Transactivation-deficient ΔTA-p73 Inhibits p53 by Direct Competition for DNA Binding

IMPLICATIONS FOR TUMORIGENESIS

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The p53 family member p73 displays significant structural and functional homology to p53. However, instead of mutational inactivation, overexpression of wild-type p73 has been reported in various tumor types compared with normal tissues, arguing against a classical tumor suppressor function. Recently, N-terminally truncated, transactivation-deficient p73 isoforms (ΔTA-p73) have been identified as a second class of p73 proteins. Because overexpression of p73 in tumors includes ΔTA-p73, we further characterized these novel p73 isoforms. We show that ΔTA-p73 retains DNA-binding competence but lacks transactivation functions, resulting in an inability to induce growth arrest and apoptosis. Importantly, ΔTA-p73 acts as a dominant-negative inhibitor of p53 and full-length p73 (TA-p73). We demonstrate that inhibition of p53 involves competition for DNA binding, whereas TA-p73 can be inhibited by direct protein-protein interaction. Furthermore, we show that up-regulation of endogenous p73 just like ectopic overexpression of ΔTA-p73 confers resistance to p53-mediated apoptosis induced by the chemotherapeutic agent II-7. Because inhibition of p53 is a common theme in human cancer, our data strongly support a role of ΔTA-p73 expression for tumor formation.

The TP53 gene was the first tumor suppressor gene to be identified and is still considered the prototypical tumor suppressor. In more than half of human tumors the TP53 gene is inactivated directly by mutations, and in many others p53 is functionally compromised epigenetically by various mechanisms (1, 2). In fact, several transforming oncogenes have been shown to be potent inhibitors of p53 (1). Loss of functional p53 mechanisms (1, 2). In contrast to mice lacking p53, p73-negative mice are not prone to tumor development (8). Despite initial reports suggesting tumor-associated deletion of p73, many subsequent studies failed to demonstrate mutational inactivation of the TP73 gene in a wide variety of tumors (3). Instead, overexpression of wild-type p73 has been reported in various tumor types compared with normal tissues (9–13). High p73 expression levels were revealed as an independent marker of poor patient survival prognosis in hepatocellular carcinomas and correlated positively with higher risk stages in B-CLLs (B-cell chronic lymphocytic leukemia) (14). Together, these data raise the question about additional activities of p73 in cancer.

The molecular basis for the apparently different functions of p53 and p73 in human tumors is at present unknown but might be related to the differences in genomic organization of the TP53 and TP73 genes. Whereas TP53 does not show much splice variations, the TP73 gene encodes a complex number of isoforms with at least nine different isoforms generated by alternative splicing of the C-terminal exons (Fig. 1) (15). However, apart from a shift toward expression of the shorter C-terminal isoforms in tumor cells, little is known to support their role in tumorigenesis (12, 16, 17). Here, the recent identification of N-terminally truncated, transactivation-deficient p73 isoforms as an additional group of p73 proteins seems to be more promising (8, 18–22). In developing mice these isoforms are predominant (termed ΔN-p73) and are generated from an alternative, cryptic promoter in intron 3 (8). Murine ΔN-p73 has been shown to be a potent anti-apoptotic protein, which rescues sympathetic neurons from apoptosis induced by nerve growth factor withdrawal or p53 overexpression (18). A similar transcript with high sequence homology to murine ΔN-p73 could be identified in human cells (20–22). In addition to these “physiological” ΔN-p73 proteins with a distinct regulation via an independent promoter, aberrantly spliced transcripts (p73Δex2, p73Δex2/3 and ΔN-p73) regulated by the TA-promoter are found in human tumor cells (3, 19, 23). Because the translation start is located in exon 2, these splice variants also encode N-terminally truncated proteins termed ΔTA-p73 (Fig. 1).

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Received for publication, January 16, 2002, and in revised form, February 13, 2002
Published, JBC Papers in Press, February 13, 2002, DOI 10.1074/jbc.M200480200

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Interestingly, overexpression of p73 in tumors has been shown to include both full-length p73 (TA-p73) and N-terminally truncated p73 isoform (ΔTA-p73).1,2 Moreover, overexpression of ΔTA-p73 results in malignant transformation of NIH3T3 cells supporting a function in tumorigenesis.2 In this study we have further characterized these novel p73 isoforms. We show that ΔTA-p73 just as full-length p73 (TA-p73) and wild-type p53 is a sequence-specific DNA binding factor. Due to the lack of the N-terminal transactivation domain, however, ΔTA-p73 does not transactivate typical p53-regulated genes, resulting in an inability to induce growth arrest and apoptosis. Moreover, ΔTA-p73 acts as a dominant-negative inhibitor of p53 and full-length p73 (TA-p73). Investigating the mechanism, we demonstrate that inhibition of p53 involves competition for DNA binding, whereas TA-p73 can be inhibited by direct protein-protein interaction. Further, we show that up-regulation of endogenous p73 just like ectopic overexpression of ΔTA-p73 confers resistance to p53-mediated apoptosis induced by the chemotherapeutic agent H-7. Because inhibition of p53 is a common theme in human cancer, our data provide further evidence supporting a role of ΔTA-p73 expression for tumor formation.

EXPERIMENTAL PROCEDURES
Cell Culture and Transfections—MCF-7 (ATCC, Manassas, VA), H1299 (human bronchoalveolar carcinoma, obtained from B. Opalka, University of Essen), and NHDF cells (normal human diploid fibroblasts, obtained from M. Roggendorf, University of Essen) were maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum. Isoquinoline (1-(5-isoquinolinesulfonyl)-2-methylpiperazine, H-7; Sigma) was used at a final concentration of 50 μM for 24 h and all-trans-retinoic acid (RA; Sigma) at 10 μM for 4 days. For induction of DNA damage, cells were exposed to 3 μM Adriamycin (doxorubicin, Sigma) for 16 h, 8 h after infection with adenoviral vectors. Transfections were performed by electroporation.

Plasmids—Expression plasmids for p53 (pC53), p53R175H (pC53-175), and Gal-p53, HA-p73α, and HA-p73β were kindly provided by B. Vogelstein, J. Brady, and G. Melino. respectively. cDNAs encoding untagged p73α, p73Δex2α, p73Δex2β, p73β, p73Δex2β, and p73Δex2β were amplified by PCR using HA-p73α as a template.

RT-PCR—Semiquantitative RT-PCR was carried out on total RNA prepared with the RNeasy Mini Kit (Qiagen) essentially as described

FIG. 1. Genomic organization of the TP73 locus. A, the splicing patterns generating C-terminal isoforms p73 α, β, γ, δ, ε, and ζ and the N-terminal isoforms p73Δex2, p73Δex2β, ΔNT-p73, and ΔN’p73 are shown. The arrows indicate transcriptional start sites. The ΔN-p73 isoform is generated from a cryptic promoter within intron 3. B, the exon structure of the ΔTA-p73-encoding transcripts is shown in comparison with full-length TA-p73 (exons 1–5 only). Noncoding sequences are depicted in white. C, domain structure of full-length p73α. TA, transactivation domain; DBD, DNA-binding domain; OD, oligomerization domain; CT, C terminus.
To obtain a semiquantitative result, we used the minimum number of cycles required to obtain a clear signal in the linear range and labeling of PCR products with \([32P]dCTP\) for high sensitivity detection. The amount of PCR product was quantitated on a Phosphor-Imager. Primer sequences are available upon request.

Electrophoretic Mobility Shift Assay (EMSA), in Vitro Translation—EMSAs were performed as described (7). For supershift analysis, 1/2 of the anti-p73 monoclonal ER15 or the polyclonal anti-p73 antibody Ab-7 were used. In vitro translation was performed with the TNT translation system (Promega) according to the manufacturer’s protocol.

Immunoprecipitation, GST-Pull-down Assay, Western Blotting, and Antibodies—For co-immunoprecipitation experiments, 3 mg of lysate from transfected H1299 cells were precipitated with 1 ε of either p53 antibodies (mixture of DO-1 and PAb421) or HA antibody (F-7, Santa Cruz Biotechnology) in NET buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 10% glycerol). Immunoprecipitates were subjected to SDS-PAGE, transferred to ECL nitrocellulose (Amersham Biosciences), and immunoblotted with p73-antibera.

For GST-pull-down assays, \(^{35}\)S-labeled in vitro translated proteins were bound with recombinant GST-p53 in GST-binding buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM dithiothreitol, 0.1 mM EDTA, 10% glycerol, 0.1% Nonidet P-40) and detected by autoradiography following SDS-PAGE.

**RESULTS**

**ΔTA-p73 Isoforms Are Transactivation-defective**—Because ΔTA-p73 proteins share the DNA-binding domain with the full-length isoforms, DNA-binding properties were analyzed by EMSA. As shown in Fig. 2A, full-length proteins as well as ΔTA isoforms all form complexes with a p53 consensus oligonucleotide with comparable affinity, which can be specifically competed and supershifted with appropriate antibodies. Whereas the full-length p73 containing complexes were supershifted by antibodies directed against the C terminus and the N terminus, ΔTA-p73 DNA complexes, which lack the N-terminal epitope, were only supershifted by the C-terminal antibody.

Next we analyzed p73-mediated transactivation of p53-regulated reporters by luciferase assay by transient transfection in p53-null H1299 cells (Fig. 2, B–D). Although full-length p73α and p73β activated a p53-responsive luciferase reporter plasmid containing three p53 binding sites upstream of a TATA box, no significant transactivation was observed for ΔTA-p73α and ΔTA-p73β, consistent with the lack of the N-terminal transactivation domain. Protein expression of ΔTA-p73 was...
Fig. 3. Regulation of endogenous target genes by p73 isoforms. A, Western blot analysis of H1299 cells infected adenoviral vectors expressing the indicated transgenes using antibodies against p73, p53, p21, and actin. B, transactivation of endogenous p53/p73 target genes (CDKN1A, HDM2, 14-3-3α, PIG3, and PIDD) was evaluated by semiquantitative RT-PCR on total RNA of H1299 cells after infection with adenoviral vectors expressing the indicated transgenes.

verified by Western blot (data not shown). Furthermore, wild-type p53 has shown to inhibit transcription from several viral and cellular promoters without known p53-binding sites such as the SV40 early promoter (30). However, certain transforming p53 mutants or artificial N-terminal deletion mutants lack transrepression activity or even activate such promoters (31, 32). As shown in Fig. 2D, full-length p73β is also a potent repressor of the SV40 promoter, whereas ΔTA-p73β leads to a significant induction of promoter activity.

The effect of ΔTA-p73 on the expression of endogenous p53-regulated target genes (p21CDKN1A, HDM2, 14-3-3α, PIG3, and PIDD) was evaluated by Western blot and semiquantitative RT-PCR in p53-deficient H1299 cells. To obtain high transfection rates we used adenoviral vectors encoding p73α, ΔTA-p73α, p73β, ΔTA-p73β, p53, or GFP as a control (expression is shown in Fig. 3A). Whereas p53, p73β, and to a lesser extent p73α activated p53 target genes, ΔTA-p73 consistently failed to induce or even repressed the majority of target genes (Fig. 3, A and B).

ΔTA-p73 Proteins Fail to Induce Cell Cycle Arrest and Apoptosis—The inability to induce growth arrest and cell death-associated target genes suggests that ΔTA-p73 lacks the cytotoxic activity considered to be the primary mechanism for tumor suppression by p53 and perhaps TA-p73. Indeed, compared with p53 and especially with full-length p73, both ΔTA-p73α and ΔTA-p73β show a significantly reduced ability to suppress colony formation (Fig. 4A). To investigate the underlying causes, we analyzed the cell cycle profile of H1299 cells infected with recombinant adenoviral vectors expressing p53, p73β, or ΔTA-p73β. As shown in Fig. 4B, both p53 and p73β induced growth arrest and apoptosis, whereas ΔTA-p73β induced no significant cell cycle aberrations. Consistently, only p53, p73β, and to a lesser extent p73α induced DNA fragmentation as an indicator of apoptotic cell death (Fig. 4C). These data demonstrate that N-terminally truncated p73 proteins retain the DNA-binding properties of full-length p73 but due to the lack of the transcriptional activation domain in the N terminus fail to activate typical p53 target genes and consequently lack the ability to induce cell cycle arrest and apoptosis.

ΔTA-p73 Isoforms Act as Dominant-negative Inhibitors of p53 and TA-p73—The ability to target p53 DNA binding sites in the absence of transactivation functions suggests that ΔTA-p73 might compete with transcription-competent isoforms of p73 and wild-type p53. As shown in Fig. 5A, expression of increasing amounts of ΔTA-p73 significantly inhibited transactivation of a p53-regulated luciferase reporter by both p53 and transactivation-competent TA-p73β. Consistently, induction of p53 target genes by adenoviral expression of p53 in H1299 cells was completely inhibited by coinfection with ΔTA-p73-expressing vectors (Fig. 5B). In addition, ΔTA-p73 inhibited the transactivation function of endogenous p53 in MCF-7 cells following treatment with adriamycin, despite efficient stabilization of the p53 protein independently of ΔTA-p73 expression (Fig. 4, C and D). As a consequence of the induction of proapoptotic genes, adenoviral expression of p53 led to a rapid loss of cell viability in H1299 cells, which could be significantly inhibited by ΔTA-p73 expression (Fig. 5E). These data indicate that overexpression of ΔTA-p73 inhibits p53- and TA-p73-induced target gene activation, thereby blocking apoptotic cell death as a major tumor suppressor function of p53.

Mechanism of Dominant-negative Activity—In general, two basic mechanisms for inhibition of p53 are conceivable. First, ΔTA-p73 may physically interact with and sequester p53 to form hetero-oligomers that are transactivation-incompetent (33). Second, as ΔTA-p73 retains the core DNA-binding domain and exhibits binding specificity for p53 binding sites, simple competition for DNA sites might prevent p53 or TA-p73 from binding to target gene promoters. To determine whether ΔTA-p73 inhibits p53 function by interfering with sequence-specific DNA binding of p53, we used a fusion protein of p53, the Gal4 DNA-binding domain, and a Gal4-dependent luciferase reporter construct (Gal-TK-Luc). Transfection of increasing amounts of ΔTA-p73 did not lead to substantial inhibition of Gal4-p53-induced reporter activity as was observed for p53 on a p53-dependent reporter (Fig. 5A), suggesting that interference with the sequence-specific DNA binding of p53 might be the primary mechanism of inhibition (Fig. 6A).

This view is further supported by a competition EMSA (Fig. 6B). Both p53 and ΔTA-p73β formed specific DNA complexes that could be supershifted with appropriate antibodies (ER15 for ΔTA-p73β and DO-1 for p53), whereas a DNA binding-defective mutant of ΔTA-p73 (ΔTA-p73βR292H) proved unable to bind DNA. The p53 complexes could be efficiently competed by increasing amounts of ΔTA-p73 but not the DNA binding-defective mutant of ΔTA-p73β, which underlines the importance of an intact DNA-binding domain of ΔTA-p73 to inhibit p53 function.

To investigate whether ΔTA-p73 interacts with and sequesters p53, we performed in vitro and in vivo protein interaction assays. A GST-pull-down assay with recombinant GST-p53 and various 35S-labeled in vitro-translated p53 and p73 proteins demonstrated strong homotypic interaction between GST-p53 and both wild-type and mutant p53 (Fig. 6C). However, neither full-length TA-p73 nor truncated ΔTA-p73 isoforms significantly bound to GST-p53. These data could be confirmed by in vivo immunoprecipitation. ΔTA-p73α co-precipitated only with the p53 mutant p53R175H, which was previously shown to interact with full-length p73 but not with wild-type p53 (Fig. 6D). The failure of ΔTA-p73 to directly interact with wild-type
p53 precludes formation of inactive p53/ΔTA-p73 hetero-oligomers and confines the mechanism of p53 inhibition to competition on the promoter level.

Fig. 4. ΔTA-p73 is unable to induce growth arrest and apoptosis. A, long-term cytotoxicity of ΔTA-p73 expression was analyzed by colony formation assay in comparison with p53 and full-length p73. The absolute colony number was obtained from duplicate experiments. Bars, S.D. Flow cytometric analysis (B) and DNA fragmentation analysis (C) of H1299 cells after infection with adenoviral vectors expressing the indicated transgenes are shown. The sub-G₁ region is marked as M₁.

DISCUSSION

The identification of the p53-homologous TP73 gene on chromosome 1p36, a genomic region frequently deleted in a variety of human cancers, suggested that p73 has tumor suppressor activity similar to that of the classical tumor suppressor, p53. However, p73 is not commonly mutated in all tumor entities analyzed so far. Instead, overexpression of wild-type p73 has been reported frequently and positively correlates with prognostically relevant parameters. Considering that oncogene-induced up-regulation of p73 expression causes apoptosis, sustained overexpression of p73 would therefore require inhibition of its inherent proapoptotic activity (24, 35). On one hand, p53 mutants were demonstrated to inhibit the proapoptotic activity of full-length p73 in a dominant-negative fashion by generating defective hetero-oligomers with wild-type p73. On the other hand, the TP73 gene itself might encode anti-apoptotic isoforms. The first evidence for the latter mechanism was based on the analysis of murine p73, which encodes N-terminally truncated p73 proteins, consistent with recent reports on induction of p73 by RA in other neuroblastoma and myeloid leukemic cells (Fig. 7A) (16, 34). Up-regulation of p73 expression by RA therefore mimics p73 overexpression in cancer cells. Treatment of SH-SY5Y cells with RA conferred resistance to p53-dependent apoptosis induced by the protein kinase inhibitor H-7 (Fig. 7B) without interfering with p53 protein accumulation (Fig. 7B), suggesting that RA inhibits apoptosis signaling via p53, possibly by induction of ΔTA-p73 (27). In fact, overexpression of ΔTA-p73 enhanced resistance to H-7 similar to RA itself (Fig. 7C). These findings strongly suggest that induction of endogenous p73 is able to inhibit apoptosis signaling by endogenous p53.

Inhibition of p53-mediated Apoptosis by Up-regulation of Endogenous p73—To demonstrate the physiological relevance of the dominant-negative activity of ΔTA-p73, we analyzed the effect of endogenous p73 on p53 function. Treatment of wild-type p53 expressing SH-SY5Y neuroblastoma cells with RA resulted in the induction of both endogenous full-length and N-terminally truncated p73α proteins, consistent with recent reports on induction of p73 by RA in other neuroblastoma and myeloid leukemic cells (Fig. 7A) (16, 34). Up-regulation of p73 expression by RA therefore mimics p73 overexpression in cancer cells. Treatment of SH-SY5Y cells with RA conferred resistance to p53-dependent apoptosis induced by the protein kinase inhibitor H-7 (Fig. 7C) without interfering with p53 protein accumulation (Fig. 7B), suggesting that RA inhibits apoptosis signaling via p53, possibly by induction of ΔTA-p73 (27). In fact, overexpression of ΔTA-p73 enhanced resistance to H-7 similar to RA itself (Fig. 7C). These findings strongly suggest that induction of endogenous p73 is able to inhibit apoptosis signaling by endogenous p53.

Apart from ΔN-p73, which is a physiological transcript regulated by its own independent promoter, in human tumor cells N-terminally truncated p73 proteins (ΔTA-p73) are also encoded by alternatively (aberrantly) spliced transcripts that lack exon 2 (p73Δex2) (3, 19, 23, 36). In addition, we and others (22) recently described other alternatively spliced transcripts, which either lack exons 2 and 3 (p73Δex2/3) or include exon 3B (ΔN'p73). In an analysis of ovarian cancers, expression of...
p73Δex2 was detected exclusively in cancer cell lines and invasive tumor tissues but not in semi-malignant borderline tumors (23). In an analysis of hepatocellular carcinomas, both p73Δex2 and p73Δex2/3 were shown to be up-regulated in tumor tissue compared with surrounding normal liver tissue. Considering that ectopic expression of these isoforms inhibits transactivation and apoptosis induction by p53 and TA-p73 and that many inhibitors of p53 act as transforming oncogenes, they might as well be involved in tumorigenesis (19). This hypothesis was supported by our own findings demonstrating that expression of p73Δex2/3 promotes anchorage-independent growth of NIH3T3 cells and tumor growth in nude mice.6

In this study we further characterized the effects of ΔTA-p73 expression and analyzed the mechanism of p53/p73 inhibition in more detail. Sequence-specific DNA binding is a prerequisite for regulation of specific genes, we first confirmed that ΔTA-p73 retains its DNA-binding competence and specificity for p53 binding sites. Due to the lack of the N-terminal transactivation domain, ΔTA-p73 acts as a DNA-binding factor without transactivation function, thereby acting as a dominant-negative inhibitor by blocking the proapoptotic activity of p53 and full-length p73.

In general, two basic mechanisms for inhibition of p53 are conceivable. First, ΔTA-p73 may physically interact with and sequester p53 to form hetero-oligomers that are transactivation-incompetent (33). In support of this theory, Kaghad et al. (3) demonstrated weak interactions between full-length p73 and p53 in a yeast two-hybrid assay. However, others failed to find an interaction between p53 and p73 using purified oligomerization domains or to detect hetero-oligomeric complexes of full-length p73 and wild-type p53 in coimmunoprecipitation and GST pull-down assays (37–40). Consistently, we were unable to detect a physical interaction of ΔTA-p73 and wild-type p53. As shown by Gaiddon and colleagues (40), interaction between mutant p53 and wild-type p73 is mediated by the p53 core domain and correlates with recognition of p53 by the conformation-sensitive monoclonal antibody PAb240. These data indicate that physical interaction between p53 and p73 requires
a special (mutant) conformation of the p53 core domain and explains specific interaction of \( \Delta T\alpha \)-p73 with mutant but not wild-type p53.

Second, as \( \Delta T\alpha \)-p73 retains the core DNA-binding domain and exhibits binding specificity for p53 binding sites, simple competition for DNA sites might prevent p53 or TA-p73 from binding to target gene promoters. This mechanism is supported by our competition EMSA, which demonstrates efficient dis-
ruption of p53 complexes by increasing amounts of ΔTA-p73. This competition is absolutely dependent on the DNA binding ability of ΔTA-p73, as shown by experiments with the DNA binding-defective mutant, R292H. Consistently, p53-mediated transactivation was inhibited by ΔTA-p73 but not ΔTA-p73R292H. In addition, inhibition of p53 targeted by fusion to the Gal4 DNA-binding domain to a Gal4-regulated promoter was significantly reduced. We could observe only a 50% reduction with the highest amount of ΔTA-p73. This might hint at additional inhibitory functions of ΔTA-p73 unrelated to interference with sequence-specific DNA-binding or simply be due to unspecific quenching effects. In summary, the data support strongly a model in which ΔTA-p73 exerts a dominant-negative effect by displacing p53 from target gene promoters (Fig. 8A).

In contrast, inhibition of full-length p73 appears to be more complex. Because both TA-p73 and ΔTA-p73 efficiently bind to target DNA, simple competition for DNA binding will certainly be involved. However, whereas ΔTA-p73 does not interact with wild-type p53, we observed protein-protein interaction between TA-p73 and ΔTA-p73 by in vivo immunoprecipitation. This interaction, which results in the formation of transactivation-defective hetero-oligomers, appears to be sufficient to inhibit transactivation by TA-p73. In fact, there is no difference in inhibition of TA-p73 by wild-type ΔTA-p73 and the DNA binding-defective mutant ΔTA-p73R292H, which still interacts with TA-p73 (data not shown). Our experiments therefore clearly demonstrate that the dominant-negative effect of ΔTA-p73 involves different mechanisms for inhibition of p53 and TA-p73 (Fig. 8B).

All of the experiments on ΔTA-p73 function by us and others relied on ectopic overexpression of ΔTA-p73. To explain the increased expression level of p73 in tumor tissues with the described dominant-negative effect of ΔTA-p73, it is therefore important to demonstrate that increased expression of endogenous p73 has a similar inhibitory effect on p53 function. It has recently been described that p73 is up-regulated during differentiation of neuroblastoma cells induced by RA (34). Consistently, treatment of wild-type p53-expressing SH-SY5Y cells with RA resulted in increased expression of p73. Because tumor tissues usually also show concomitant up-regulation of full-length and ΔTA-p73 expression,2 this system is an appropriate model to assess the net function of p73 overexpression. SH-SY5Y cells undergo p53-dependent apoptosis when treated with the protein kinase inhibitor H-7 (27). Although p53 protein was stabilized by H-7

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**Fig. 7.** Inhibition of p53 by up-regulation of endogenous p73. A, Western blot demonstrating induction of p73α in SH-SY5Y neuroblastoma cells by treatment with RA for 4 days. In vitro translated p73α proteins are shown for comparison. B, Western blot of whole cell extracts showing an increase in p53 protein levels by treatment with H-7 for 24 h in SH-SY5Y cells cultured for 4 days in the absence or presence of RA. C, MTT assay showing H-7 induced loss of cell viability in RA-treated and AdΔTA-p73-infected SH-SY5Y cells in comparison with mock and AdGFP-infected cells.

**Fig. 8.** Model for the dominant-negative mechanism of ΔTA-p73. A, inhibition of p53 by direct competition for promoter binding. B, inhibition of full-length TA-p73 by competition for promoter binding and/or formation of transactivation-defective hetero-oligomers.
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in both untreated and RA-treated cells, apoptosis was significantly reduced by RA treatment. Because ectopic expression of ΔTA-p73 had an effect similar to RA treatment on cell survival, it can be assumed that up-regulation of endogenous ΔTA-p73 is responsible for this effect.

Together, our data show that ΔTA-p73 proteins are effective inhibitors of p53 and TA-p73 function. Consistently, increased expression of endogenous p73 including expression of ΔTA-p73 provides a possible explanation for the high level of p73 expression in human cancer cells, even in the absence of p73 mutations, although additional work is needed to further investigate the role of ΔTA-p73 for tumor development and progression.

Acknowledgments—We thank A. Eggert for providing the neuroblastoma cell line and K. Lennars for support in flow cytometry analysis.

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J. Biol. Chem. 2002, 277:14177-14185.
doi: 10.1074/jbc.M200480200 originally published online February 13, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200480200

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