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New antibodies recognizing p73: Comparison with commercial antibodies

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Abstract

p73, unlike p53, is expressed as a number of isomeric forms. Alternative splicing at the 3′ end of p73 transcript, together with the usage of a second promoter downstream of exon 3, can generate up to 24 p73 isoforms. Variants lacking the TA domain (ΔN isoforms) are induced by TAp73 and by p53, and inhibit their transcriptional activity. However, understanding the complex biology of p73 has been handicapped by the lack of high affinity specific antibodies for the different isoforms. Here, we report the characterization, by Western blotting and immunoprecipitation, of three new polyclonal antisera recognizing all p73 isoforms, only ΔN isoforms or only p73α, and which have advantages of affinity and specificity over previously available antibodies.

Keywords: p53 family; p53; p73; TP73; Apoptosis; DNA damage

p73, together with p63, is a member of the p53 tumour suppressor family [1–3]. Although all three family members, p53, p63, and p73, have a similar modular structure, with a high degree of sequence homology particularly in the DNA binding domain, their detailed biochemistry and function show significant differences [1,4]. Unlike p53, p73 is expressed in at least six C-terminal isomeric forms, derived by alternative splicing [5,6]. In addition, these C-terminal p73 isomers can also be transcribed from a second promoter located in intron 3 [7,8]. These N-terminal (ΔN) variants therefore lack the TA domain, but because they retain the ability to bind to cognate promoter DNA, they inhibit the transactivational activity of the full-length TA isoforms and of p53 [7–9]. ΔNp73 isoforms are induced by p53 and TAp73, thereby forming a negative feedback loop [8]. Similar N-terminally truncated forms of TAp73, lacking one or more of the N-terminal exons, also appear to arise spontaneously in cancers [10].

In terms of function, although both p53 and TAp73 activate an overlapping set of genes, including those involved in cell cycle arrest and apoptosis, p73 null mice show developmental defects, particularly in the nervous system, whereas p53 null animals have an increased incidence of spontaneous and experimentally induced tumours [7]. Moreover, p73 mutations in human cancers are extremely rare, although mutational inactivation of p53 is found in approximately 50% of human malignancies [4]. However, the functional distinction between developmental p73 and tumor suppressor p53 may not

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be absolute, since high expression of ΔNp73 has been correlated with poor prognosis in a number of human tumor types [11–13].

Research on p73 in both development and cancer has so far been handicapped by the lack of available high affinity, high isoform specificity antibodies. Since intracellular levels of p73 are low, currently available antibodies mostly require subcellular fractionation and analysis of changes in nuclear p73 protein. Moreover, some of the available antibodies are of questionable specificity and few detect more than one p73 isoform. In an attempt to overcome these problems, we have generated a new set of polyclonal p73 antibodies, and here compare their characteristics with a range of widely used antibodies available commercially.

Materials and methods

Antibody. The N-terminal 12 aminoacid peptide of ΔNp73 was synthesized (Moravian Biotechnology, Czech Republic) and coupled at its C-terminus to keyhole limpet hemocyanin (KLH) with glutaraldehyde and/or carbodiimide. Full-length TAp73α (amino acids 1–636) and the SAM domain of p73 (amino acids 487–554) were cloned into the pET101/D vector with an N-terminal 6 histidine tag. Recombinant proteins were expressed in *Escherichia coli* (BL21) and purified under denaturing conditions using Ni–NTA resin. Proteins were then refolded and buffer exchanged to PBS using NAP buffer exchange columns (Amersham). Finally, proteins were concentrated using Centripreps (Millipore). Antisera were generated by subcutaneous injection of peptide conjugates or recombinant proteins emulsified with Titermax adjuvant (Stratech Scientific, UK) into New Zealand White rabbits at 4 week intervals. Serum was collected prior to the first immunization (pre-immune serum) and after the third immunization with peptide conjugate. The immune sera obtained are referred to as p73FL (anti-full-length TAp73), p73SAM (anti-SAM domain, i.e., anti-p73α), and p73DN (anti-ΔNp73).

Cell culture and transfection. All cell lines were grown in Dulbecco's modified essential medium supplemented with 10%(v/v) fetal bovine serum (FCS), 1.2 g of bicarbonate per liter, 1% non-essential amino acids, and 15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid at 37 °C in a humidified atmosphere of 5%(v/v) CO_2 in air. SK-N-SH cells were differentiated with 5 μM all-*trans*-retinoic acid for 5 days in the dark. SaOs-2-inducible cells were grown in RPMI medium with tetracycline free FCS and induced with 2 μg/ml deoxycycline for 24 h. All of the p73 and p63 isoforms were HA-tagged whereas p53 was untagged. SaOs-2 cells were transfected with 27 μg of the indicated plasmids using Lipofectamine 2000 transfection reagent (Life Technologies) according to the manufacturer's protocol. We have also used TAp73α, β, γ, and ΔNp73α SaOs-2-inducible cell lines [14]. HEK293 cells were drug treated for 24 h. The drugs and their concentrations were: 5-fluorouracil (5-FU, 10 μg/ml), tricostatin A (TSA, 10 μM), tunicamycin (Tun, 5 μg/ml), 5-methyl azacytidine (5-Aza, 4.4 μM), *N*-acetyl cysteine (NAC, 100 μg/ml), etoposide (Etop, 20 μM), and staurosporine (Stau, 20 μg/ml). p53-deficient H1299 cells were treated with 20 μm etoposide and 2.5 μg/ml 5-fluorouracil (5-FU) for 24 h.

Western blotting. For overexpression analysis, induced (p53, TAp73α-HA, Tap73β-HA, and Tap73γ-HA) and transfected (p53-HA, ΔNp73α-HA, TAp63α-HA, and ΔNp63α-HA) SaOs-2 cells were trypsinized, pelleted, and washed with PBS. The pellet was then resuspended in lysis buffer (50 mM Tris–HCl, pH 7.5, 500 mM NaCl, 1% Triton X-100, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM DTT, 1 mM EDTA, and 1 mM AEBSF) and sonicated. The homogenate was kept for 1 h in ice and then centrifuged at 10,000*g* for 30 min at +4 °C. The supernatant was recovered and used for Western blot analysis.

Ten micrograms of protein was loaded when using p73FL and p73SAM while 50 μg of protein was loaded when using Ab2 and Ab4 commercial antibodies. Proteins were separated on 10% SDS–polyacrylamide gels and blotted onto PVDF sheets. Filters were blocked with 10% non-fat dried milk and 5% bovine serum albumin for 2 h, and then incubated for 2 h with new p73α antibodies p73FL and p73SAM (diluted 1:5000 in blocking solution) or Ab2 and Ab4 antibodies (1:100 in blocking solution). After three washes with PBS + 0.1% Tween 20, filters were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:20,000 in blocking solution) for 1 h. Detection was performed using West Dura Chemiluminescence System (Pierce).

To test the ΔNp73 antibody (p73DN), SaOs-2 cells were transfected with TAp73 (α, β, ε, γ) and ΔNp73 (α, β, ε, γ) constructs without an HA tag. Cells were resuspended in 6 ml of hypotonic lysis buffer (10 mM Tris–HCl, pH 7.5, 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, and 1 mM AEBSF) and kept for 1 h in ice. After lysis, 9 ml of 30% sucrose solution in hypotonic lysis buffer was added and the mixture was centrifuged at 2000*g* for 15 min. Pellets were washed with hypotonic lysis buffer, and purified nuclei were collected in the final pellet and resuspended in a lysis buffer containing detergents. Forty micrograms of nuclear proteins was loaded onto 10% SDS–polyacrylamide gels and blotted onto PVDF sheets. Filters were blocked with 10% non-fat dried milk and 5% bovine serum albumin for 2 h, and then incubated for 2 h with p73DN (diluted 1:1000 in blocking solution). After three washes with PBS + 0.1% Tween 20, filters were incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:20,000 in blocking solution) for 1 h. Detection was performed as above.

Evaluation of commercial antibodies in Western blotting was performed following the protocols of suppliers. The antibodies evaluated were: Ab2, Ab4, and Ab5 from Neomarkers; Ab-1 (OP181) from Oncogene Research; IMG 246 and IMG 259 from Imgenex; and E4, H79, C17, and C20 from Santa Cruz (see also Table 1).

Nuclei from SK-N-SH and HaCat cells, for analysis of endogenous p73 proteins, were purified as described above. One hundred micrograms of nuclear protein was loaded onto 10% SDS–polyacrylamide gels and blotted onto PVDF sheets. Filters were blocked with 10% non-fat dried milk and 5% bovine serum albumin for 2 h, and then incubated for 2 h with p73FL (diluted 1:3000 in blocking solution). Detection was performed as above.

Total proteins from HEK293 cells were extracted by suspending the drug treated or untreated cells in lysis buffer (50 mM Tris–HCl, pH 7.5, 500 mM NaCl, 1% Triton X-100, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM DTT, 1 mM EDTA, and 1 mM AEBSF), followed by sonication and centrifugation at 13K rpm for 15 min at 4 °C. The proteins were separated on an 8% gel and transferred onto PVDF filters. Then the filters were blocked and incubated with p73SAM (1:5000 dilution). Detection was performed as above.

Immunoprecipitation. TAp73α SaOs-2-inducible cells were induced with doxycycline for 24 h and nuclei were purified as described above. Two hundred micrograms of nuclear protein was diluted in binding buffer (25mM Tris–HCl, pH 7.5, 250 mM NaCl, 0.5% TX-100, 0.25% NP-40, 0.25% sodium deoxycholate, 1 mM DTT, 1 mM EDTA, and 1 mM AEBSF) and incubated overnight with 25 μl of equilibrated protein A agarose-conjugated beads and with 1–2 μl p73FL or 10 μl (2 μg) Ab4 antibody. Beads were then pelleted at 10,000*g* for 1 min and washed three times with binding buffer. Immunoprecipitates were eluted by resuspending agarose beads with 50 μl Laemmli buffer and by boiling at 100 °C for 10 min.

For evaluating commercial antibodies in immunoprecipitation, 150 μg of overexpressed TAp73α (for C17, H79, E4, Ab4, Ab5, Ab6, IMG 246, and IMG 259) or TAp73β (for C20) or ΔNp73α (for OP181) was made up to 1 ml with binding buffer, pre-cleared with protein A

Table 1
Evaluation of p73 antibodies in different techniques

Antibody	Epitope	Species of origin	Species specificity	Detected p73 isoforms	Detection of p73 in overexpression			Detection of endogenous p73			References
					WB	IP	IF	WB	IP	IF	
New antibodies											
p73FL	Full-length p73	Rabbit	Human, mouse, and monkey	All p73 isoforms	+	+	+	+	+	+	
p73SAM	aa 487–534	Rabbit	Human, mouse, and monkey	TAp73α and ΔNp73α	+	+	+	+	+	+	
p73DN	aa 1–12	Rabbit	Human	All ΔNp73 isoform	+	+	+	nt	nt	nt	
Commercial antibodies											
Ab4	aa 380–637	Mouse	Human	TAp73, ΔNp73, α, β	+	+	+	+/−	+/−	+/−	[15]
Ab2	aa 380–499	Mouse	Human, mouse	α isoforms	+	+	+	−	nt	nt	[15]
OP181	aa 1–12 of ΔNp73	Mouse	Human, mouse	All ΔNp73 isoform	−	+	+	−	nt	nt	
IMG 246	TA domain of p73	Mouse	Human, mouse	All TAp73 isoforms	+	+	+	+	nt	+	
IMG 259	DBD of p73	Mouse	Human, mouse	All p73 isoforms	+	+/−	+	−	nt	nt	
C17	C-terminal of p73α	Goat	Human, mouse	TAp73 and ΔNp73α	+/−	+	nt	−	−	−	[27–29]
C20	C-terminal of p73β	Goat	Human, mouse	All p73 isoforms	+/−	+	nt	−	−	−	[28]
E4	N-terminal 80 aa of TAp73	Mouse	Human, monkey	All TAp73 isoforms	+/−	+/−	nt	−	−	−	
H79	N-terminal 80 aa of TAp73	Rabbit	Human, mouse	All TAp73 isoforms	+/−	+	nt	−	−	−	[29,30]
Ab5	aa 1–15 of TAp73	Rabbit	Human	All TAp73 isoforms	+/−	+	nt	−	−	−	
Ab6	aa 436–450 of p73	Rabbit	Human	All p73 isoforms	+/−	+/−	nt	−	−	−	

TA domain, transactivation domain; DBD, DNA binding domain; aa, amino acid; +, working, +/−, weakly detecting; −, not working; and nt, not tested.

alone (1 h at 4 °C), and incubated with the appropriate primary antibody (2–5 μg) for 1 h at 4 °C. Then 25 μl of protein G was added to the mixture and left at 4 °C for another hour. The immune complexes were then precipitated and eluted as described above. All of the eluate was loaded to an 8% SDS–PAGE gel together with 10 μg overexpressed protein as a positive control, electrophoresed, and transferred to PVDF filters following the protocols above. The filter was then probed with p73FL (1:5000) dilution, washed and treated with HRP-conjugated anti-rabbit secondary antibody.

Indirect immunofluorescence and confocal analysis. H1299 and HaCat cells were incubated with p73FL antibody (1:2000) overnight at 4 °C. After washing three times in PBS, sections were incubated for 1 h with secondary antiserum (goat anti-rabbit Alexa Fluor 488 or Alexa Fluor 568) diluted in 10% BSA solution 1:1000. Slides were then mounted using Prolong Antifade kit. Fluorescence was evaluated with a confocal microscope (Nikon Instruments Spa, Eclipse TE200), exciting at 488 nm with an argon laser and at 542 nm with a helium laser. The software used was EZ2000 for PCM2000.

Results and discussion

Characterization of the new polyclonal antibodies

Inducible SaOs-2 cell lines containing hemagglutinin (HA)-tagged TAp63α, ΔNp63α, TAp73α, TAp73β, TAp73γ, ΔNp73α or non-tagged p53 plasmids were induced with doxycycline for 24 h. Total proteins were extracted as described in Materials and methods, run on SDS–PAGE gels, transferred to PVDF membranes, and blotted with the new anti-pan-p73 (p73FL) and anti-HA antibodies. Since the p53 protein is non-tagged, it was probed with DO-1 antibody (Fig. 1B). p73FL showed strong reactivity to all forms of TAp73 and ΔNp73 (Figs. 1B and C) but not TAp63α, ΔNp63α, and p53. Anti-HA and DO-1 probing of the same membranes showed the presence of all p53 family member proteins, and anti-tubulin probing showed equal protein loading (Fig. 1B). p73FL reacted more strongly with TAp73α and ΔNp73α, as compared to p73β and p73γ, suggesting that the anti-p73α epitope is more strongly represented in the polyclonal sera.

The same p53 family proteins were induced and blotted as described above and subjected to Western blotting with new anti-p73α (p73SAM) and anti-ΔNp73 (p73DN) antibodies (Figs. 1C and D). p73SAM, which was raised using the SAM domain of p73, reacted only with TAp73α and ΔNp73α but not with other p73 isoforms or with TAp63α as expected (Fig. 1C). p73DN detected specifically the ΔN isoforms (Fig. 1D). The anti-pan-p73 antibody (p73FL) but not the anti-ΔNp73 antibody (p73DN) recognized a doublet in all transfections (Fig. 1D). This could be the result of a post-translational modification which could not be recognized by the anti-ΔNp73 antibody, or due to a cryptic Kozak sequence yielding a protein that is lacking several amino acids from the N-terminus of ΔNp73. Indeed, after the predicted translation start site of ΔNp73, there are several in-frame methionine residues which could act as alternative translation initiation sites.

Evaluation of commercial antibodies in Western blotting

For comparing the detection limit of p73FL with commercial antibodies, we have performed Western

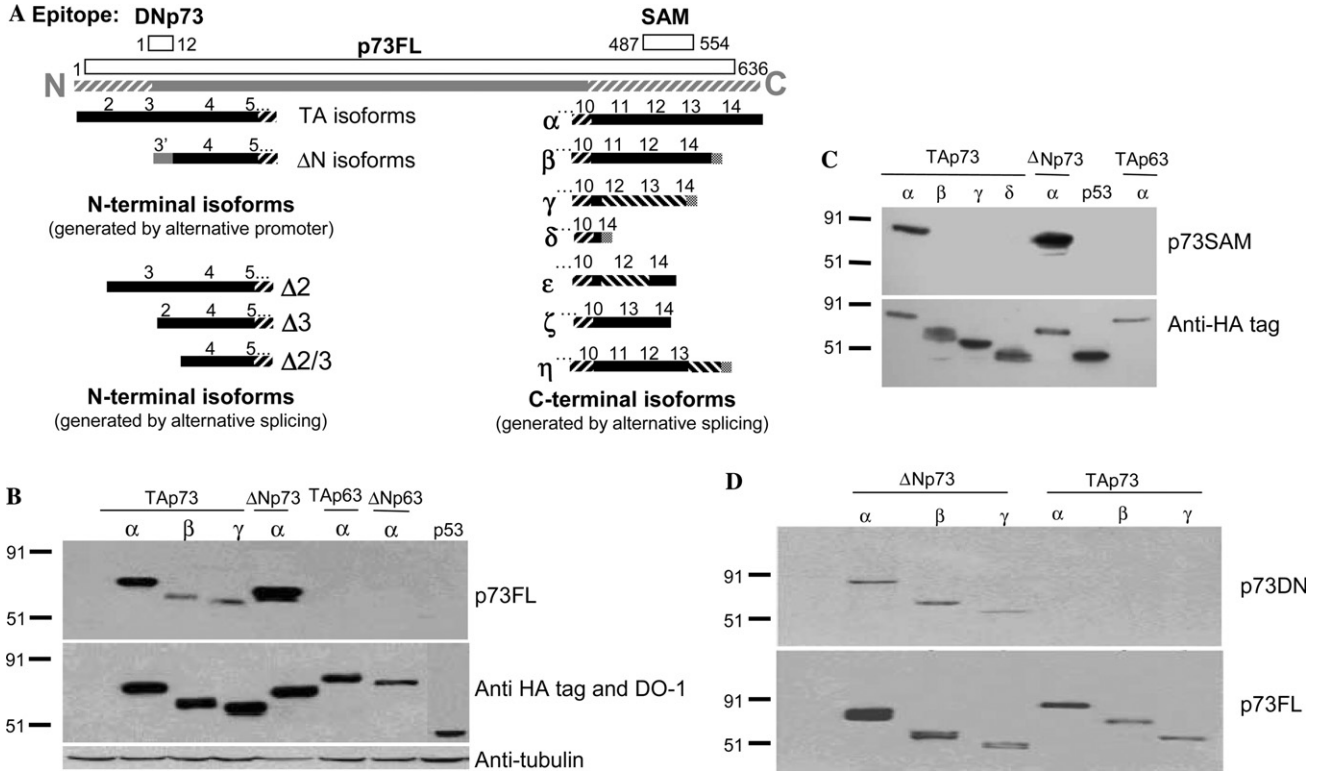

Fig. 1. Characterization of new p73 antibodies. (A) Schematic representation of the TA and ΔN isoforms of the p73 gene. Splicing events at the C-terminus end give rise to the β–η splicing variants. Splicing events at the N-terminus end give rise to the Δ2, Δ3, and Δ2/3 variants. Localization of the epitopes is shown by the white bars, and the corresponding residues are indicated. The proteins (TAp73α, TAp73β, TAp73γ, ΔNp73α, ΔNp63α, TAp63α and p53) obtained either from inducible or transfected SaOs-2 cells were subjected to Western blotting using anti-pan-p73 (p73FL; B), anti-p73α antibodies (p73SAM; C), and anti-ΔNp73 (p73DN; D) antibodies. The expression of all p73 and p63 isoforms and p53 was shown by anti-HA blot or DO-1 antibody (to detect untagged p53). Equal protein loading was confirmed by blotting with anti-β-tubulin antibody.

blotting under the same conditions and using the same amounts of protein used for Fig. 1. The antibodies evaluated were: Ab2 Ab4, and Ab5 from Neomarkers, Ab-1 (OP181) from Oncogene Research, IMG 246 and IMG 259 from Imgenex, and E4, H79, C17, and C20 from Santa Cruz (see Table 1 for the overall results). To compare the specificity of the new anti-p73 polyclonal antibody with those of the well-described commercial Ab2 and Ab4 anti-p73 antibodies, replicate membranes were prepared as described above for Figs. 1B and C, and probed with these antibodies following the instructions of the supplier. The result of Ab2 probing yielded a weak signal only for the TAp73α and ΔNp73α isoforms, and no signal for other p73 isoforms, p63 isoforms or p53 (Fig. 2A). Ab4 is a cocktail of three monoclonal antibodies (including Ab2), all raised against the C-terminal region of p73 [15]. It recognized TAp73α (weakly), ΔNp73α, and TAp73β isoforms, and did not cross-react with either p63 isoforms or p53 (Fig. 2A). Imgenex 246 reacted with all tested TAp73 isoforms but not ΔNp73, and Imgenex 259 showed reactivity against all p73 isoforms, including ΔN (as also suggested by the supplier), although additional bands were also detected. The p73γ transcript lacks exon 11, and therefore translates exon 12 in a different frame, truncating after 75 amino acids [5]. With the exception of Imgenex 246 and 259 antibodies, and FL1, none of the commercial antibodies recognized the p73γ isoform.

Among these 10 antibodies, Ab2, Ab4, IMG 246, and IMG 259 produced a relatively specific signal although the others either yielded very weak signal, unspecific bands or no signal (Table 1—see Figs. 2A and B for Ab2, Ab4, IMG 246, IMG 259, C17, and Ab5; data not shown for the others). IMG 246 was the strongest commercial antibody, giving a detectable but weak signal with TAp73 isoforms within 5 min of exposure. For the rest of the commercial antibodies, the exposure time had to be extended to at least 30 min even for a weak signal. These results are summarized in Table 1.

Immunoprecipitation of p73

For evaluating the affinity of p73FL in pull-down assays, immunoprecipitation experiments were performed with different dilutions of primary antibody in TAp73α-induced cell lysates. Ab4 was used as a positive control. As shown in Fig. 3A, p73FL antibody immunoprecipitates p73 protein very efficiently in 1:250 and 1:500

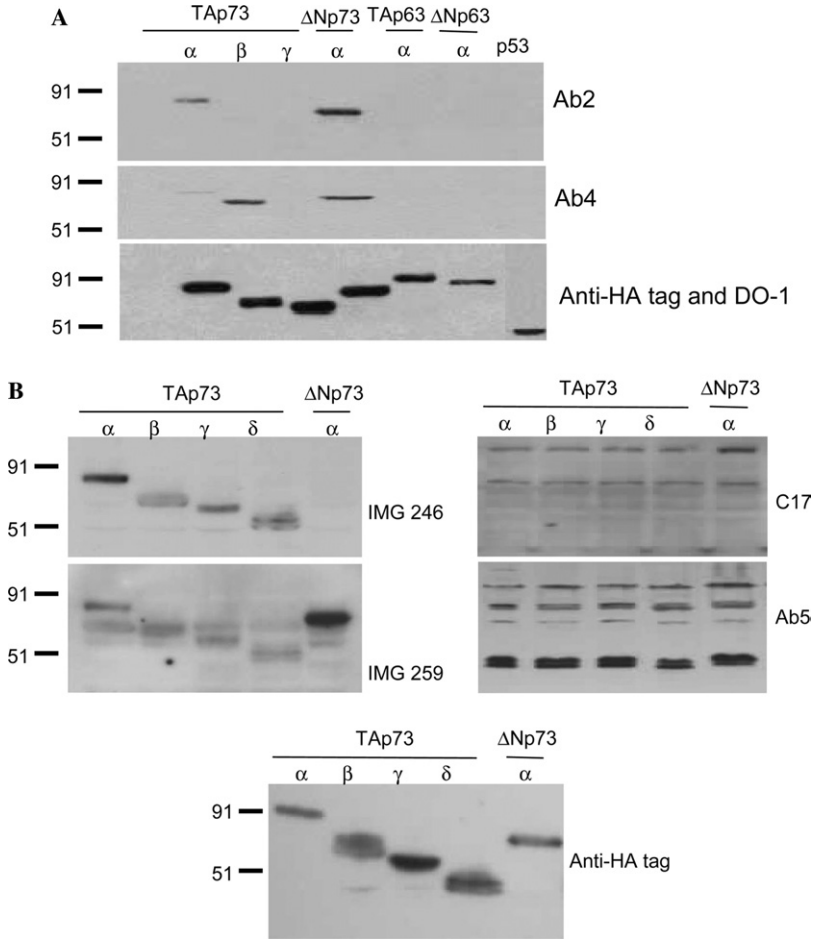

Fig. 2. Evaluation of commercial anti-p73 antibodies by Western blotting. The proteins obtained as described in Fig. 1 were subjected to Western blotting using the commercially available antibodies Ab-2 and Ab-4 (A) and Imgenex 246, Imgenex 259, C17, and Ab5 (B). The expression of all p73 and p63 isoforms and p53 was assessed as in Fig. 1.

dilutions as compared to the Ab4, which was used at 1:50 dilution, following the instructions of the supplier.

Using commercial antibodies, immunoprecipitation of 150 μg overexpressed TAp73α (for C17, H79, E4, Ab4, Ab5, Ab6, IMG 246, and IMG 259), TAp73β (for C20) or ΔNp73α (for OP181) was performed in parallel with p73FL (Fig. 3B; see Table 1 for the overall results). As shown in Figs. 3A and B, Ab4, C17, IMG 246,

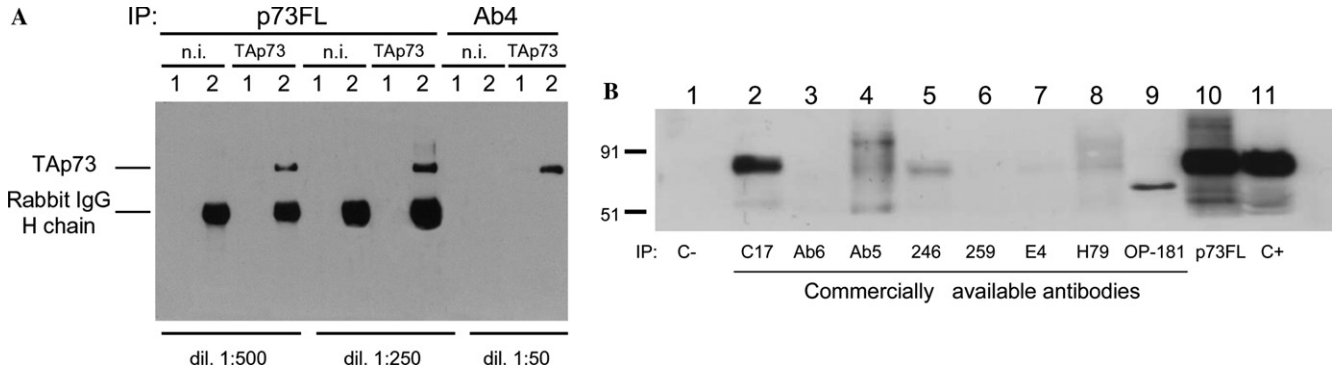

Fig. 3. Immunoprecipitation assay. TAp73-inducible SaOs-2 cells were induced for 24 h. p73FL immunoprecipitates the overexpressed HA-TAp73α in a dose-dependent manner (lane 2 for each dilution). As a positive control we also performed the immunoprecipitation using Ab4 antibody (A: n.i., not induced). One hundred and fifty micrograms of TAp73α (and ΔNp73α for OP181) was subjected to immunoprecipitation with commercial anti-p73 antibodies (B). The negative control (lane 1 for each antibody or dilution in (A) and C− in (B)) is without any primary antibody and the positive control (C+ in (B)) is 10 μg overexpressed TAp73α.

and p73FL immunoprecipitated TAp73α, p73FL being the strongest, whereas other antibodies did not give any significant signal or a very weak signal. The ΔNp73 specific antibody OP181 could also precipitate ΔNp73 (see Fig. 3B). C20 (Santa Cruz Biotechnology) precipitated TAp73β but did not give any signal with TAp73α (data not shown).

Analysis of endogenous p73 protein

Among p53 family members, the function of p73 protein is the least characterized because it is expressed at very low levels in specific tissues. The common method to bypass this problem is laborious cell fractionation and isolation of nuclei since p73 proteins are mainly nuclear. However, this approach is hard to perform with samples such as fresh frozen tissues and not very effective for studying the function of some p73 alternative splicing variants. These restrictions led tumor biologists to study the expression of p73 RNA levels, mainly by RT-PCR. We used proliferating versus differentiating HaCaT (keratinocyte) and SK-N-SH (neuroblastoma) cell lines and drug treated H1299 (lung cancer) and HEK293 cells to assess the new p73 antibodies under endogenous conditions in Western blotting and immunofluorescence. Antibodies, that were not detecting overexpressed p73 isoforms, were not tested in endogenous conditions.

Retinoic acid causes differentiation of neuroblastoma cell lines via induction of at least TAp73α and TAp73β [16,17], in keeping with a neuro-developmental role of p73 [18]. In parallel with previous observations, there is a slight increase of p73 protein detectable with p73FL in retinoic acid-induced SK-N-SH cells compared to the untreated control (Fig. 4A). Endogenous p73 protein in the HaCaT cell line is also readily detected with p73FL in Western blotting (Fig. 4A). Since HaCaT cells gave a strong signal in the Western, we also studied the localization and staining pattern of endogenous p73 protein by immunofluorescence. Proliferating HaCaT cells, as previously described [16,19], produced a uniform nuclear staining of all cells whereas calcium-induced differentiation of the same cell line resulted in only occasional nuclear staining, probably representing a downregulation of p73β (Fig. 4B). p73 protein accumulates upon DNA damage [20]. We have treated human embryonic kidney cells (HEK293) with different DNA damaging or apoptosis-inducing drugs to see if we can detect changes in the levels of endogenous p73α with p73SAM antibody (see Materials and methods for details on drug treatments). p73 protein accumulation was detected with different drug treatments (Fig. 4C). We also tested p73FL in cells after DNA damaging drug treatments. As shown in Fig. 4D, p73FL staining revealed nuclear induction and accumulation of p73 protein after treating H1299 cells with 5-FU and etoposide. Please note that the magnification of the images is the same, but drug treatment induced an increase in the size of nucleus.

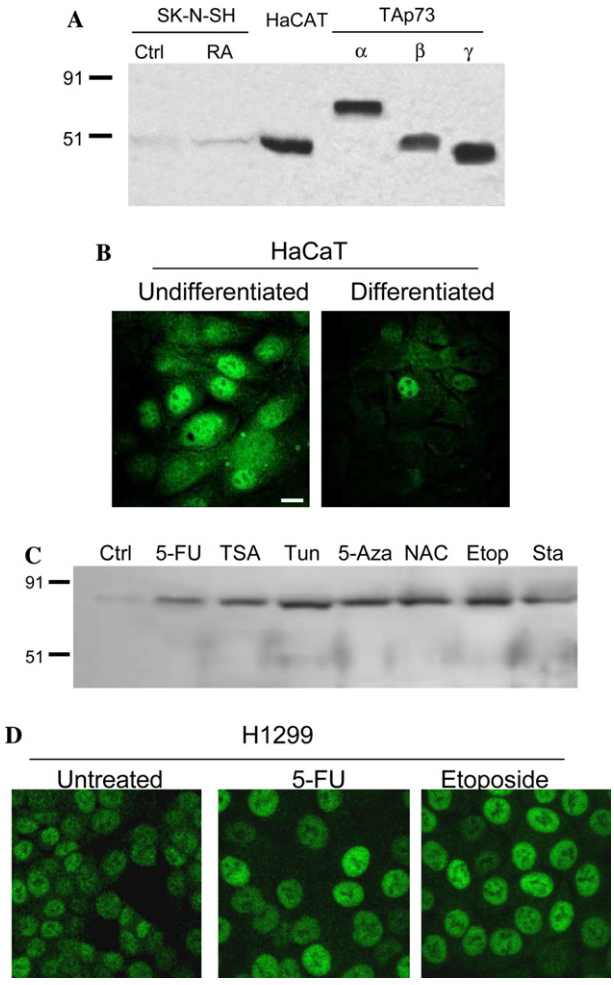

Fig. 4. Detection of endogenous p73 by Western blotting and immunofluorescence. (A) Western blot analysis on SK-N-SH (a human neuroblastoma cell line) and HaCaT (human keratinocytes) cells were performed with p73FL. For a size comparison, proteins extracted from TAp73α, β, and γ transfected SaOs-2 were loaded on the gel. (B) Immunofluorescence and confocal analysis with p73FL of HaCat cells kept in culture under proliferating and differentiating conditions. (C) The p73α protein levels of drug treated versus untreated HEK293 cells (see Materials and methods for details on drug treatments). The filter was probed with p73SAM antibody for only detecting the α form TA and ΔNp73. (D) H1299 cells were treated with 5-FU and etoposide for 24 h and visualized by immunofluorescence using p73FL.

Therefore, these results demonstrate that p73FL and p73SAM recognize endogenous p73 both by Western blotting and immunofluorescence.

Conclusion

Detection of p73 proteins is very important topic both for functional and descriptive studies since the proteins are the functional end products of the genomic

information. In general, protein detection with immunoaffinity based methods such as immunofluorescence, immunohistochemistry, and Western blotting are the most used methods employed in research methodologies. Although p73 gene was identified more than 7 years ago, very few antibodies were raised recognizing the endogenous levels of p73 proteins and only some of them are commercialized [3,15,21–23]. Thus, most of the studies, even the ones questioning the stability of p73 proteins, were done using overexpression systems producing different results. The half-life of TAp73α was described to be 2.25, 2, and 12 h whereas the half-life of ΔNp73α was described to be 4, 8 h, and half an hour in three different publications, respectively [24–26]. Moreover, p73 gene derived proteins are very hard to study since p73 gene produces at least 24 different transcripts yielding proteins with different amino- and carboxyl termini. These proteins are very close in molecular weight thus it is almost impossible to differentiate just concluding from the migration pattern on SDS–PAGE. The presence of new, strong, and domain specific antibodies is vital for the p73 field to describe the function of p73 protein in endogenous levels. Together, these three new antibodies, with some degree of p73 isoform specificity, and of high affinity, are likely to prove useful reagents in unrevealing the complexities of the biological functions of p73.

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