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TAp63γ 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 TAp63α/β/γ and TAp73α/β/γ isoforms, were employed. Overexpression of TAp63γ 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 Soas2 cells. The enhancement of NER of UV-induced DNA damage by TAp63γ was also confirmed in H1299 cells with overexpression of TAp63γ. Consistently, knockdown of endogenous TAp63 decreases NER of UV-induced DNA damage in H1299 cells. TAp63α/β and TAp73α/β/γ isoforms do not have a clear effect on NER in Saos2 or H1299 cells. TAp63γ overexpression clearly induces the expression of DDB2, XPC and GADD45 at both RNA and protein levels. Furthermore, luciferase reporter assays show that TAp63γ transcriptionally activates DDB2, XPC and GADD45 genes through the regulation of the p53 binding elements in these genes. These results demonstrate that TAp63γ 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.

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 prolin-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 (ΔN) isoforms that lack the TA domain. TAp63 and TAp73 are transcriptionally proficient, and often have biological functions similar to p53. ΔN 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 ΔN 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

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.

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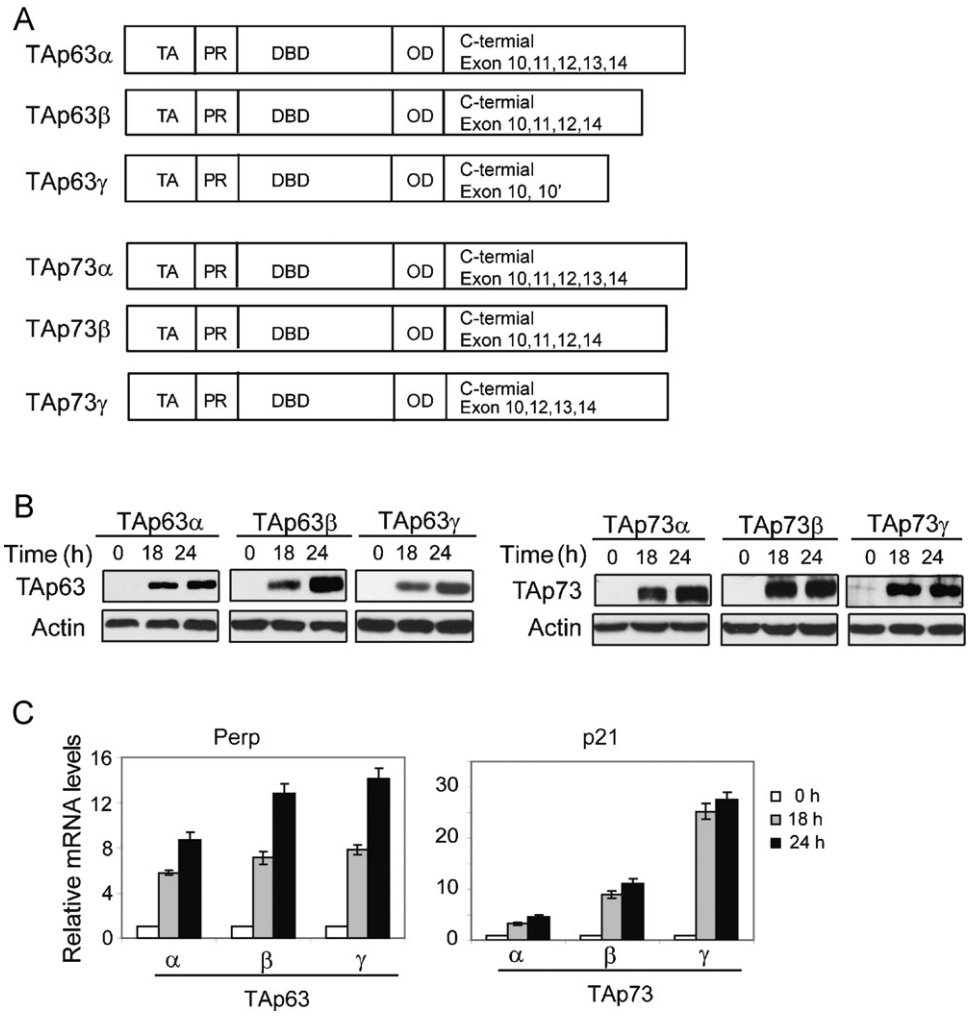

Fig. 1. The expression of α, β and γ isoforms of TAp63 and TAp73 proteins in Saos2-TAp63α/β/γ and Saos2-TAp73α/β/γ cells. (A) Schematic structure of α, β and γ isoforms of TAp63 and TAp73 proteins. TA: transactivation domain; PR: prolin-rich domain, DBD: DNA binding domain; OD: oligomerization domain. (B) The expression of α, β, and γ isoforms of TAp63 and TAp73 proteins in Saos2-TAp63α/β/γ and Saos2-TAp73α/β/γ cells treated with Dox. Saos2 cells, which are deficient for p53, p63 and p73, were stably transfected with Tet-on expression vectors for TAp63α, TAp63β, TAp63γ, TAp73α, TAp73β, or TAp73γ (Saos2-TAp63α/β/γ and Saos2-TAp73α/β/γ cells). These 6 cell lines were treated Dox (2 μg/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-TAp63α/β/γ and Saos2-TAp73α/β/γ cells were treated with or without Dox (2 μg/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 ± SD ($n = 3$).

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 (DBS) 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 TAp63γ in cells clearly enhances NER of UV-induced DNA damage, whereas TAp63α/β and TAp73α/β/γ isoforms do not show clear effect on NER. TAp63γ also enhances cell survival after UV irradiation. Furthermore, TAp63γ displays a strong transcriptional induction of DDB2, XPC and GADD45 at both mRNA and protein levels, whereas TAp63α/β and TAp73α/β/γ isoforms show limited or no induction of these genes. Luciferase reporter assays demonstrate that TAp63γ transcriptionally activates DDB2, XPC and GADD45 genes through the regulation of the p53 binding elements in these