saville MK, Lane DP. p53 isoforms can regulate p53 transcriptional activity. *Genes Dev*. 2005; 19:2122–2137. [PubMed: 16131611]
6. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsch V, Andrews NC, Caput D, McKeon F. p63, a p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol Cell*. 1998; 2:305–316. [PubMed: 9774969]
7. Moll UM, Slade N. p63 and p73: roles in development and tumor formation. *Mol Cancer Res*. 2004; 2:371–386. [PubMed: 15280445]
8. Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ*. 2006; 13:962–972. [PubMed: 16601753]
9. Lin YL, Sengupta S, Gurdziel K, Bell GW, Jacks T, Flores ER. p63 and p73 transcriptionally regulate genes involved in DNA repair. *PLoS Genet*. 2009; 5:e1000680. [PubMed: 19816568]
10. Adimoolam S, Ford JM. p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA Repair (Amst)*. 2003; 2:947–954. [PubMed: 12967652]
11. Terrinoni A, Ranalli M, Cadot B, Leta A, Bagetta G, Vousden KH, Melino G. p73-alpha is capable of inducing scotin and ER stress. *Oncogene*. 2004; 23:3721–3725. [PubMed: 15116103]
12. Hu W, Feng Z, Tang MS. Chromium(VI) enhances (+/-)-anti-7beta, 8alpha-dihydroxy-9alpha, 10alpha-epoxy-7,8,9,10-tetrahydro benzo[a]pyrene-induced cytotoxicity and mutagenicity in mammalian cells through its inhibitory effect on nucleotide excision repair. *Biochemistry*. 2004; 43:14282–14289. [PubMed: 15518579]
13. Hu W, Feng Z, Ma L, Wagner J, Rice JJ, Stolovitzky G, Levine AJ. A single nucleotide polymorphism in the MDM2 gene disrupts the oscillation of p53 and MDM2 levels in cells. *Cancer Res*. 2007; 67:2757–2765. [PubMed: 17363597]
14. Katoh I, Aisaki KI, Kurata SI, Ikawa S, Ikawa Y. p51A (TAp63gamma), a p53 homolog, accumulates in response to DNA damage for cell regulation. *Oncogene*. 2000; 19:3126–3130. [PubMed: 10871867]
15. Cistulli CA, Kaufmann WK. p53-dependent signaling sustains DNA replication and enhances clonogenic survival in 254 nm ultraviolet-irradiated human fibroblasts. *Cancer Res*. 1998; 58:1993–2002. [PubMed: 9581844]
16. Ford JM, Baron EL, Hanawalt PC. Human fibroblasts expressing the human papillomavirus E6 gene are deficient in global genomic nucleotide excision repair and sensitive to ultraviolet irradiation. *Cancer Res*. 1998; 58:599–603. [PubMed: 9485006]
17. Smith ML, Kontny HU, Zhan Q, Sreenath A, O'Connor PM, Fornace AJ Jr. Antisense, GADD45 expression results in decreased DNA repair and sensitizes cells to U.V.-irradiation or cisplatin. *Oncogene*. 1996; 13:2255–2263. [PubMed: 8950993]
18. Hwang BJ, Ford JM, Hanawalt PC, Chu G. Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc Natl Acad Sci USA*. 1999; 96:424–428. [PubMed: 9892649]
19. Fitch ME, Cross IV, Turner SJ, Adimoolam S, Lin CX, Williams KG, Ford JM. The DDB2 nucleotide excision repair gene product p48 enhances global genomic repair in p53 deficient human fibroblasts. *DNA Repair (Amst)*. 2003; 2:819–826. [PubMed: 12826282]
20. Venema J, van Hoffen A, Karcagi V, Natarajan AT, van Zeeland AA, Mullenders LH. Xeroderma pigmentosum complementation group C cells remove pyrimidine dimers selectively from the transcribed strand of active genes. *Mol Cell Biol*. 1991; 11:4128–4134. [PubMed: 1649389]

21. Fitch ME, Cross IV, Ford JM. p53 responsive nucleotide excision repair gene products p48 and XPC, but not p53, localize to sites of UV-irradiation-induced DNA damage, in vivo. *Carcinogenesis*. 2003; 24:843–850. [PubMed: 12771027]
22. Smith ML, Ford JM, Hollander MC, Bortnick RA, Amundson SA, Seo YR, Deng CX, Hanawalt PC, Fornace AJ Jr. p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol Cell Biol*. 2000; 20:3705–3714. [PubMed: 10779360]
23. Carrier F, Georgel PT, Pourquier P, Blake M, Kontny HU, Antinore MJ, Gariboldi M, Myers TG, Weinstein JN, Pommier Y, Fornace AJ Jr. Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. *Mol Cell Biol*. 1999; 19:1673–1685. [PubMed: 10022855]
24. Patel S, Bui TT, Drake AF, Fraternali F, Nikolova PV. The p73 DNA binding domain displays enhanced stability relative to its homologue, the tumor suppressor p53, and exhibits cooperative DNA binding. *Biochemistry*. 2008; 47:3235–3244. [PubMed: 18260640]
25. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature*. 1999; 398:708–713. [PubMed: 10227293]
26. Yang A, Walker N, Bronson R, Kaghad M, Oosterwegel M, Bonnin J, Vagner C, Bonnet H, Dikkes P, Sharpe A, McKeon F, Caput D. p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature*. 2000; 404:99–103. [PubMed: 10716451]
27. Flores ER, Sengupta S, Miller JB, Newman JJ, Bronson R, Crowley D, Yang A, McKeon F, Jacks T. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. *Cancer Cell*. 2005; 7:363–373. [PubMed: 15837625]
28. Park BJ, Lee SJ, Kim JI, Lee CH, Chang SG, Park JH, Chi SG. Frequent alteration of p63 expression in human primary bladder carcinomas. *Cancer Res*. 2000; 60:3370–3374. [PubMed: 10910040]
29. Nylander K, Coates PJ, Hall PA. Characterization of the expression pattern of p63 alpha and delta Np63 alpha in benign and malignant oral epithelial lesions. *Int J Cancer*. 2000; 87:368–372. [PubMed: 10897041]
30. Crook T, Nicholls JM, Brooks L, O’Nions J, Allday MJ. High level expression of deltaN-p63: a mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma (NPC)? *Oncogene*. 2000; 19:3439–3444. [PubMed: 10918601]
31. Casciano I, Mazzocco K, Boni L, Pagnan G, Banelli B, Allemanni G, Ponzoni M, Tonini GP, Romani M. Expression of DeltaNp73 is a molecular marker for adverse outcome in neuroblastoma patients. *Cell Death Differ*. 2002; 9:246–251. [PubMed: 11859407]
32. Concin N, Becker K, Slade N, Erster S, Mueller-Holzner E, Ulmer H, Daxenbichler G, Zeimet A, Zeillinger R, Marth C, Moll UM. Transdominant DeltaTAp73 isoforms are frequently up-regulated in ovarian cancer. Evidence for their role as epigenetic p53 inhibitors in vivo. *Cancer Res*. 2004; 64:2449–2460. [PubMed: 15059898]
33. Puig P, Capodiceci P, Drobnjak M, Verbel D, Prives C, Cordon-Cardo C, Di Como CJ. p73 Expression in human normal and tumor tissues: loss of p73alpha expression is associated with tumor progression in bladder cancer. *Clin Cancer Res*. 2003; 9:5642–5651. [PubMed: 14654547]
34. Urist MJ, Di Como CJ, Lu ML, Charytonowicz E, Verbel D, Crum CP, Ince TA, McKeon FD, Cordon-Cardo C. Loss of p63 expression is associated with tumor progression in bladder cancer. *Am J Pathol*. 2002; 161:1199–1206. [PubMed: 12368193]
35. Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, Leung ML, El-Naggar A, Creighton CJ, Suraokar MB, Wistuba I, Flores ER. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature*. 2010; 467:986–990. [PubMed: 20962848]
36. Tomasini R, Tsuchihara K, Wilhelm M, Fujitani M, Rufini A, Cheung CC, Khan F, Itie-Youten A, Wakeham A, Tsao MS, Iovanna JL, Squire J, Jurisica I, Kaplan D, Melino G, Jurisicova A, Mak TW. TAp73 knockout shows genomic instability with infertility and tumor suppressor functions. *Genes Dev*. 2008; 22:2677–2691. [PubMed: 18805989]
37. Ferguson-Yates BE, Li H, Dong TK, Hsiao JL, Oh DH. Impaired repair of cyclobutane pyrimidine dimers in human keratinocytes deficient in p53 and p63. *Carcinogenesis*. 2008; 29:70–75. [PubMed: 17984111]

38. Petitjean A, Ruptier C, Tribollet V, Hautefeuille A, Chardon F, Cavard C, Puisieux A, Hainaut P, Caron de Fromental C. Properties of the six isoforms of p63: p53-like regulation in response to genotoxic stress and cross talk with DeltaNp73. *Carcinogenesis*. 2008; 29:273–281. [PubMed: 18048390]
39. Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, Wang Z, Freidberg EC, Evans MK, Taffe BG, et al. p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nat Genet*. 1995; 10:188–195. [PubMed: 7663514]

Fig. 1.

The expression of α , β and γ isoforms of TAp63 and TAp73 proteins in Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells. (A) Schematic structure of α , β and γ isoforms of TAp63 and TAp73 proteins. TA: transactivation domain; PR: prolin-rich domain, DBD: DNA binding domain; OD: oligomerization domain. (B) The expression of α , β , and γ isoforms of TAp63 and TAp73 proteins in Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells treated with Dox. Saos2 cells, which are deficient for p53, p63 and p73, were stably transfected with Tet-on expression vectors for TAp63 α , TAp63 β , TAp63 γ , TAp73 α , TAp73 β , or TAp73 γ (Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells). These 6 cell lines were treated Dox (2 μ g/ml) for 18 or 24 h before Western-blot assays. (C) The regulation of Perp and p21 by α , β and γ isoforms of TAp63 and TAp73, respectively, in Saos2 cells. Perp and p21 are known target genes for TAp63 and TAp73, respectively. Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells were treated with or without Dox (2 μ g/ml) for 18 or 24 h. mRNA levels of Perp and p21 were determined by Taqman real-time PCR assays and normalized with the levels of β -actin in cells. Data are represented as mean $\pm$ SD ($n = 3$).

Fig. 2.

TAp63 γ enhances the repair of CPD and 6-4 PP in Saos2 cells. Saos2-TAp63 α / β / γ and Saos2-TAp73 α / β / γ cells were treated with Dox (2 μ g/ml) for 24 h to induce the expression of α , β , or γ isoforms of TAp63 and TAp73 proteins. Cells without Dox treatment were used as controls. Cells were then irradiated with UV (20 J/m²), and collected immediately (0 h) or cultured in fresh medium with Dox (2 μ g/ml) for different hours (4, 6, 9, or 24 h) before assays. (A) TAp63 γ enhances the repair of CPD in cells. The levels of CPD in genomic DNA of cells were analyzed by a cellular CPD DNA-damage detection ELISA kit. (B) TAp63 γ enhances the repair of 6-4 PP in cells. The levels of 6-4 PP in genomic DNA of cells were analyzed by a cellular 6-4 PP DNA-damage detection ELISA kit. Data are represented as mean $\pm$ SD ($n = 3$). * $p < 0.05$.

Fig. 3.

TAp63 γ promotes host cell reactivation of UV-irradiated luciferase reporter plasmids in cells. (A) TAp63 γ promotes host cell reactivation of luciferase reporter plasmids damaged by UV irradiation in Saos2-TAp63 γ cells. The pGL3 luciferase reporter plasmids irradiated with different doses of UV (300 and 1000 J/m²) were transfected into Saos2-TAp63 $\alpha/\beta/\gamma$ cells which were treated with Dox (2 μ g/ml) for 24 h before transfection. Non-irradiated pRL-SV40 plasmid was co-transfected as an internal control to normalize the transfection efficiency. After transfection, cells were cultured in fresh medium with Dox (2 μ g/ml) for 24 h and luciferase activities were then measured. (B and C) TAp63 γ promotes host cell reactivation of luciferase reporter plasmids damaged by UV in Saos2 (B) and H1299 (C)

cells. The pGL3 luciferase reporter plasmids irradiated with UV (1000 J/m²) were transfected into Saos2 cells (B) and H1299 (C), respectively, together with pcDNA-TAp63 α , pcDNA-TAp63 β , or pcDNA-TAp63 γ expression plasmids. The pRL-SV40 plasmid was co-transfected as an internal control. (D) Knockdown of endogenous TAp63 decreases host cell reactivation of luciferase reporter plasmids damaged by UV in H1299 cells. H1299 cells were transfected with siRNA against TAp63 or TAp73. Twenty-four hours after transfection, the pGL3 luciferase reporter plasmids irradiated with UV (1000 J/m²) were then transfected into cells. Relative mRNA expression levels of TAp63 and TAp73 were determined by real-time PCR assays and normalized with β -actin. The pRL-SV40 plasmid was co-transfected as an internal control. The luciferase activities were measured at 24 h after transfection. The relative luciferase activity, which represents the relative extent of NER repair of UV-induced DNA damage, was determined as the percentage of luciferase activity expressed from UV-irradiated plasmids to that from untreated plasmids. The data are represented as mean $\pm$ SD ($n = 3$). * $p < 0.05$. (E) UV induces the TAp63 γ protein accumulation in cells. Saos2-TAp63 γ cells were pretreated with Dox (50 ng/ml) for 8 h before they were exposed to UV (20 J/m²). The cells were collected at different time points after UV irradiation (0–24 h), and the TAp63 γ protein levels were determined by Western-blot assays.

Fig. 4. TAp63γ increases cell survival after UV irradiation. Saos2-TAp63α, Saos2-TAp63β, Saos2-TAp63γ, Saos2-TAp73α, Saos2-TAp73β, Saos2-TAp73γ cells with (closed triangle) or without (closed circle) Dox treatment (2 μg/ml) were irradiated with different doses of UV (2, 4, and 6 J/m²), and their colony formation abilities were measured. The survival (%) was calculated as a percentage of colony formation ability of UV-irradiated cells compared with non-irradiated cells. The data are represented as mean ± SD (*n* = 3). **p* < 0.05.

Fig. 5. Differential transcriptional regulation of DDB2, XPC and GADD45 by TAp63 and TAp73 isoforms. Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells were cultured in medium containing Dox (2 μ g/ml) for different hours (0, 18, and 24 h) to induce the expression of specific isoforms of TAp63 and TAp73. The expression of TAp63 $\alpha/\beta/\gamma$ and TAp73 $\alpha/\beta/\gamma$ isoforms were shown in Fig. 1B. The mRNA and protein expression levels of DDB2, XPC and GADD45 expression levels were measured by real-time PCR and Western-blot assays, respectively. (A) The protein levels of DDB2, XPC and GADD45 in Saos2-TAp63 $\alpha/\beta/\gamma$ and Saos2-TAp73 $\alpha/\beta/\gamma$ cells treated with Dox. (B) The relative mRNA expression levels of DDB2, XPC and GADD45 in Saos2-TAp63 β , Saos2-TAp63 γ , Saos2-TAp73 β and Saos2-TAp73 γ cells treated with Dox. (C) The relative mRNA expression levels of DDB2, XPC and GADD45 in H1299 cells with ectopic TAp63 γ expression. H1299 cells were transiently transfected with TAp63 γ expression plasmids. The mRNA levels of genes were normalized with β -actin. Data are presented as mean $\pm$ SD ($n = 3$).

Fig. 6. TAp63 γ transactivates the luciferase reporter genes containing p53-consensus DNA binding elements in human DDB2 and XPC genes. (A) p53-consensus DNA binding elements in human DDB2, XPC and GADD45 genes. N, any nucleotide; Pu, purine; Py, pyrimidine. Positions of p53-consensus DNA binding elements relative to the ATG site are indicated. (B) TAp63 γ transactivates the luciferase reporter genes containing p53-consensus binding elements in human DDB2, XPC and GADD45 genes. Saos2 and Saos2-TAp63 γ cells were treated with or without Dox (2 μ g/ml) for 24 h before being transfected with pGL2 luciferase reporter plasmids containing one copy of p53-consensus binding elements in human DDB2, XPC or GADD45 genes. pRL-SV40 plasmid was co-transfected as an internal control. Cells were then cultured in medium with or without Dox (2 μ g/ml) for another 24 h after transfection, and the luciferase activities were measured. The reporter activity was calculated as luciferase activity of reporter plasmids in cells induced with Dox compared with that in cells without Dox treatment. Data are presented as mean $\pm$ SD ($n = 3$).