Different intracellular compartmentalization of TA and ΔNp73 in non-small cell lung cancer

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Received May 27, 2008; Accepted August 7, 2008

DOI: 10.3892/ijo_00000169

Abstract. The p53 homologue p73 is overexpressed in many tumors, including lung cancer. We have evaluated the differential expression and subcellular localization of the functionally distinct apoptotic (TA) and anti-apoptotic (ΔN) isoforms of p73 in non-small cell lung cancer (NSCLC), their possible association with p53 expression and determined the methylation status of the two p73 gene promoters (P1 and P2) in this tumor type. Immunohistochemical analysis showed that both isoforms are expressed in the majority of cases. However, the oncogenic ΔN variant, derived from the transcripts Δ*N'p73* (from P1) and/or Δ*Np73* (from P2), is localized mainly in the nucleus, while the anti-oncogenic TAp73 isoform (derived from a P1 transcript) is sequestered in the cytoplasm in almost all cases analyzed. Significant correlation was found between p53 and Δ*Np73* expression (p=0.041). Methylation analysis conducted on 41 tumor samples showed that the P1 promoter is almost invariably unmethylated (39/41 cases) whereas P2 was found completely methylated in 17 cases and partially or totally unmethylated in 24 samples. No correlation was found between the methylation status of P1 and P2 and p73 expression. Our results demonstrate that both isoforms contribute to p73 overexpression in NSCLC and suggest that their different intracellular localization may reflect an alteration of the functional p53-p73 network that might contribute to lung cancer development.

Introduction

The *p73* gene is a *p53* homologue that transactivates several *p53* target genes, induces apoptosis and inhibits cell proliferation (1-3). Many p73 splicing variants (both at the -NH_2 and -COOH terminus) have been detected (3). Among these, the N-terminal deleted isoforms (collectively indicated as ΔTAp73) are able to block the transactivating activities of TAp73 and p53 and their ability to induce apoptosis, by acting as direct competitors for the DNA binding-sites and/or by heteroduplex formation with p53 and TAp73 variant, thus functionally acting as oncogenes (3-7). Moreover, ΔTAp73 isoforms confer drug resistance on wild-type p53 and TAp73 harboring tumor cells (8) and act as oncogenes transforming NIH3T3 cells and making them tumorigenic in nude mice (4). In normal human tissues, TAp73 is expressed at low levels and, in general, ΔNp73 is undetectable (9). Many primary tumors of different origin, including lung cancer, overexpress p73 (10-14), however, only few studies have specifically determined the different extent of contribution of TA or ΔTA isoforms to the overexpression of p73 in the same set of samples and their prognostic role (15-18).

Intracellular compartmentalization may be a mechanism through which the functionality of the p53 family network can be altered. The p73 proteins, similarly to its siblings p53 and p63, are normally localized in the nucleus and the shuttle of p73 from nucleus to cytoplasm is associated with its degradation (19). The functions of p73 are controlled by the interactions with their binding partners and, in turn, the pro- or anti-apoptotic activities of the p73 isoforms can be modulated by their recruitment to specific subcellular compartments (20).

Indeed, several tumor types have shown an altered protein topography: in neuroblastoma, cytoplasmic sequestration of p53 is considered a mode of inactivation of this gene alternative to point mutations that are remarkably absent in this tumor type (21). Similarly, the cytoplasmic localization of the non-mutated p16 protein in breast and NSCLC cells has been proposed as a mode of inactivation of the *CDKN2A* gene in the absence of mutations (22-23).

The *p73* gene has two distinct promoters. The p73-P1 (P1), upstream of exon 1, drives the transcription of the full-length and of the 3' splicing variants (*TAp73α-θ*) as well as of several 5' variants (Δ*Ex2p73*, Δ*Ex2-3p73* and Δ*N'p73*). The internal

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Key words: methylation, lung cancer, p73

p73-P2 promoter (P2), within intron 2 and upstream of an alternative exon 3 (exon 3') regulates the transcription of Δ*Np73*, another 5' variant whose protein product is indistinguishable from that of Δ*N'p73*. The expression of Δ*Np73* from the P2 promoter is upregulated by TAp73 and p53 through a complex feed-back regulatory loop (3-6).

The expression of the *p73* gene is in some cases regulated by epigenetic factors. Indeed, a large canonical CpG island (CGI) characterizes the P1 promoter and its hypermethylation is associated with p73 silencing in haematopoietic malignancies but rarely in other tumors (24-29). A smaller and less evident CGI is present at the P2 promoter and it is almost always fully methylated in most of the normal tissues examined (27,28 and unpublished observations). In malignant neuroblastic tumors, P2 was found unmethylated or partially methylated whereas it was fully methylated in benign ganglioneuroma. Thereafter, the hypomethylation of P2 was put in relation with the inappropriate expression of Δ*Np73* in rapidly progressing neuroblastic tumors (18,27)

Along these lines we have evaluated the pattern of expression of TA and ΔNp73 as well as of p53, in tumor tissue and in the surrounding morphologically normal tissues of NSCLC as well as the methylation status of P1 and P2 in this tumor type.

Materials and methods

Tissue samples and cell lines. We analyzed 41 cases of primary NSCLC at stage I to III and the corresponding peritumoral tissue from 21 of the cases, that were collected and snap-frozen from January to December 2004 at the Thoracic Unit of the Department of Surgical Sciences (University of Insubria, Varese, Italy). The group of patients enrolled in the study had 32 males and 9 females and included adenocarcinoma (n=15), squamous cell carcinoma (n=21), large cell carcinoma (n=3), and bronchialveolar carcinoma (n=2).

Control tumor cell lines for methylation analysis (U937, GI-ME-N, HL60) were obtained from the American Type Culture Collection (Rockville, MD, USA).

For DNA extraction, the samples were lysed overnight at 55˚C in a buffer containg 100 mM NaCl 100 mM Tris pH 5.0, 10 mM EDTA, 0.5% SDS and proteinase K at 0.2 mg/ml. DNA was purified from the lysate with the Phase Lock Gel kit (Eppendorf, Milano, Italy). RNA was extracted with TRIzol (Invitrogen, San Giuliano Milanese, Italy) following the manufacturer's protocol.

Histological and immunohistochemical study. The forty-one cases of resected carcinoma of the lung were retrieved from the Surgical Pathology archives at the Department of Pathology (University of Insubria, Varese). Formalin-fixed, paraffin-embedded tissue blocks were selected and tissue sections were stained with hematoxylin and eosin (H&E) and Alcian Blue, periodic acid Schiff stain (AB-PAS) and the slides were reviewed to confirm the diagnosis according to the WHO classification (2004). Immunoperoxidase studies were performed on sections prepared from formalin-fixed and paraffin-embedded specimens that were dewaxed and rehydrated using Bio-Clear (Bio-Optica, Milan, Italy) and graded alcohol. Endogenous peroxidase was blocked dipping sections in 3% aqueous hydrogen peroxide for 10 min and antigen retrieval was performed with trypsin digestion for 20 min at 37˚C (for ΔNp73) and with microwave treatment in 10 mM citrate buffer, pH 6.0 (20 min for TAp73 and 10 min for p53). The immunostaining was performed with the avidin-biotin-peroxidase complex technique using diaminobenzidine as chromogen. The sections were incubated overnight at 4˚C with the mouse monoclonal antibodies against TA and ΔNp73 (both from Imgenex, San Diego, CA) utilized at a dilution of 1:80 and with the mouse monoclonal antibodies against p53 (clone DO-7, Dako, Denmark) utilized at a dilution of 1:500. The antibody against TAp73 (clone 5B429) was raised against NH2- terminus of the protein and does not cross-react against ΔNp73; the antibody against ΔNp73 (clone 38C674.2) was raised against a peptide corresponding to AA 2-13 unique to this isoform and does not cross-react against TAp73 whereas the antibody against p53 was raised against NH2- terminus of the protein and reacts with both wild-type and mutated form of p53. Antibodies were certified for Western blot analysis and immunostaining by the manufacturer. Sections were lightly counterstained with hematoxylin and then observed with Olympus B40 microscope. A case was considered positive when either the nucleus or the cytoplasm or both were stained. Scoring was: <10% of cells (+), between 10 and 50% of cells (++) and >50% of neoplastic cells (+++). Negative controls, where primary antibody was omitted, were included.

Methylation analysis. The methylation status of the *p73* P1 and P2 promoters was determined by methylation-specific PCR (MSP) (30) and for P2, to confirm the MSP results, also by Bisulfite-Restriction Enzyme analysis (BRE) (31). The MSP primer sequences specific for the methylated or unmethylated templates, their annealing temperature and PCR conditions are indicated in Table I.

For BRE, after DNA modification, the primers (Table I) were designed to amplify a 351 bp promoter fragment from P2 independently from its methylation status. The amplification product was digested with *Rsa*I and *Pvu*I.

HL60 cells were used as positive control for unmethylated P1, U937 as positive control for methylated P1 and P2 and GI-ME-N as positive control for unmethylated P2. Platinum Taq polymerase (Invitrogen) was used in all the PCR reactions. All assays were conducted at least in triplicate.

RT-PCR and sequencing analysis. Total RNA from four lung cancer fragments was retrotranscribed utilizing the specific exon 4 primer: 5'-TGCTGGAAAGTGACCTCAAA-3' that amplifies all the known 5' variants of *p73*. Two μl of the RT product were utilized for the PCR amplification. The primers and PCR conditions for the RT analysis are reported in Table I. A nested protocol was employed to amplify Δ*Np73*; in this reaction 2 μl of RT product was amplified first with primers RT ΔNp73 Fw1 and Rev1 and 2 μl of the first amplification product was subjected to a second round of PCR with the internal set of primers RT ΔNp73 Fw2 and Rev2. Amplification of the ΔN'p73 transcript was carried out with the primer set and PCR conditions reported in Table I. A schematic representation of the 5' end of the *p73* gene with the localization of the RT-PCR primers utilized to discriminate

Table I. Primers and PCR conditions for expression, methylation and sequencing analyses.

Primer	Sequence	Annealing T.˚C
RT ΔNp73 Fw 1	AAGCGAAAATGCCAACAAAC	55
RT ΔNp73 Rev 1	GGTCCATGGTGCTGCTCAGC	
RT ΔNp73 Fw 2	ACTAGCTGCGGAGCCTCTC	56
RT ΔNp73 Rev 2	TGCTCAGCAGATTGAACTGG-3	
RT ΔN'p73 Fw	GATTCCAGCATGGACGTCTTC	54
RT ΔN'p73 Rev	GAGAGGCTCCGCAGCTAGT	
P1 Met Fw	GGACGTAGCGAAATCGGGGTTC	60
P1 Met Rev	ACCCCGAACATCGACGTCCG	
P1 UnMet Fw	AGGGGATGTAGTGAAATTGGGGTTT	60
P1 UnMet Rev	ATCACAACCCCAAACATCAACATCCA	
P2 Met Fw	GTTGTCGGGCGGTTACGATC	63
P2 Met Rev	TCACACCTACCGTAACGAAATACCG	
P2 UnMet Fw	GGTTTATGTTGTTGGGTGGTTATGATTG	65
P2 UnMet Rev	CACATCACACCTACCATAACAAAATACCATAC	
P2 BRE Fw	TTAGTTGATAGAATTAAGGGAGATGG	59
P2 BRE Rev	AAAAAATACCCCTCTAAACCCTACA	
Nuclear Export Fw	TCTGGTTAGACCTGCTTCTTG GG	59
Nuclear Export Rev	TGATGCCTTCTGGAGGGGTTTC	
Nuclear Import Fw	GGAGGAGAAGGGGACACATT	59
Nuclear Import Rev	GAAGATGACTCCCAGCCAAG-3	

PCR conditions for RT analysis: 2 min denaturation at 94˚C; 35 cycles each consisting for 30 sec 94˚C, 30 sec annealing at the appropriate temperature, 30 sec extension at 72˚C. PCR conditions for methylation analysis: 2 min denaturation at 94˚C; 35 cycles each consisting for 20 sec 94˚C, 10 sec annealing at the appropriate temperature, 30 sec extension at 70˚C. PCR conditions for Nuclear Import/Export motif sequencing: 2 min denaturation at 94˚C; 35 cycles each consisting for 30 sec 94˚C, 30 sec annealing at 59˚C, 30 sec extension at 72˚C.

between Δ*N'* and Δ*Np73* is shown in Fig. 1. The Nuclear Import and Export signals within the p73 gene were sequenced by automated Sanger sequencing on PCR products generated with the primers reported in Table I.

Statistical analysis. Survival curves were computed according to the Kaplan-Meier method (32) and were compared by means of the log-rank test.

Statistical significance was evaluated using the Pearson's χ^2 test. The statistical difference was considered significant at $P<0.05$. Data were analyzed with the Statistical Package for the Social Sciences version 13.0 (SPSS Inc., Chicago, IL).

Results

Expression and intracellular localization of TAp73, ΔNp73 and p53. We have determined by immunohistochemistry the pattern of TAp73, ΔNp73 and p53 expression in a cohort of 41 NSCLC patients.

As shown in Table II, the expression of TAp73 was detected in 28/40 cases and was, in 24 of them, localized exclusively in the cytoplasm as representatively shown in Fig. 2. On the contrary, immunostaining with the antibody specific for ΔNp73 showed that the expression of this protein, detected in 35/40 cases (Table II) was predominantly confined to the nucleus, although eight cases displayed both nuclear and cytoplasmic reactivity. Only in two cases the protein was localized exclusively in the cytoplasm.

In the peritumoral lung tissue, both TA and ΔNp73 were expressed at low level or were below the level of detection in the majority of the sections (Fig. 2).

P53 expression was detected in 26 out of 39 samples and, in all cases, was confined to the nucleus of cancer cells.

In our clinical series, the high expression of p53, defined as immunostaining in >10% of the cells, was significantly associated ($p=0.041$) with similarly high expression of ΔNp73. Conversely, no association was found between the expression of TA and ΔNp73

We next evaluated if the expression of the ΔNp73 or p53 protein had an influence on the survival in the cohort of patients included in our study and we did not observe differences in the overall survival among the patients that expressed ΔNp73 or p53 as compared to those that did not express these proteins (p-values for the log-rank test was 0.63, HR=1.28 for ΔNp73 and 0.59, HR 1.32 for p53).

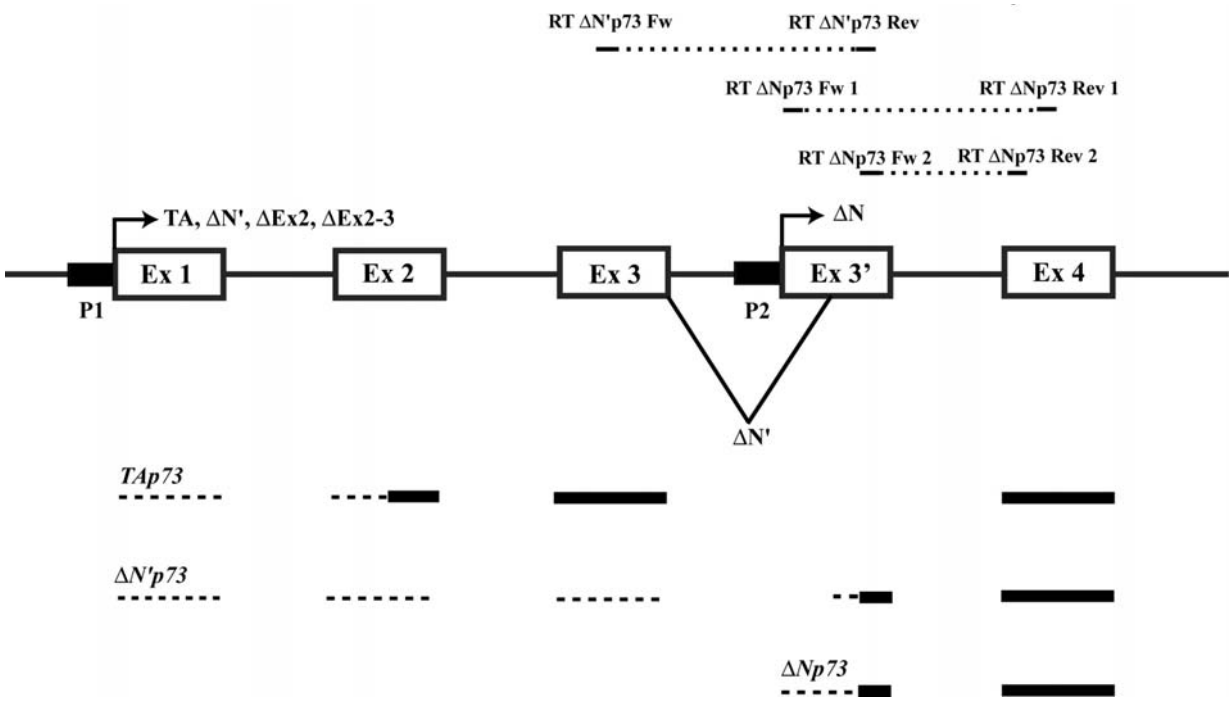

Figure 1. Structure of the 5' end of the p73 gene. Exons 1-4 of the p73 gene are indicated (Ex1-Ex4), the two promoters (P1 and P2) are represented by filled boxes, the intronic sequences are represented by thin lines between the exons. The transcription starts for the 5' end variants of p73 and the alternative splicing leading to Δ*N'p73* are indicated. Below the diagram of the genomic region the structure of the transcripts is reported. The dotted line indicate the 5' UTR and the thick, continuous line the coding region. In the upper part of the figure the localization of the RT-PCR primers utilized for the identification of the transcripts coding for the ΔNp73 protein is reported.

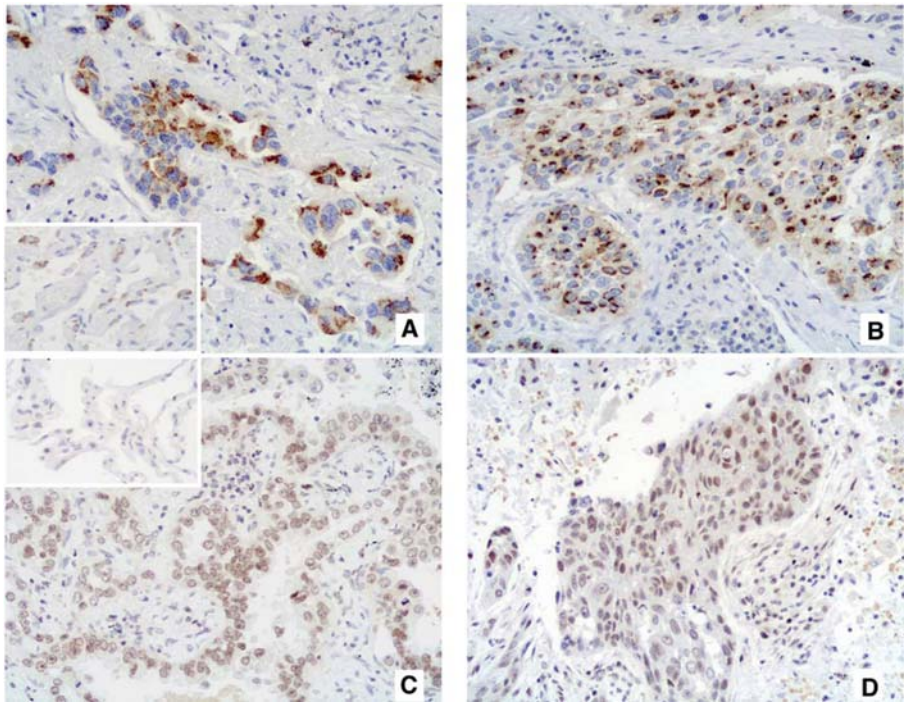

Figure 2. Immunohistochemical expression of TAp73 showing cytoplasmic expression in lung adenocarcinoma (A), in scattered cells of peritumoral tissue (inset down left) and in squamous cell carcinoma (B). Immunostaining with the anti-ΔNp73 antibody showing nuclear localization in lung adenocarcinoma (C), in adjacent non tumoral tissue (inset top left) and in squamous cell carcinoma (D). Immunoperoxidase with hematoxylin counterstain, original magnification, x200.

Methylation pattern of P1 and P2 promoters in NSCLC. We have analyzed the pattern of methylation of the two p73 promoters in 41 NSCLCs and in the corresponding peritumoral tissue that was available for 21 of the samples. The results of these experiments, summarized in Table II and representatively illustrated in Fig. 3, showed that the P1 promoter (that drives the transcription of TA and Δ*N'p73*) is rarely hypermethylated in the malignant tumor samples (2/41 cases) and never in the

Table II. Expression and methylation analysis of TA, ΔNp73 and p53.

Case N.	TAp73	ΔNp73	p53	Methylation T-P1	Methylation N-P1	Methylation T-P2	Methylation N-P2
		IHC					
1	+ C	+++ N	+++N				
2	++ C	++ N	++N		ND		ND
3	-	+ N	-				
4	-	+++ N	-				
5	+ C	++ N	-				
6	++ C	+++ N, + C	+++N				
7	-	++ N	+++N				
8	+++ C	-	+++N		ND		ND
9	+++ C	+ N	-				
10	++ C	-	++N				
11	+C	+ C, +N	+++N				
12	+ N	-	+N				
13	++ C	++ N, ++C	ND		ND		ND
14	-	+ C	+++N				
15	-	+++ N	++N		ND		ND
16	+ C	++ N	++N				
17	+++ C	+ N	-		ND		ND
18	+ C	-	-		ND		ND
19	++ N, ++C	+++ N	+++N		ND		ND
20	+ C	-	-				
21	+ C	+++ N	++N		ND		ND
22	+ C	+ N	+++N				
23	+ C	++ N	ND		ND		ND
24	ND	ND	-				
25	-	+ N	+N				ND
26	-	+ C	+N				
27	+++ C	+++ N	-				
28	++ C	+ C, +N	-				
29	-	+++ N	+++N				
30	+ N, +C	++ N	-		ND		ND
31	-	++ N	+++N		ND		ND
32	+ C	+ N	-				ND
33	-	+N	+++N		ND		ND
34	+N, +C	+++N	-		ND		ND
35	+C	++N, +C	+++N		ND		ND
36	-	+N	+++N		ND		ND
37	-	+N, ++C	+++N		ND		ND
38	++C	++N	+++N		ND		ND
39	+C	++N, +C	+N		ND		ND
40	++C	++N, +C	+++N		ND		ND
41	+C	++N	+N		ND		ND

Unmethylated
Partially Methylated
Completely Methylated

T, Tumor; N, morphologically normal peritumoral tissue; M, methylated; U, unmethylated; C, cytoplasmatic staining; N, nuclear staining; -, no staining; scoring of the IHC results was: +, <10% of the cells; ++, 10-50% of the cells and +++, >50% of the cells.

morphologically normal lung tissue surrounding the lesion. It is therefore evident that P1 methylation status is not in strict relationship with TA or Δ*Np73* expression since the two methylated cases express both isoforms.

The P2 promoter (driving the transcription of Δ*Np73*) that is generally fully methylated in non-cancer tissues was completely or partially unmethylated in 24/41 (58%) tumor samples and in 9/19 (47%) peritumoral tissue samples (Table II and Fig. 3). No significant correlation was observed between demethylation of P2 promoter and overexpression of the ΔNp73 protein.

The methylation status of P2 was verified by BRE in a subset of samples and the results obtained with this technique were concordant with those generated by MSP (data not shown).

c-Promoter functionality in NSCLC. The ΔN isoform of the p73 protein is generated from two distinct transcripts, Δ*N'p73*

Table III. ΔNp73 mRNA and protein expression and P2 promoter methylation.

Case No.	RT-Δ*Np73*	RT-Δ*N'p73*	IHC-ΔNp73	P2 Methylation
5	+	+	++ N	M + U
6	+	+	+++ N + C	M + U
27	+	-	+++ N	M + U
29	-	+	+++ N	M + U

N, nuclear; C, cytoplasmatic; M, methylated and U, unmethylated.

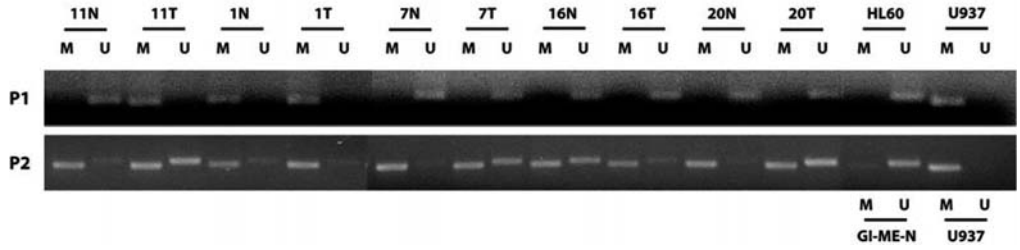

Figure 3. Methylation analysis of the P1 and P2 promoter in a subset of NSCLC. DNA from paired tumor/morphologically normal samples were utilized for methylation analysis along with DNA from cell lines whose methylation status was assessed independently by sequencing. M and U are methylated and unmethylated target sequence and N and T are the peritumoral and tumoral tissue, respectively.

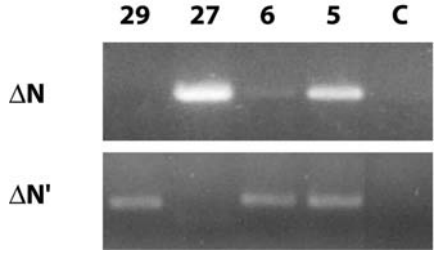

Figure 4. Expression analysis for Δ*Np73* and Δ*N'p73* RT-PCR analysis with primer sets discriminating the P1 and P2 transcripts.

and Δ*Np73*, whose expression is controlled by P1 and P2, respectively. These two promoters respond to a partially distinct set of transcription factors. In this respect, to understand the pathway leading to the production of this oncogenic variant, it was of importance to determine if, in NSCLC, both promoters are functionally active and if the overexpression of ΔNp73 could be attributed to either P1 or P2 or both. To answer this question we extracted total RNA from four tumor samples expressing high or intermediate levels of ΔNp73 protein and we utilized variant-specific primers in RT-PCR assay to unambiguously distinguish between Δ*Np73* and Δ*N'p73* transcripts (Fig. 1). As shown in Table III and Fig. 4, two samples expressed either Δ*Np73* or Δ*N'p73* whereas in two other samples both transcripts were detected indicating that ΔNp73 could be derived from transcripts driven by both promoters.

Discussion

The *p73* gene is a member of the *p53* family that, like its sibling *p63*, can act as an oncogene and as an antioncogene (1-4). The function of the p53 family members largely depends on their nuclear localization and recent evidence indicates that the export to the cytoplasm is an important determinant for p53 functionality. Indeed, cytoplasmic sequestration has been considered as a mechanism for the inactivation of p53 alternative to point mutations (21). Cytoplasmic redistribution of p73 in neuroblastoma has been observed and was put in relation with neuroblastic differentiation (33). At that time, however, it was not possible to distinguish the different p73 isoforms since antibodies with distinct specificities were not yet available.

In the present study the immunohistochemical assessment of p73 expression in NSCLC showed that the anti-oncogenic isoform is sequestered in the cytoplasm of tumor cells while the ΔN oncogenic variant remains localized in the nucleus.

The abnormal sequestration of a nuclear protein in the cytoplasm has been attributed to a variety of mechanisms, including mutation of the relevant localization motifs (34,35). Although inactivating mutations of the p73 gene are exceedingly rare, we have investigated this possibility in our samples and found no evidence of mutations in the nuclear export/import signals (19) (data not shown). Thus, other mechanisms should be taken into account to explain this finding.

Dissimilar results are found in the literature on the subcellular localization of p73 isoforms in different tumor types. In a series of hepatocellular and cholangiocellular carcinomas, p73 was found confined to cell nucleus; however, the antibody utilized in those studies could not discriminate the different p73 isoforms (36,37). The localization of p73 in breast cancer is unclear: Dominguez *et al* (38) reported that all the cases included in their study showed negative cytoplasmic immunostaining for TAp73 and that the majority of them, showed only wery weak ΔNp73 cytoplasmic staining.

On the contrary, Bozzetti *et al* (39) showed the cytoplasmic localization of TAp73 and the predominant cytoplasmic localization of ΔNp73.

In lung cancer, the expression of ΔNp73 was found mainly in the cytoplasm of tumor cells (17), while our results, showed that ΔNp73 is prevalently localized in the nucleus in ~85% of the positive cases. The comparison of these conflicting results is made difficult by the utilization of different antibodies raised against slightly different peptides. Interestingly, in both studies, ΔNp73 was not detectable in the morphologically normal peritumoral tissue suggesting that the aberrant expression of ΔNp73 is a cancer-related phenomenon. However, in our cohort of patients the expression of the ΔNp73 variant was not associated with a shorter survival.

Similarly to that observed in breast cancer (39) we have found a significant association between p53 immunostaining, considered as evidence of *TP53* mutation and ΔNp73 expression. This may indicate that these alterations are not mutually exclusive and could confer additional growth advantage to cancer cells.

In Burkitt lymphomas and lymphoblastic leukemias TAp73 is frequently silenced by hypermethylation of the P1 promoter (24,25) whereas in other tumors the methylation of P1 is uncommon (40,41). Differently from P1, the systematic survey of the methylation status of P2 in normal and cancer tissues has yet to be conducted. A preliminary analysis revealed that this promoter is generally hypermethylated in many non-cancer cells and in benign ganglioneuroma, whereas is unmethylated or partially methylated in neuroblastic tumors expressing the ΔN variant, indicating that demethylation of P2 might be a characteristic of certain malignant tumor cells (27,28 and our unpublished observations).

In the samples included in our study, we have observed a heterogeneous pattern of P2 promoter methylation in both the tumor and the corresponding peritumoral tissue. This suggests that the P2 promoter undergoes methylation changes in a subset of NSCLC. Differently from neuroblastic tumors, these changes do not seem to have a role in ΔNp73 expression. The significance of P2 hypo-methylation in our series of NSCLCs is unclear, however, it may reflect the alteration of the methylation machinery taking place in the early stages of lung carcinogenesis or may be the consequence of exposure to cigarette smoke or other carcinogens.

We have observed that in NSCLC, ΔNp73 can be produced by transcripts originating from P1, P2 or both. The P2 promoter is characterized by the presence of three p53/p73 responsive elements (3,5). Indeed, Δ*Np73* transcription is strongly induced by both p53 and TAp73 that, in turn, are inhibited by ΔNp73. Intuitively this feedback loop is functional only for ΔNp73 driven by P2 and the biological significance of the pathway leading to the production of ΔNp73 also from P1 has yet to be understood. It is likely however that ΔNp73 expression depends on redundant regulatory mechanisms that are controlled, at least in part, from different sets of transcription factors.

In conclusion, this study demonstrates, for the first time, that in NSCLC, both TA and ΔNp73 contribute to p73 overexpression and that they may have a different intracellular compartmentalization. This finding leads us to hypothesize that the cytoplasmatic sequestration of the pro-apoptotic isoform of p73 might have an influence in NSCLC and other tumors by altering the physiological interaction between the oncogenic and anti-oncogenic variants of p73 and negatively influencing the apoptotic pathway.

Acknowledgements

B.B. is a Fellow of the Italian Foundation for Neuroblastoma Research. This study was supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC), by grants from the Fondazione San Paolo, from Regione Liguria and from the Italian Health Ministry.

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