Differential effects of diverse p53 isoforms on TAp73 transcriptional activity and apoptosis

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The p53 activities are due, at least in part, to its ability to form oligomers that bind to specific DNA sequences and activate transcription. Since some mutant p53 proteins and ΔNp73 isoforms form heterocomplexes with TAp73, we asked whether p53 isoforms can do the same and potentially act as dominant-negative inhibitors of TAp73. Moreover, it has already been found that some isoforms form complex with wt53 and some of them inhibit p53 tumor-suppressor functions. Therefore, we studied the complex formation and co-immunoprecipitation assays show that all six p5 isoforms examined can form complexes with TAp73β, whereas only Δ133p53αβγ isoforms form complex with TAp73α. All p53 isoforms counteract TAp73β transactivation function but with different efficiency and in a promoter-dependent manner. Furthermore, apoptotic activity of TAp73β was augmented by coexpression of p53β, whereas Δ133p53αβ and β inhibit its apoptotic activity most efficiently. We have determined the half-life of different p53 isoforms: p53γ isoform has the shortest half-life, whereas Δ133p53γ has the longest half-life. Inhibitory interactions of two proteins in complex often lead to their stabilization. However, only three isoforms (Δ133p53α, Δ133p53β and Δ40p53α) stabilize TAp73β. We are convinced that defining the interactions between p53/p73 would give a new insight into how the p53 isoforms modulate the p73 functions in tumorigenesis.

Introduction

The tumor-suppressor protein p53 regulates cellular response to stress signals, by inducing DNA repair and, if DNA is not repairable, transient or permanent cell-cycle arrest, apoptosis or senescence (1). p53 function is frequently compromised in cancer cells, as over 50% of the human cancers contain mutations and other alterations of the gene (2), p53 specifically binds to response elements and coordinates transcription of numerous target genes.

In the past 15 years, two genes, p63 and p73, have been found to encode several proteins whose structures and functions are similar to those of p53 but not identical. As a matter of fact, p63 and p73 are more similar to each other than each is to p53 (3,4). p53 is important in the prevention of cancer, whereas p63 and p73 are in addition crucial for normal development (5).

p53 family members share significant similarity at the protein level within three functional domains: transactivation domain, DNA-binding domain and tetramerization domain. All family members contain at least one proline-rich domain, containing PXXP motifs, p53 and p73 have a C-terminal basic domain, whereas both p63 and p73 contain a C-terminal sterile-α-motif. The complexity of the family has been enriched by the use of alternative promoters, splicing and translational sites (6,7). Consequently, several protein isoforms with distinct N- and C-termini are encoded.

Transcribing from P1 promoter principally gives rise to full-length isoforms with transactivation domain (p53, TAp63 and TAp73), whereas using the alternative P2 promoter produces aminoterminal truncated isoforms without transactivation domain (ANp53 (Δ133/160p53), ΔNp63, ΔNp73). Combining the alternative splicing of the 5′ end with different promoter usage, additional protein isoforms of p53, p63 and p73 arise (6,8,9). Most of the alternative splicing, however, occurs at the 3′ end which creates isoforms that have different C-termini.

Discovery of p53 homologues certainly has changed the assumptions about this tumor suppressor. Mutation of p53 tumor-suppressor gene is a very important factor in tumorigenesis (10), whereas p73 member of the family is rarely mutated (6). However, there is an important cross-talk between family members in tumors. Any p53 family member can bind specifically to p53 response element and modulate the expression of p53 target genes. Therefore, p53 family members can regulate the expression of genes involved in cell cycle, apoptosis and senescence. Through binding to p53 response element, ΔNp63/ANp73 can exert dominant-negative effects on the activity of p53, p63 and p73.

Certain isoforms of p53/p63/p73 proteins can form heterotetramers. The formation of such mixed heterocomplexes correlates with functional transdominance—loss of transactivation of target genes and proapoptotic abilities (Figure 1, (11,12)).

A subset of p53 mutant proteins negatively regulates TAp73 through direct interaction which is mediated through DNA-binding domain of mutant p53 proteins that bind to both DNA-binding domain and oligomerization domain of TAp73 (11,13,14). Functionally, such stable heterocomplexes cause loss of TAp73 tumor-suppressor functions comparable with abrogated wild-type p53 functions by p53 mutants (Figure 1, (6)). In tumors that express both TAp73 and mutant p53, the function of TAp73 might be inactivated (6). Furthermore, ΔNp73, the dominant-negative inhibitor of both TAp73 and wild-type p53 interferes with TAp73 or p53 activity by direct binding to the proteins or by competing for promoter sites, respectively (6,12,15). ΔNp73 efficiently counteracts transactivation function, apoptosis and growth suppression mediated by wild-type p53 and TAp73, and confers drug resistance to wild-type p53-harboring tumor cells. When coexpressed in various cell types, hetero-oligomerization with dominant-negative ΔNp73 mediates the significant stabilization/accumulation of TAp73α and β simultaneously with inactivation (16).

Interestingly, although p53 is induced by a variety of anticancer drugs due to the increased transcription and protein stabilization upon DNA damage, it cannot induce apoptosis without the presence of either p63 or p73 (17). Besides the protein interactions among them or regulating promoters of common target genes, p53/p63/p73 proteins can interact by regulating each other’s promoters (18). Recently, it was shown that p53 regulates the transcription of Δ133p53 isoform (19,20). In response to DNA damage, p53 directly binds to p53 response element within P2 internal promoter inducing Δ133p53 expression. This isoform modulates p53-induced apoptosis and p53-dependent G1 arrest without inhibiting p53-dependent G2 arrest (20). In addition, it was recently found that it also counteracts p53-dependent growth suppression in clonogenic assays (19,20) and inhibits replicative cellular senescence (21). Moreover, it was also recently reported that in addition to p53, at least four p63/p73 isoforms (p63β, ΔNp63α, ΔNp63β and ΔNp73γ) regulate Δ133p53 expression at transcriptional level through direct DNA binding to the internal TP53 promoter (22). Proliferation assays indicated that Δ133p53 regulates the antiproliferative activities of these p63/p73 isoforms. Although that was the first paper which addressed the interplay between p53, p63 and p73 isoforms, it is still unknown whether p53 isoforms interfere with TAp73 activity. Here, we report the inhibition of TAp73β-mediated transcriptional activity and apoptosis through the formation of heterocomplexes between p53 isoforms (p53β, p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ40p53α) and TAp73β.
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Materials and methods

Cell lines

The p53 null human lung cancer cell line H1299 cells were maintained in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum and 2 mM l-glutamine, with 5% CO2, at 37°C (Gibco BRL, Carlsbad, CA, USA). The antibodies used in this study are as follows: ER15 (Calbiochem, EMD Chemicals, Gibbstown, NJ, USA) and IMG-246 (Ingenex, CA, USA) for p73α, GC15 (Calbiochem, EMD Chemicals, Gibbstown, NJ, USA) for p73β, DO-1 (Santa Cruz Biotechnology, Inc., CA, USA) for p53, p53β and p53γ, PAb421 (Calbiochem, EMD Chemicals, Gibbstown, NJ, USA) for Δ133p53α and Δ40p53α, KJC8 (kindly provided by J.C. Bourdon) for Δ133p53β, DO-12 (kindly provided by B. Vojtesek) for Δ133p53γ, anti-GFP (JL-8; BD Biosciences, San Jose, CA, USA), and anti-vimentin (Lab Vision Corporation, CA, USA) as loading control. Generally, 50 μg of total cell lysates for exogenous and 100 μg for endogenous proteins was used from transfected cells to monitor steady-state levels of proteins. Proteins were visualized using Western Lightening Chemiluminescence Reagent Plus (Perkin Elmer, CA, USA), SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL, USA) or SuperSignal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology, Rockford, IL, USA).

Co-immunoprecipitation

H1299 cells were transiently transfected in 60 mm plates with 8 μg of DNA and harvested 24 h after transfection. Extracts were centrifuged at 15 000g for 20 min at 4°C to remove cell debris. Protein concentration was determined by the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). One mg of total protein extract was immunoprecipitated with 1 μg of anti-p73 or anti-p53 antibody. For co-immunoprecipitation assay, we used Dynabeads® Protein G (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol. Immunoprecipitates were subjected to immunoblot analysis as described above.

Luciferase assay

H1299 cells were transiently transfected in 24-well plates in duplicate with 500 ng of DNA in total. To test the effect of the dominant-negative inhibitor, each well was transfected with 100 ng of TAp73β expression vector plus 400 ng of empty vector pcDNA3 or with 100 ng of TAp73β expression vector plus 400 ng of individual p53 isoform together with the 80 ng of Firefly luciferase construct (p53-responsive PG13-Luc, Bax-Luc or p21-Luc) and 8 ng of Renilla luciferase vector (pRL-TK). To test p21 and Bax activity, camptothecin of 5 μM concentration was added 6 h after transfection. After 24 h, cells were harvested and the luciferase activity was measured at Fluoroscan as FL luminometer (Thermo, Finland) using Dual-Glo Luciferase Assay System (Promega Corporation, WI, USA) as described by the supplier. Luciferase activity was normalized for Renilla activity.

Apoptosis assays

H1299 cells were transfected in six-well plates with 4 μg of expression vector containing TAp73, wild-type p53 or p53 isoforms. The pcDNA3 empty vector was used as a negative control. In the experiments of coexpression of p73β with p53 isoforms, H1299 cells were cotransfected in proportion 1:2. Six hours after transfection, 5 μM camptothecin was added. To detect the apoptotic phenotype, cells were stained using Annexin-V-FLUOS Staining Kit (Roche Diagnostics, Indianapolis, IN, USA) and subjected to fluorescence-activated cell sorting (FACS) analysis on FACS Calibur™ (Becton Dickinson, San Jose, CA, USA). Data were analyzed using CELLQuest software (Becton Dickinson, San Jose, CA, USA). For fluorescence microscopy analysis, cells were seeded in eight-well chambers and transfected with 1 μg of the indicated expression vector or empty vector pcDNA3. After 48 h, apoptotic cells were determined using Annexin-V-FLUOS Staining Kit (Roche Diagnostics, Indianapolis, IN, USA) and evaluated by fluorescence microscopy.

Half-life determination

H1299 cells were treated with 40 μg/ml of cycloheximide 24 h posttransfection in six-well plates and harvested at different time points. Rates of degradation were assessed by immunoblotting with corresponding anti-p53 antibody. The percentage of p53 remaining was quantified by ImageMaster VDS Software (Pharmacia Biotech) and was normalized to α-vimentin load.

Results

Heterotetramer analysis between TAp73α or TAp73β and p53 isoforms

The physical interaction between family members was demonstrated as a transdominant mechanism inhibiting the suppressor functions of wt p53 and TAp73 (12, 15, 23). To determine whether proteins form complexes with each other, we performed the co-immunoprecipitation analyses.

Fig. 1. Dominant-negative effects of ΔNp73, mutant p53 and p53 isoforms on TAp73 activity. Upper panel: TAp73 isoforms bind p53-specific DNA sequences and recruit the transcription machinery. Lower panel: (A) TAp73 and ΔNp73; (B) TAp73 and mtp53; (C) TAp73 and p53 isoforms can form hetero-oligomers, potentially deficient in their capacity to recruit the transcription machinery.
assay with ectopic proteins. The existence of heterocomplex was examined in both directions. The results are summarized in Table I and representative immunoblots are shown in Figure 2. Altogether, all examined p53 isoforms form mixed complex with Tap73β (Table I, Figure 2B). In contrast, Tap73α tetramerizes only with α133 isoforms (α, β and γ, Table I, Figure 2A).

**Effect of p53 isoforms on Tap73β transactivation**

To test the hypothesis that some p53 isoforms could be dominant-negative inhibitors of human Tap73, we performed reporter assays. First, we determined the transactivation activity of p53 isoforms (p53β, p53γ, α133p53α, α133p53β, α133p53γ, Δ40p53α). Therefore, we cotransfected H1299 cells (do not express any p53, Dominant-negative inhibitors of human TAp73, we performed reporter examination in both directions. The results are summarized in Table I and representative immunoblots are shown in Figure 2. Altogether, all examined p53 isoforms form mixed complex with Tap73β (Table I, Figure 2B). In contrast, Tap73α tetramerizes only with α133 isoforms (α, β and γ, Table I, Figure 2A).

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amounts of certain p53 isoform (p53β, p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ40p53α). The steady-state levels of both exogenous/ectopic and endogenous TAp73β increased proportionally with cotransfected Δ133p53α, Δ133p53β, Δ133p53γ, Δ40p53α isoforms (Figure 5C and D showing representative immunoblots). In contrary, isoforms p53β, p53γ and Δ133p53γ did not stabilize TAp73β protein. For exogenous stabilization, the cells were cotransfected with GFP and the transfection efficiency was normalized according to GFP expression (Figure 5C).

Discussion

All three p53 family members encode multiple variants that are transcribed from two different promoters and are the result of alternative splicing. Earlier studies have shown that the functions of p73 and p63 isoforms are distinct and specific (6). Biological functions of p53 isoforms are still being investigated. Physical protein–protein interaction between oncogenic and anti-oncogenic family members has been demonstrated previously (11,12,14,15,24). This is one mechanism for

Fig. 3. The effect of p53 isoforms on TAp73β transcriptional activity. (A) Transcriptional activity of p53 isoforms on p53/TAp73-responsive reporter construct PG13-Luc in p53 null H1299 cells. Luciferase activity is normalized for Renilla luciferase activity. (B) H1299 cells were cotransfected with p53/TAp73-responsive reporter PG13-Luc and plasmid expressing TAp73β alone or together with p53 isoform (p53β, p53γ, Δ133p53α, Δ133p53β, Δ133p53γ, Δ40p53α) in 1:4 ratio. About 6h after transfection, 5 µM camptothecin was added. Cells were harvested 24h after cotransfection and luciferase activity was measured. Results are represented as fold induction of luciferase activity compared with control cells transfected with pcDNA3 empty vector. Standard deviation is calculated from the data of two independent experiments performed in triplicates. (C,D) The effect of p53 isoforms on p21 and Bax promoters. H1299 cells were cotransfected with the plasmids p21-Luc or Bax-Luc containing the natural promoters driving the luciferase reporter gene, pRL-TK Renilla luciferase as an internal control and TAp73 or p53 isoform, or both in 1:4 ratio. The results shown are the average of three independent experiments performed in duplicate. Standard deviations are indicated as error bars. (D) The cells were treated with 5 µM camptothecin 6h after transfection.
transdominant interference of N-terminally truncated p73 isoforms with the suppressor functions of wtP53 and TAp73 (12). Functionally, formation of such stable complexes leads to loss of p53- and TAp73-mediated transactivation and proapoptotic abilities. It was already shown that p53β and Δ40p53α are co-immunoprecipitated with full-length p53 (8, 25).

Based on systemic analysis using co-immunoprecipitation assay, we confirmed the heterocomplex formation between p53 isoforms and TAp73α/β isoforms. All isoforms can form heterocomplex with TAp73α, whereas only Δ133p53α, Δ133p53β and Δ133p53γ form complex with TAp73α. These data expand the set of previously discussed differences between TAp73α and TAp73β (11). Although TAp73α is expressed at higher levels, only small amounts of TAp73α are sufficient to activate transcription better than TAp73α (11).

The luciferase assay has shown that p53 isoforms inhibit TAp73β transcriptional activity with a different effect. Δ133p53α and Δ133p53γ are the strongest inhibitors, isoforms Δ133p53β and Δ40p53α inhibit 35 and 38% of transcriptional activity, respectively, whereas p53β and p53γ isoforms inhibit only 28 and 21% of transcriptional activity of TAp73β (Figure 3B). So far no one has studied the inhibitory effect of p53 isoforms on the TAp73 activity. However, several studies have investigated the effect of p53 isoforms on wtP53 activity. The study by Goldsteiner et al. (26) has shown that several C-terminal truncated forms cloned from neuroblastoma cells (including p53β) enhance p53-mediated transcriptional activity. However, Graupner et al. (27) have shown that both C-terminal truncated forms p53β and p53γ are unable to influence p53-dependent transcriptional activity in luciferase reporter gene assays using different p53-responsive elements, explaining it with inability to associate with p53 and hence not binding to p53-responsive sites. However, in co-immunoprecipitation assays, we have found that TAp73β forms complex with all these isoforms, no matter of truncated C-terminus. Therefore, the inhibitory activity of p53 isoforms observed in our experiments is related to their ability to associate with TAp73β.

Interestingly, when we examined the transcriptional activity of individual isoforms (Figure 3A), we have found that p53β has extremely strong transcriptional activity, even stronger than TAp73β. The transcriptional activity of p53β and p53γ with respect to wtP53 was so far measured only by Graupner et al. (27). The study revealed that wtP53
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has stronger transcriptional activity than both p53β and p53γ (27). However, we are the first to explore transcriptional activity of p53 isoforms with respect to TAp73.

To determine the specific effect of p53 isoforms on transcription, we cotransfected H1299 cells with TAp73β and p53 isoform expression vector and the luciferase reporter plasmids driven by the p21 or Bax promoter. Transfection of an individual p53 isoform (p53γ, Δ133p53α, Δ133p53β, Δ133p53γ) in the absence of TAp73β has no effect on p21/Bax promoter transactivation, regardless of camptothecin treatment (Figure 3C and D). However, p53β slightly induces p21/Bax promoter (~30% of TAp73β value) and Δ40p53α isoform induces transcription of p21 promoter only with same capacity as p53β but exclusively upon cell treatment with camptothecin. p53γ cotransfected with TAp73β followed by camptothecin treatment enhances p21 promoter activity. TAp73β-mediated Bax promoter activity was elevated with p53γ, but with Δ133p53α and Δ40p53α as well. Cotransfection of Δ133p53β with TAp73β has no effect on TAp73β-mediated p21 transcription; however, p53γ, Δ133p53α, Δ133p53βγ and Δ40p53α reduce it. Only Δ133p53β isoform was a strong inhibitor of TAp73β-mediated Bax transcription.

Cell treatment with camptothecin, an activator of TAp73, does not increase TAp73 transcriptional activity. This is probably because transfection already induced cellular stress sufficiently to activate TAp73 transcriptional activity (28). However, the inhibition efficiency of some p53 isoforms differs; p53β did not enhance TAp73β-mediated p21/Bax promoter activity and neither did other isoforms. Moreover, all isoforms strongly inhibited TAp73β-mediated p21/Bax promoter (Figure 3C). The luciferase data was validated by reverse transcription–real-time PCR. TAp73β-mediated p21 expression was the most impaired when cotransfected with Δ133p53α, and Bax expression when cotransfected with Δ40p53α. Couple of studies have been done with p53, but so far none with p73. Those investigating the effect of p53β on p53 function have shown conflicting data. Whereas one study presents evidence that p53β selectively enhances the p53 transcriptional activity on Bax promoter strongly in stressed cells, it has no effect on p21 promoter even after stress (8), and the other study demonstrates that p53β enhances p53-dependent activity of both p21 and Puma promoters regardless of stress induction (27). Graupner et al. (27) have demonstrated the inability of p53β and p53γ, as well as Δ133p53β to influence the p53-mediated Bax promoter transactivation beside p53-responsive elements (27). Therefore, according to previous publications, p53β has low transcriptional activity both on Bax and p21 promoter in the absence of p53, or TAp73 as we demonstrated (8,27,29).

Furthermore, we investigated the biological relevance of heterocomplex formation in response to DNA damage. One of the cellular functions of p53 is to promote apoptosis in response to stress (DNA damage, hypoxia or activation of oncogenes (30)). Overexpressed TAp73 also induces apoptosis and thus prevents the growth of tumor cells (31). Our results have shown that the proapoptotic activity of all individual p53 isoforms is reduced in comparison with TAp73β, but in a different ratio. When cotransfected with TAp73β p53 isoforms affected TAp73β apoptotic activity:

![Image](https://academic.oup.com/carcin/article-abstract/34/3/522/2463022)
only p5β enhanced TAp73-mediated apoptosis, all other isoforms reduced it with different ability. TAp73-mediated apoptosis is most impaired in cells cotransfected with TAp73β and p5γ, Δ133p53α or Δ133p53β. Our data is consistent with previous reports showing that Δ133p53α can act in a dominant-negative manner toward p5β. First, Bourdon et al. (8) have explored the proapoptotic activity of p53γ and Δ133p53α finding the same trend as we did. Furthermore, it was published that Δ133p53α, the zebrafish homologue of human Δ133p53α, prevents p53-mediated apoptosis in response to embryonic defects or DNA damage (32). Further publications have shown that induced Δ133p53α inhibits p53-dependent apoptosis (20) and that human Δ133p53α can inhibit replicative senescence (21). Early publications reported that Δ40p53 can also act, after transfection, in a dominant-negative manner toward wild-type p53, inhibiting both p53 transcriptional activity and p53-mediated apoptosis (26,33). Here, we report the inhibiting effect of Δ40p53 toward TAp73β-mediated apoptosis.

Under normal conditions, p53 protein has a short half-life due to rapid degradation by proteasome mediated by MDM2 (34,35). Recently, it was shown that MDM2 differentially controls the modification of p53 isoforms and all isoforms co-immunoprecipitate with MDM2, although MDM2 is not able to promote degradation of p53β and p53γ (36). In this paper, we analyze and compare the stability of p53 isoforms after transfection into p53-null H1299 cells treated with cycloheximide. Compared with p53, the rapid degradation of p53γ and Δ40p53α was found. In contrast, p5β, Δ133p53α and Δ133p53β isoforms are long-living. Δ133p53γ isoform is relatively stable but has shorter half-life in comparison with p53 and p5β. MDM2 has distinct roles in posttranslational modifications in the regulation of p53 isoforms (36).

Inhibitory interaction of two proteins in complex often leads to their stabilization. We have found that transfection of increasing amounts of Δ133p53αc, Δ133p53β and Δ40p53αc isoforms (not others) into H1299 leads to concomitant increase of exogenous and endogenous TAp73β protein levels. This is very interesting if we compare the structure of single isoforms. Only Δ133p53α and Δ40p53αc have the oligomerization domain, which confirmed our previous experiments showing that the ability of Anp73 to mediate TAp73 accumulation largely depends on its oligo/tetramerization domain (16). Δ133p53β isoform stabilizes TAp73β but less effectively. Thus, it is probably that the stabilization/accumulation of TAp73 by p53 isoforms occurs due to the formation of mixed oligomers/heterooligomers with TAp73, thereby stabilizing TAp73 in an inactive form. In any case, our research confirmed the previous ones suggesting again that increased TAp73 protein levels in tumors should be interpreted with caution when levels are the only criteria that can be used to deduce TAp73 activity (16). Future protein-based studies are needed to verify this relationship between TAp73 and p53 isoforms in primary tumors.

Despite many years of research in p53 field, it is still unknown how one protein, p53, with its ability to control cell cycle and apoptosis can determine cellular response and define the cell fate. In addition, it is still difficult to link all these data to cancer prognosis and cancer treatment. It is evident that p53 mutational status is not sufficient to determine therapeutic response and clinical outcome, suggesting that the answer could be found in studying p53/p63/p73 isoforms and their role in tumorigenesis. The clinical studies report the expression of p53 isoforms in several tumor types, confirming that p53 isoforms are expressed both at the messenger RNA and protein levels (37–40). Our understanding of their biological roles will greatly depend on the investigation of p53/p63/p73 expression profiles. Our results suggest (summarized in Table II) that the biochemical and biological functions of p53 family member, p73, can be mediated by the interplay between p53 isoforms and TAp73 protein. Thus, regulation of p73 function in normal and tumor human tissues is likely to be more complex than has been so far considered. It is obvious that p53 isoforms can modulate p53 family tumor-suppressor activities and the expression ratio between members of the family is probably the most important factor in determining the cell destiny.

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**References**

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