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Advances in Brief

Transactivation-deficient ΔTA-p73 Acts as an Oncogene[1]

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Abstract

The recently discovered p53-family member p73 displays significant homology to p53, but data from primary tumors and knockout mice argue against p73 being a classical tumor suppressor. We report on the overexpression of NH_2-terminally truncated, transactivation-deficient p73 proteins (ΔTA-p73) in human cancer cells. Moreover, we show that ΔTA-p73 overexpression results in malignant transformation of NIH3T3 fibroblasts and tumor growth in nude mice, thereby providing the experimental evidence for an oncogenic function of ΔTA-p73. Apparently, increased expression of NH_2-terminally truncated p73 isoforms conveys the *TP73* gene with oncogenic activity that appears to be actively selected for during tumor development.

Introduction

The *TP53* gene was the first tumor suppressor gene to be identified and is still considered to be the prototype tumor suppressor. In more than half of human tumors the *TP53* gene is directly inactivated by mutations, and in many others, p53 is functionally compromised epigenetically by various mechanisms (1). Although the recently discovered p53 family member p73 shows remarkable structural and functional similarities to p53, several pieces of evidence argue against p73 being a classical tumor suppressor. In contrast to mice lacking p53, p73-negative mice are not prone to tumor development (2). 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, 4). Instead, overexpression of wild-type p73 in various tumor types compared with normal tissues has been reported and shown to correlate with a poor patient prognosis (5–8). Together, these data raise the question about additional activities of p73 in cancer. In this study we report that p73 overexpression is associated with increased levels of NH_2-terminally truncated p73 proteins (ΔTA-p73). Moreover, we show that overexpression of ΔTA-p73 protein results in malignant transformation of NIH3T3 cells supporting an oncogenic function of ΔTA-p73. Therefore, our data provide a possible explanation for the observed overexpression of p73 in human cancers.

Materials and Methods

Cell Culture. Unless otherwise indicated, cell lines were obtained from American Type Culture Collection, Manassas, VA. HeLa, C-33A, H1299 (human bronchoalveolar carcinoma; obtained from B. Opalka, University of Essen, Essen, Germany), A549, AsPC-1, Capan-1, Capan-2, MZA (human pancreas carcinoma; obtained from D. I. Smith, Mayo Clinic, Rochester, MN), HT29, MCF-7, SK-OV-3, Saos-2, 293, NHDF cells (normal human diploid fibroblasts; obtained from M. Roggendorf, University of Essen), and NIH3T3 cells were maintained in DMEM supplemented with 10% FCS. K-562 ,and LAMA-84 chronic myeloid leukemia cell lines (obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and TT cells were grown in RPMI 1640 plus 15% FCS. The 4-OHT[3] inducible Saos-2 ER-E2F1 cells have been described previously (9). CHX (Sigma, Taufkirchen, Germany) was used at a final concentration of 10 μg/ml. For genotoxic treatment, NIH3T3 cells were exposed to 10 μg/ml Adr, 20 μg/ml CP, 250 ng/ml Act, or 400 μM Eto for 3 h. For gene expression analysis, cells were harvested 9 h later. Transfection of NIH3T3 cells was performed using Polyfect (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

HCC Tissue Samples. Tissue samples from HCC tissue and adjacent normal liver were obtained from patients undergoing partial hepatectomy at the Department of General and Transplantation Surgery at the University of Essen, Essen, Germany. The tissues were frozen and stored in liquid nitrogen until RNA extraction. Histological analysis showed >75% tumor cells in all of the tumor sections.

Plasmids and Recombinant Retroviruses. cDNAs encoding NH_2-terminally truncated p73 were amplified by PCR using HA-p73α as a template (kindly provided by G. Melino, University Tor Vergata, Rome, Italy), cloned into pcDNA3.1 and sequence-verified. ΔTA-p73β (aa72–499) was cloned into the retroviral vector pLXRN (Clontech, Palo Alto, CA). Retroviruses pseudotyped with the envelope glycoprotein from the vesicular stomatitis virus were produced as described (9). The luciferase-reporter plasmids (pGL3-p53 and pGL3basic) have been described (9).

IP and Western Blotting. Endogenous p73 from ~5 mg of radioimmunoprecipitation assay cell extract (50 mM Tris/HCL, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS) was immunoprecipitated with 1 μg of anti-p73 (ER15) antibody. Immunoprecipitates were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with various p73 antisera. Murine anti-p73 monoclonals ER15 and ER13 were obtained from Oncogene Science. Goat polyclonal anti-p73 (Ab-7) and rat monoclonal anti-H-ras (sc-35) were obtained from Santa Cruz Biotechnology, Santa Cruz, CA.

Two-dimensional Electrophoresis. For two-dimensional Western blot analysis, the anti-p73 immunoprecipitates were solubilized in 125 μl isoelectric focusing rehydration buffer [8 M urea, 1 mM DTT, 1% 3-[(3-cholamidopropyl)dimethylammonio]-1 -propanesulfonic acid, 0.25% Bio-Lyte (pH 3–10), and 5 mM Pefabloc] and subjected to isoelectric focusing using IPG strips (pH 3–10; Bio-Rad, Munich, Germany) with a total of 20,000 Vh. IPG strips were equilibrated in SDS equilibration buffer [6 M urea, 30% glycerol, 2% SDS, and 50 mM Tris-HCl (pH 8.8)] and subjected to second dimension separation on 8% SDS-PAGE.

RT-PCR, Southern Blotting, and Luciferase Assays. Semiquantitative RT-PCR was carried out on total RNA prepared with the RNeasy Mini kit (Qiagen) essentially as described (9). 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 α-[^{32}P]dCTP for high-sensitivity detection. The amount of PCR product was quantitated on a phosphorimager. Primer sequences were as follows: p73: 5′-GACGGAATTCAC-CACCATCCT-3′ and 5′-CCAGGCTCTCTTTCAGCTTCA-3′; TA-p73: 5′-GGCTGCGACGGCTGCAGAGC-3′ and 5′-GCTCAGCAGATTGAACTG-GGCCATG-3′; p73Δex2 and p73Δex2/3: 5′-GGCTGCGACGGCTGCA-

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[3] The abbreviations used are: 4-OHT, 4-hydroxytamoxifen; CHX, cycloheximide; RT-PCR, reverse transcription-PCR; Adr, Adriamycin; IP, immunoprecipitation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCC, hepatocellular carcinoma; Act, actinomycin D; CP, cisplatin; Eto, etoposide; ER, estrogen receptor.

GGGA-3′ and 5′-GGCTGCGACGGCTGC AGGCC-3′ as the sense primers and 5′- CAGGCGCCGGCGACATGG-3′ as the antisense primer; ΔN′-p73: 5′-TCGACCTTCCCCAGTCAAGC-3′ and 5′-TGGGACGAGGCATGGATCTG-3′; ΔN-p73: 5′-CAAACGGCCCGCATGTTCCC-3′ and 5′-TTGAACTGGGCCGTGGCGAG-3′; and p73–1/4: 5′-ACGCAGCGAAACCGGGGCCCG-3′ and 5′-GCCGCGCGGCTGCTCATCTGG-3′. For Southern blotting, RT-PCR products were separated on 2% agarose gels, transferred to Hybond-N+ nylon membranes (Amersham Pharmacia Biotech), and probed with the [^{32}P]dCTP end-labeled oligonucleotide p73-exon4 5′-TTCATGGTGCTGCTCAGCAGATTGAACTGGGC-3′. Luciferase assays were essentially performed as described (9).

Real-Time RT-PCR. MDM2 and p21 transcripts levels were quantitated on a LightCycler (Roche Diagnostics) using SYBR Green I. Primer sequences were as follows: MDM2: 5′-GGATCCTTTGCAAGCGCCAC-3′ and 5′-TCAAAGGACAGGGACCTGCG-3′; and p21: 5′-TTTCAGCCACAGCGACCATG-3′ and 5′-AAAGTTCCACCGTTCTCGGG-3′. Transcript levels were normalized to GAPDH expression and presented as the fold induction relative to untreated control cells.

Tumorigenicity Assays. For soft agar analysis, 10^4 cells in DMEM/10% FCS were mixed with an equal volume of 2% methylcellulose medium and poured onto a bed of 0.6% agarose. After 3 weeks, colonies were microscopically counted and measured. All of the experiments were performed in triplicate. Tumor growth in nude mice was assayed by injecting 5×10^6 cells in 200 μl of DMEM s.c. into the flanks of nude mice. Tumor dimensions were measured in two perpendicular diameters. Care of experimental animals was in accordance with institutional animal care and use committee guidelines.

Results and Discussion

Identification of NH_2-terminally Truncated p73 Protein in Human Tumor Cells. Analysis of p73 protein in a panel of human tumor cell lines showed various expression levels ranging from absence of p73 protein in MCF-7 cells to high-level expression in 293 cells (Fig. 1*A*). p73 expression in 293 cells is consistent with p73 induction by the adenoviral *E1A* oncogene (10). Interestingly, high-level p73 expression correlated with a complex isoform pattern in MZA, K562, and 293 cells. All of the detected bands share the epitope of the ER15 antibody in exon 11 (data not shown), which is only present in the p73α and β-isoforms, but not in the other COOH-terminal variants described thus far, suggesting that the additional bands might be because of either novel COOH-terminal variants or NH_2-terminally truncated forms. These can be differentiated by the Ab-7 antibody, which was raised against amino acids 1–15 and detects only full-length p73α and p73β (Fig. 1*B*). A detailed analysis of p73 immunoprecipitates from 293 cells reveals four distinct isoforms (Fig. 1*C*). In addition to the previously described full-length p73 proteins TA-p73α and TA-p73β, we could identify two novel isoforms, which lack the NH_2-terminal epitope recognized by the Ab-7 antibody. Consistent with the lack of the acidic NH_2-terminal transactivation domain they have a more basic isoelectric point as determined by two-dimensional gel electrophoresis (Fig. 1*D*). They appear as a series of spots differing in isoelectric point, potentially reflecting post-translational modifications. Because of the lack of the major transactivation domain, these two isoforms are termed ΔTA-p73α and ΔTA-p73β. These results demonstrate that several human tumor cells express NH_2-terminally truncated p73 proteins.

Transcripts Encoding ΔTA-p73. Using RT-PCR analysis, we detected an alternative transcript derived from a cryptic promoter in intron 3, which is termed ΔN-p73 because of its homology to murine ΔN-p73 (11–13). It encodes a NH_2-terminally truncated p73 protein in which the 61 NH_2-terminal amino acids are replaced by 13 amino acids encoded by the alternative exon 3B. In addition, we detected three alternatively spliced transcripts (p73Δex2, p73Δex2/3, and ΔN′-p73) derived from the TA promoter. p73Δex2 and p73Δex2/3 aberrantly lack either exon 2, or exons 2 and 3. Because the translation start of the full-length transcript is located in exon 2, both alternatively spliced transcripts encode ΔTA-p73 proteins starting with amino acids 49 and 72, respectively (Fig. 2, *A* and *B*). The third TA promoter-derived transcript, ΔN′-p73, aberrantly includes the 3′-portion (198 bp) of the alternative exon 3B and encodes for the same protein as the ΔN-p73 transcript (Fig. 2*B*; Ref. 13). In the following, all of the NH_2-terminally truncated isoforms (p73Δex2, p73Δex2/3, ΔN-p73, and ΔN′-p73) are collectively termed "ΔTA-p73."

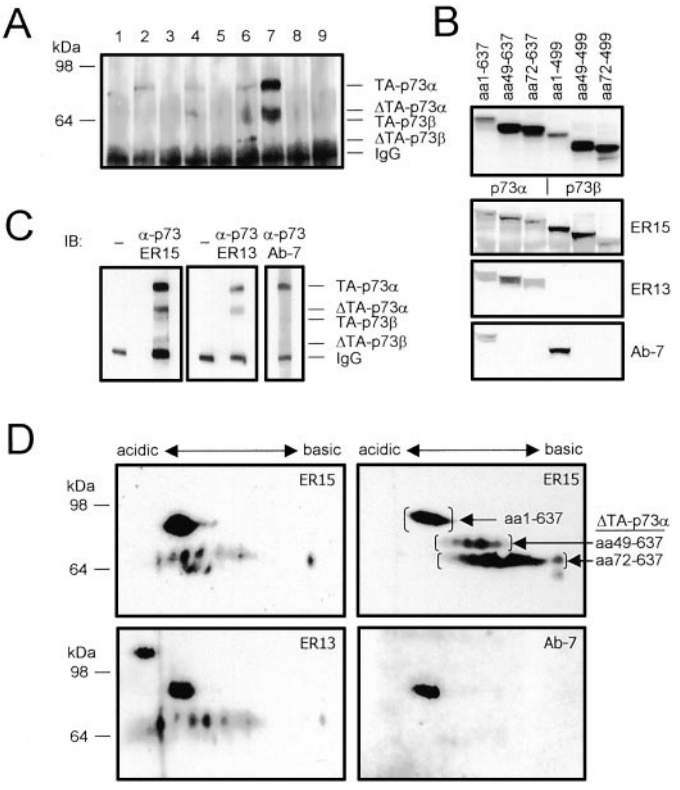

Fig. 1. Identification of NH_2-terminally truncated p73 proteins in human cancer cells. *A*, IP-Western blot analysis of p73 in various cancer cell lines. The observed bands are labeled according to their reactivity with specific antibodies as determined in *C*. *Lanes 1*, HeLa; *2*, C-33A; *3*, H1299; *4*, MZA; *5*, MCF-7; *6*, K-562; *7*, 293; *8*, HepG2; *9*, NHDF (normal human diploid fibroblasts). *B*, Western blot analysis of *in vitro* translated, ^{35}S-labeled NH_2-terminal deletion mutants of p73. The *upper panel* shows an autoradiography, the *lower panels* immunoblots with three different p73 antibodies. *C*, IP-Western blot analysis of 293 cell extracts with the indicated p73 antibodies. The *first* and *third lane* show appropriate controls without the primary antibody. The bands are labeled according to their reactivity with the various antibodies. *D*, two-dimensional IP-Western blot analysis. Analysis of recombinant proteins TA-p73α, ΔTA-p73 (amino acids 49–637), and ΔTA-p73 (amino acids 72–637) expressed in H1299 cells is shown in the *upper right panel* with *brackets* denoting the location of the individual proteins. Analyses of endogenous p73 proteins from 293 cells is shown in the other panels.

Semiquantitative RT-PCR analysis of a broad panel of human tumor cell lines showed that p73 was either barely detectable or abundantly expressed (Fig. 2*C*). Interestingly, those cell lines showing high-level expression of p73 also expressed high amounts of all of the various ΔTA-p73 isoforms described above. Simultaneous amplification of various NH_2-terminal transcripts showed that expression of the truncated transcripts is comparable with the amount of full-length transcripts with slight variations between the different cell lines (Fig. 2*D*). This indicates that overexpression of p73 in tumors may not be limited to expression of the full-length, transactivation-competent TA-p73 isoforms but also includes expression of the various ΔTA-p73 isoforms (p73Δex2, p73Δex2/3, ΔN′-p73, and ΔN-p73). However, the relative contribution of these transcripts to the observed overexpression of ΔTA-p73 protein in tumor tissues is not clear at present. Nevertheless, our analysis showed a concomitant increase in mRNA levels for all four of the ΔTA-p73-encoding transcripts, suggesting

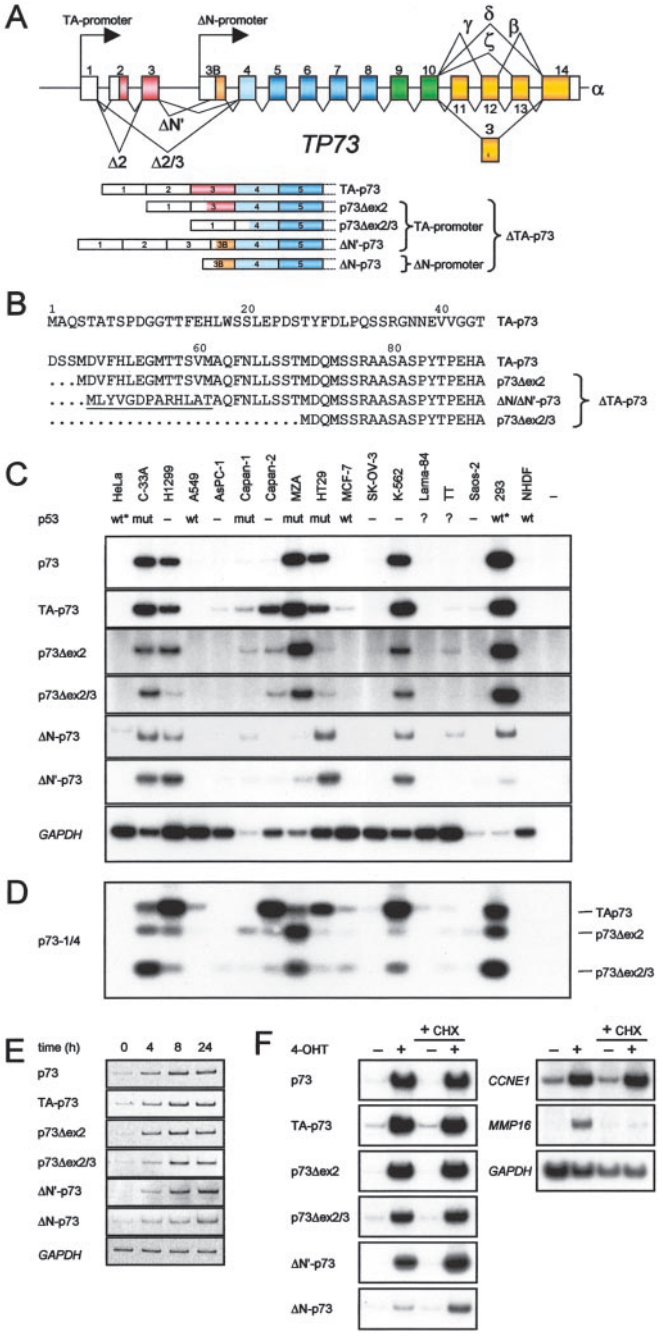

Fig. 2. Transcripts encoding ΔTA-p73. *A*, genomic organization of the *TP73* locus. The splicing patterns generating COOH-terminal isoforms p73 α, β, γ, δ, ϵ, and ζ, and the NH_2-terminal isoforms p73Δex2, p73Δex2/3, and ΔN′-p73 are shown. Transcriptional start sites are indicated by *arrows*. The ΔN-p73 isoform is generated from a cryptic promoter within intron 3. Color coding: *red*, transactivation domain; *orange*, exon 3B-derived coding sequence; *blue*, DNA-binding domain; *green*, oligomerization domain; *yellow*, COOH terminus; *white*, noncoding sequences. *B*, alignment of p73 NH_2-terminal isoform amino acid sequences. The *underlined* amino acids are unique to the ΔN-p73 and ΔN′-p73 isoforms, and are derived from the alternative exon 3B located in intron 3. *C*, semiquantitative RT-PCR analysis of p73 expression in various cancer cell lines. p73 transcripts were amplified with specific primer pairs and the radioactively labeled RT-PCT products visualized by autoradiography. *NHDF*, normal human diploid fibroblasts; −, no template control. The p53 status is indicated; ∗, inhibition of wild-type p53 by HPV E6 or adenoviral E1B-55K; ?, p53 status unknown; −, p53-deleted. *D*, Southern blot of RT-PCR products. NH_2-terminally truncated p73 transcripts were simultaneously amplified with primers corresponding to sequences in exon 1 and 4, respectively. Probing of a Southern blot of the RT-PCR products with an oligonucleotide from exon 4 revealed distinct transcripts with similar expression levels depending on the cell line analyzed. Lanes are labeled as in *C*. *E*, induction of ΔTA-p73 by E2F1. E2F1 activity was induced with 4-OHT in ER-E2F1-expressing Saos-2 cells for the time indicated. Total RNA was analyzed for the various ΔTA-p73 encoding transcripts by semiquantitative RT-PCR. *F*, TA promoter-derived ΔTA-p73 transcripts are direct targets of E2F1. E2F1 activity was induced in the absence or presence of CHX. Total RNA was analyzed by semiquantitative RT-PCR. *CCNE1* is shown as a control for a direct, *MMP16* for an indirect E2F1 target gene.

that all four might contribute to increased ΔTA-p73 protein levels in tumor cells.

Induction of ΔTA-p73 by E2F1. Expression of p73 is regulated by E2F, which shows deregulated activity in most tumors (9, 14). Therefore, increased levels of p73 in cancer tissues might be attributable to increased transcriptional activation of the *TP73* gene by E2F. To analyze the regulation of the various p73 isoforms by E2F, we used Saos-2 cells that constitutively express E2F1 fused to a modified, 4-OHT-responsive version of the ligand binding domain of the mouse ER as described previously (9). Conditional activation of E2F1 in the presence of 4-OHT resulted not only in an increase of total p73 mRNA levels but also in a parallel induction of both full-length and NH_2-terminally truncated p73 isoforms (Fig. 2*E*). Activation of the ER-E2F1 fusion protein in the presence of the protein synthesis inhibitor CHX allows us to discriminate a direct effect of E2F1 on p73 isoform expression from secondary effects because of E2F1-mediated cell cycle stimulation. All of the TA promoter-derived transcripts (TA-p73, p73Δex2, p73Δex2/3, and ΔN′-p73) were strongly induced by E2F1 (~23.7-fold) even in the presence of CHX, whereas the ΔN-promoter-regulated ΔN-p73 transcript showed only a modest induction (~2.2-fold). Therefore, at least the TA promoter-derived ΔTA-p73 isoforms are direct transcriptional targets of E2F1 (Fig. 2*F*) suggesting that increased expression levels of ΔTA-p73 in tumor cells are also attributable to increased E2F activity. Depending on the cellular context E2F1 apparently exhibits a dual function as an oncogene and a tumor suppressor by stimulating both proliferation and apoptosis (15). Therefore, activation of both proapoptotic full-length and transforming ΔTA-p73 isoforms by E2F1 appears to be intriguingly similar.

Increased Expression Levels of ΔTA-p73 in HCCs. Importantly, the correlation between increased expression of p73 and ΔTA-p73 is not only found in cell lines but also in primary tumor samples. As has been reported, p73 overexpression is a common finding in HCCs and is significantly correlated with a poor patient survival prognosis (7). Consistent with their data, our analysis of the various NH_2-terminal p73-isoforms by RT-PCR clearly showed enhanced expression of p73 in most HCC samples compared with adjacent normal liver tissue (Fig. 3). Of note, all of the samples with high level p73 overexpression (samples #1, #3, #5, and #6) demonstrated tumor-specific up-regulation of the TA promoter-derived alternatively spliced ΔTA-p73 isoforms (p73Δex2, p73Δex2/3, and ΔN′-p73), whereas the ΔN-p73 transcript was barely detectable (data not shown). These data are consistent with findings by Sayan *et al.* (11), who also failed to detect tumor-specific up-regulation of the ΔN-p73 transcript. This underlines the importance of the TA promoter-derived alternatively spliced tran-

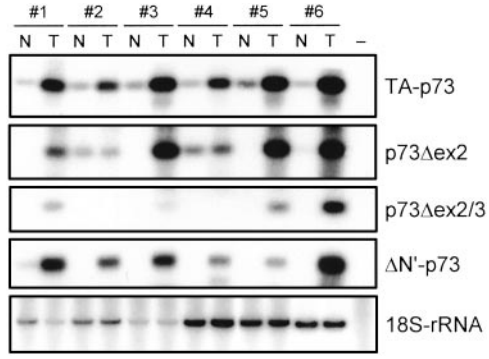

Fig. 3. Increased expression levels of ΔTA-p73 in HCCs. Expression of NH_2-terminal p73 isoforms was analyzed by semiquantitative RT-PCR in six matched pairs of tumor (*T*) and adjacent normal (*N*) liver tissue. Amplification of 18S-rRNA demonstrates use of equal amounts of total RNA. −, no template control.

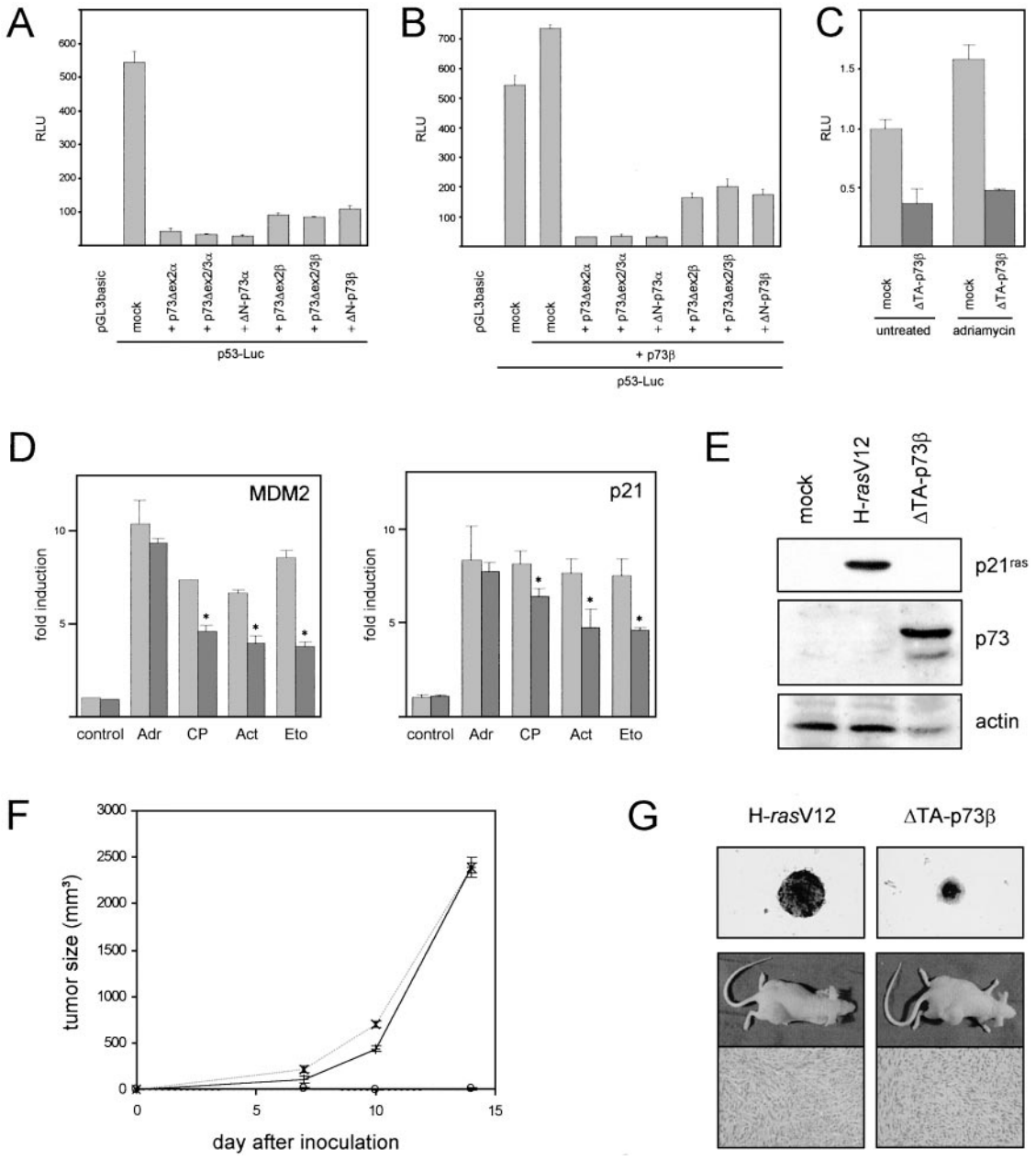

Fig. 4. ΔTA-p73 acts as a transforming oncogene in NIH3T3 cells. *A*, luciferase assay demonstrating the dominant-negative function of ΔTA-p73. Four hundred ng of a reporter plasmid together with 150 ng of the indicated ΔTA-p73 plasmid were transfected into NIH3T3 cells and assayed for luciferase activity after 48 h. *B*, ΔTA-p73 acts as a dominant-negative inhibitor of full-length p73β. Transfections including 50 ng p73β plasmid were performed as described in *A*. *C*, suppression of p53-dependent reporter activity by ΔTA-p73β following genotoxic damage induced by Adr. Mock or ΔTA-p73β-expressing NIH3T3 cells were transfected with 100 ng p53-reporter plasmid and treated 24 h later with 3 μM Adr as indicated for another 24 h before luciferase measurement. *D*, induction of endogenous p53 targets genes by various genotoxic agents (Adr, CP, Act, and Eto) in parental (*light gray*) or ΔTA-p73β-expressing (*dark gray*) NIH3T3 cells. Transcript levels for MDM2 and $p21^{Waf1}$ were assessed by quantitative RT-PCR and normalized to GAPDH expression. Fold induction was calculated in relation to untreated control cells. Data are expressed as mean. $*P < 0.05$ (*t* test). *E*, Western blot of stably transduced NIH3T3 cells with antibodies against $p21^{ras}$, p73, and actin. *F*, tumor growth kinetics of mock- (○), ΔTA-p73β- (+), or H-*ras*V12- (×) transduced NIH3T3 cells inoculated s.c. into the flank of athymic nude mice. Shown is the average from three mice per cell population. *G*, anchorage-independent growth in semi-solid medium of ΔTA-p73β or H-*ras*V12 expressing NIH3T3 cells. Mock-infected cells did not form any colonies. Representative colonies from both cell populations are depicted in the *upper panel*. Photographs of nude mice 2 weeks after injection of ΔTA-p73β- or H-*ras*V12-infected NIH3T3 cells (*middle panels*). Corresponding formalin-fixed and paraffin-embedded tumor sections stained with H&E show a typical sarcomatous morphology (*lower panels*); *bars*, ± SD.

scripts and questions the relevance of the ΔN-promoter for hepatocarcinogenesis.

Overexpression of ΔTA-p73 Results in Malignant Transformation of NIH3T3 Cells. Recently, we and others have shown that NH_2-terminally truncated p73 isoforms act as dominant-negative inhibitors of both full-length p73 and wild-type p53 (12, 16–18). As shown in Fig. 4, *A* and *B*, expression of the various ΔTA-p73 isoforms significantly reduced the activity of a p53/p73-dependent luciferase reporter in NIH3T3 cells. In addition, ΔTA-p73β expression markedly reduced the p53/p73-response to genotoxic damage induced by various chemotherapeutic agents. Both induction of a p53/p73-dependent luciferase reporter (Fig. 4*C*) and the endogenous p53/p73 target genes *MDM2* and $p21^{Waf1}$ (Fig. 4*D*) were reduced significantly in the presence of ΔTA-p73β. Because many potent inhibitors of p53 act as transforming oncogenes, we examined the possibility that ΔTA-p73 might promote anchorage-independent growth as a hallmark of tumor cells. We stably transduced NIH3T3 cells with retroviral vectors expressing ΔTA-p73β or H-*ras*V12 as a positive control.

We verified expression of the transgenes by Western blot (Fig. 4*E*). As shown in Fig. 4*G*, colony formation in semi-solid medium was detectable in ΔTA-p73β and H-*ras*V12-expressing cell populations, whereas controls did not form any colonies. Although colonies from H-*ras*V12-expressing cells were significantly larger than colonies from ΔTA-p73β-expressing cells (Fig. 4*G*), the total number of colonies was equivalent for both cell populations (ΔTA-p73β: 105 ± 8, H-*ras*V12: 108 ± 9). After injection into nude mice they formed rapidly growing tumors of sarcomatous morphology within 2 weeks comparable with cells transformed with the *bona fide* oncogene H-*ras*V12 (Fig. 4, *F* and *G*). In contrast, control vector-transduced cells were nontumorigenic. Interestingly, our analysis of primary HCC samples revealed expression of wild-type p53 in all of the cases except for sample #6, which is negative for p53 expression (data not shown), suggesting that high levels of ΔTA-p73 might serve to suppress wild-type p53 function and, thus, remove the selection pressure for loss of p53 function. However, it remains speculative whether inhibition of p53 is the major mechanism of transformation by ΔTA-p73. Several studies on primary tumor samples failed to establish a significant correlation between overexpression of p73 and the p53 status (5, 7, 19, 20), raising the possibility that overexpression of p73 or ΔTA-p73, respectively, may also result in a p53-independent enhancement of oncogenicity. Together, our data strongly support that ΔTA-p73 acts as an oncogene by triggering intracellular signaling cascades leading to cell transformation and tumorigenicity, and provide one possible explanation for high-level p73 expression in human cancer in the absence of p73 mutations.

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References

1. Vogelstein, B., Lane, D., and Levine, A. J. Surfing the p53 network. Nature (Lond.), *408:* 307–310, 2000.
2. Yang, A., Walker, N., Bronson, R., Kaghad, M., Oosterwegel, M., Bonnin, J., Vagner, C., Bonnet, H., Dikkes, P., Sharpe, A., McKeon, F., and Caput, D. p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. Nature (Lond.), *404:* 99–103, 2000.
3. Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Lelias, J. M., Dumont, X., Ferrara, P., McKeon, F., and Caput, D. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell, *90:* 809–819, 1997.
4. Stiewe, T., and Putzer, B. M. p73 in apoptosis. Apoptosis, *6:* 447–452, 2001.
5. Zaika, A. I., Kovalev, S., Marchenko, N. D., and Moll, U. M. Overexpression of the wild type p73 gene in breast cancer tissues and cell lines. Cancer Res., *59:* 3257–3263, 1999.
6. Yokomizo, A., Mai, M., Tindall, D. J., Cheng, L., Bostwick, D. G., Naito, S., Smith, D. I., and Liu, W. Overexpression of the wild type p73 gene in human bladder cancer. Oncogene, *18:* 1629–1633, 1999.
7. Tannapfel, A., Wasner, M., Krause, K., Geissler, F., Katalinic, A., Hauss, J., Mossner, J., Engeland, K., and Wittekind, C. Expression of p73 and its relation to histopathology and prognosis in hepatocellular carcinoma. J. Natl. Cancer Inst., *91:* 1154–1158, 1999.
8. Novak, U., Grob, T. J., Baskaynak, G., Peters, U. R., Aebi, S., Zwahlen, D., Tschan, M. P., Kreuzer, K. A., Leibundgut, E. O., Cajot, J. F., Tobler, A., and Fey, M. F. Overexpression of the p73 gene is a novel finding in high-risk B-cell chronic lymphocytic leukemia. Ann. Oncol., *12:* 981–986, 2001.
9. Stiewe, T., and Putzer, B. M. Role of the p53-homologue p73 in E2F1-induced apoptosis. Nat. Genet., *26:* 464–469, 2000.
10. Zaika, A., Irwin, M., Sansome, C., and Moll, U. M. Oncogenes Induce and Activate Endogenous p73 Protein. J. Biol. Chem., *276:* 11310–11316, 2001.
11. Sayan, A. E., Sayan, B. S., Findikli, N., and Ozturk, M. Acquired expression of transcriptionally active p73 in hepatocellular carcinoma cells. Oncogene, *20:* 5111–5117, 2001.
12. Grob, T. J., Novak, U., Maisse, C., Barcaroli, D., Luthi, A. U., Pirnia, F., Hugli, B., Graber, H. U., De, L. V., Fey, M. F., Melino, G., and Tobler, A. Human ΔNp73 regulates a dominant negative feedback loop for TAp73 and p53. Cell Death Differ., *8:* 1213–1223, 2001.
13. Ishimoto, O., Kawahara, C., Enjo, K., Obinata, M., Nukiwa, T., and Ikawa, S. Possible oncogenic potential of [δ]Np73: a newly identified isoform of human p73. Cancer Res., *62:* 636–641, 2002.
14. Irwin, M., Marin, M. C., Phillips, A. C., Seelan, R. S., Smith, D. I., Liu, W., Flores, E. R., Tsai, K. Y., Jacks, T., Vousden, K. H., and Kaelin, W. G. Role for the p53 homologue p73 in E2F-1-induced apoptosis. Nature (Lond.), *407:* 645–648, 2000.
15. Phillips, A. C., and Vousden, K. H. E2F-1 induced apoptosis. Apoptosis, *6:* 173–182, 2001.
16. Pozniak, C. D., Radinovic, S., Yang, A., McKeon, F., Kaplan, D. R., and Miller, F. D. An anti-apoptotic role for the p53 family member, p73, during developmental neuron death. Science (Wash. DC), *289:* 304–306, 2000.
17. Stiewe, T., Theseling, C. C., and Putzer, B. M. Transactivation-deficient ΔTA-p73 inhibits p53 by direct competition for DNA-binding: implications for tumorigenesis. J. Biol. Chem., *277:* 14177–14185, 2002.
18. Fillippovich, I., Sorokina, N., Gatei, M., Haupt, Y., Hobson, K., Moallem, E., Spring, K., Mould, M., McGuckin, M. A., Lavin, M. F., and Khanna, K. K. Transactivation-deficient p73α (p73Δexon2) inhibits apoptosis and competes with p53. Oncogene, *20:* 514–522, 2001.
19. Ng, S. W., Yiu, G. K., Liu, Y., Huang, L. W., Palnati, M., Jun, S. H., Berkowitz, R. S., and Mok, S. C. Analysis of p73 in human borderline and invasive ovarian tumor. Oncogene, *19:* 1885–1890, 2000.
20. Chen, C. L., Ip, S. M., Cheng, D., Wong, L. C., and Ngan, H. Y. P73 gene expression in ovarian cancer tissues and cell lines. Clin. Cancer Res., *6:* 3910–3915, 2000.

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