A differential proteomic approach to identify proteins associated with thyroid cell transformation

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Abstract

Tumour suppressor p53 is a transcription factor essential for DNA damage checkpoints during cellular response to stress. Mutations in the p53 gene are the most common genetic alterations found in human tumours; most pathogenetic modifications are missense mutations that abolish the p53 DNA-binding function. In the same cell type, distinct p53 missense mutations may determine different phenotypes. The PC Cl3 cell line retains several markers of thyroid differentiation *in vitro*. Introduction of the V143A mutant p53 allele, which abolishes the p53 DNA-binding function, leads to loss of differentiation markers as well as TSH dependency for growth. Conversely, PC Cl3 cells transfected with the S392A mutant p53 allele, presenting the mutation located outside the DNA-binding domain, show only loss of TSH dependency for growth. To identify molecular differences existing between PC Cl3 cell lines transformed by the V143A and the S392A mutant alleles, a differential proteomic approach was used. Two-dimensional gel electrophoresis analyses indicated that expression of a significant portion of protein species was modified by both p53 mutants. In fact, compared with wild-type PC Cl3 cells, modification of expression in V143A mutant cells occurred in 23.6% of the entire protein species. Conversely, modification of S392A mutant cells affected 14.0% of total proteins. Among these components, 8.3% were common to both mutants. Several of these proteins were identified by mass spectrometry procedures; some proteins, such as HSP90 and T-complex proteins, are already known to be related to p53 function.

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Introduction

Identification of molecules associated with cell transformation induced by oncogenes or tumour suppressor genes is an important task of cancer research. These findings will contribute to two relevant issues: delineation of molecular mechanisms by which oncogenes or oncosuppressors induce the malignant phenotype and selection of candidate markers to detect cancer cells or evaluate aggressiveness of human tumours. For this aim, analysis of cell transformation by the differential proteomics approach, usually combining two-dimensional gel electrophoresis (2-D PAGE) and mass spectrometry analysis, is a well-established procedure (Simpson & Dorow 2001). Compared with nucleic acids-based approaches, such as DNA microarrays or SAGE, proteomic analysis offers the advantage of detecting real effectors of molecular functions (proteins instead of mRNAs) (Velculescu *et al.* 1995).

To ensure control of proliferation, mammalian cells have selected several different key regulators. Among

them, tumour suppressor p53 is essential for DNA damage checkpoint and response to stress of various origins (Okorokov 2003, Sherr 2004). In stressed cells, activation of p53 leads to various biological effects, including cell-cycle inhibition and apoptosis. The p53 protein is a transcription factor that acts as a tetramer. It is present at low levels in normal cells. Upon stress, several different signalling systems induce post-translational modifications and stabilization of p53. Accumulation of p53 activates transcription of a variety of target genes. Phosphorylation plays a significant role in p53 functional regulation (Xu 2003). Mutations of p53 are the most common genetic alterations found in human tumours (Soussi & Beroud 2001). Most pathogenetic p53 gene modifications are missense mutations scattered along the entire coding sequence. A large fraction of these mutations abolishes the p53 DNA-binding function (Sigal & Rotter 2000). Thus, these p53 mutants lose the ability to activate transcription of target genes; therefore, they are unable to initiate cell-cycle arrest or apoptosis. Cell culture

experiments, in which some p53 mutants are overexpressed, indicate that missense mutations have dominant negative or gain-of-function effects (Sigal & Rotter 2000). Moreover, in the same cell type, distinct missense mutations may determine different phenotypes (Parant & Lozano 2003).

A clear example of this phenomenon has been obtained in rat thyroid cells. The PC Cl3 cell line retains *in vitro* several markers of thyroid differentiation, such as thyroglobulin (Tg) and thyroperoxidase (TPO) gene expression and iodide uptake, as well as dependency of thyroid-stimulating hormone (TSH) for proliferation (Fusco *et al.* 1987). Introduction of the V143A mutant p53 allele, which abolishes the p53 DNA-binding function (Friedlander *et al.* 1996), leads to loss of Tg and TPO gene expression as well as TSH dependency for growth (Casamassimi *et al.* 1998). Conversely, PC Cl3 cells transfected with a mutant p53 allele in which the missense mutations are located outside the DNA-binding domain, the S392A mutant, show stimulated expression of markers of thyroid differentiation and loss of TSH dependency for growth. The S392A mutation is located at the COOH of p53, which is not involved in DNA binding. This mutation abrogates a CK-II phosphorylation site (Fiscella *et al.* 1994, Keller & Lu 2002). In cells transfected with the S392A mutant, the basal cAMP levels are increased with respect to the wild-type cells (Casamassimi *et al.* 1998). Thus, the V143A and the S392A mutants induce differential phenotypic effects on thyroid cells. However, information about molecular difference between the two mutants is still limited. We may note that, in thyroid carcinomas, p53 mutations are a common feature in the anaplastic phenotype (Ito *et al.* 1992, Donghi *et al.* 1993, Fagin *et al.* 1993). In the present investigation, we have used a differential proteomic approach to identify molecular differences between PC Cl3 cells and derived cell lines transformed by V143A and S392A mutant p53 alleles.

Materials and methods

Cell cultures

The PC Cl3 is a thyroid epithelial cell line derived from Fisher rats 18 months of age (Fusco *et al.* 1987). The generation of cell lines PC Cl3 V143A and PC Cl3 S392A has been previously described (Battista *et al.* 1995, Casamassimi *et al.* 1998). PC Cl3 cells and derived cell lines were grown in Ham's F-12 medium, Coon's modification (Sigma), supplemented with 5% fetal calf serum (FCS), and six growth factors (10 nM TSH, 10 nM hydrocortisone, 100 nM insulin, 5 mg/ml transferrin, 5 nM somatostatin and 20 mg/ml glycyl-histidyllysine), as previously reported (Meucci *et al.* 1994).

2-D PAGE

Cell lines were lysed directly into buffer containing 7.0 M urea, 2 M thiourea, 2% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid, 10 mM DTT, 1% pH 4–7 L IPG Buffer (Amersham–Pharmacia Biotech, Piscataway, NJ, USA), 1% (v/v) β -mercaptoethanol and 40 mM Tris–HCl. Accurate sample normalization was performed by Bradford's protein assay (Bradford 1976), coupled to quantitative, monodimensional SDS-PAGE by Gel-Doc scanner equipment (Bio-Rad, Milan, Italy). An amount of 50–100 μ g was loaded onto 13 cm, pH 4–7 IPG strips. Isoelectric focusing (IEF) was conducted by the IPGPhor II system (Amersham–Pharmacia Biotech) according to the manufacturer's instructions. Focused strips were equilibrated with 6.0 M urea, 26 mM DTT, 4% (w/v) SDS and 30% (v/v) glycerol in 0.1 M Tris–HCl (pH 6.8) for 15 min, followed by 6.0 M urea, 0.38 M iodoacetamide, 4% (w/v) SDS, 30% (v/v) glycerol and a dash of bromophenol blue in 0.1 M Tris–HCl (pH 6.8) for 15 min. The equilibrated strips were applied directly to 10% SDS-polyacrylamide gels and separated at 130 V. Gels were fixed and stained by silver staining, as previously described (Havlis *et al.* 2003).

Image analysis

Gels were scanned with Image Master 2-D apparatus (Amersham–Pharmacia Biotech). All silver-stained gels were analysed by the Image Master 2-D system program (Amersham–Pharmacia Biotech), which allows estimation of the relative differences in spot intensities for each represented protein. Due to the different representative levels of the proteins, gel analysis was performed by cropping the region under 50 kDa. Protein spots in the cropped images were detected and matched between the different samples, and individual spot intensity values were obtained according to the program instructions. For each cropped image, the total intensity of matched spots was set equal to 1.0 with the intensity normalization function of the software.

Protein identification

Spots from 2-D PAGE were excised from the gel, triturated and washed with water. Proteins were in-gel reduced, S-alkylated and digested with trypsin (Talamo *et al.* 2003). Digest aliquots were removed and used directly or subjected to a desalting/concentration step on μ ZipTipC18 (Millipore, Bedford, MA, USA) before matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF) mass spectrometry analysis. Peptide mixtures were loaded on the MALDI target by the dried droplet technique with α -cyano-4-hydroxycinnamic as

Figure 1 Two-dimensional gel electrophoresis analysis of total protein extracts from PC Cl3, PC Cl3 V143A and PC Cl3 S392A cell lines. Molecular weight markers are indicated together with the pH range used in isoelectric focusing.

matrix, and analysed by the Voyager-DE PRO mass spectrometer (Applied Biosystems, Framingham, MA, USA). Internal mass calibration was performed with peptides derived from trypsin autolysis. The mass spectra were acquired in reflectron mode with delayed extraction. Post-source decay (PSD) fragment ion spectra were eventually acquired for intense signals after isolation of the appropriate precursor by timed ion selection. ProteinProspector and PROWL software (<http://prowl.rockefeller.edu/>) packages were used to identify spots unambiguously from independent, non-redundant sequence databases by mass fingerprint and tandem mass spectrometry experiments respectively. In mass fingerprint experiments, the following parameters were set: database: NCBIInr; taxonomy: all taxa; fixed modification: carbamidomethyl cysteine; variable modification: oxidized methionine; number of missed cleavage sites: one. Positive identification was achieved only when a 50–70 ppm mass accuracy met with a significant probability PROWL software score, and nearly all dominant signals of the spectrum were assigned to the identified protein. Candidates were further evaluated by comparison with their calculated mass and pI, using the experimental values obtained from 2-D PAGE.

Western blot analysis

The indicated amounts of protein extracts obtained from PC-Cl3 cells were electrophoresed on an SDS/10% (w/v) polyacrylamide gel. Then, proteins were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH, USA). After transfer, membranes were saturated by incubation, at 4 °C overnight, with

10% (w/v) non-fat dry milk in PBS and 0.1% (w/v) Tween 20, and then incubated with one of the following antibodies: the anticalreticulin polyclonal antibody (Affinity Bioreagents, Golden, CO, USA), the anti-HSP-90 monoclonal antibody (Stressgen Biotech, York, North Yorkshire, UK), the antivimentin monoclonal antibody clone V9 (Dako, Glostrup, Denmark) or the antigalectin-1 polyclonal antibody (Chiariotti *et al.* 1995). In all cases, filters were incubated for 3 h with each of these primary antibodies. After three washes with PBS, 0.1% (w/v) Tween 20, membranes were incubated with antirabbit or antimouse immunoglobulins coupled with peroxidase (Sigma, St Louis, MO, USA). After 60-min incubation at 20 °C, membranes were washed three times with PBS, 0.1% (w/v) Tween 20, and blots were developed by the ECL (enhanced chemiluminescence) procedure (Amersham Biosciences, Milan, Italy). Normalizations were performed with the polyclonal antiactin antibody (Sigma-Aldrich, Milan, Italy). Blots were quantified by a Gel Doc 2000 videodensitometer (Bio-Rad, Milan, Italy).

Results

Total cellular extracts from wild-type PC Cl3, PC Cl3 V143A and PC Cl3 S392A mutant cell lines were subjected to 2-D PAGE analysis. Typical silver-stained gels are shown in Fig. 1. Several hundred spots are clearly identifiable in each gel. Gels were analysed for both quantitative and qualitative differences. Quantitative difference indicates the situation in which a matched spot is present in two distinct samples, but is stained with different intensities. On the other hand, qualitative difference refers to the situation in which a spot is

Table 1 Statistical parameters of 2-D PAGE analysis

	PC CI 3 wild-type	V143A	S392A
Mean	−0.918	0.004	0.002
Median	0.010	0.008	0.015
KS	0.190	0.218	0.164
P value	>0.10	>0.10	>0.10
S.D.	0.03415	0.05570	0.5344
Passed normality test?	Yes	Yes	Yes

present in only one of two distinct samples. Therefore, in this situation, matching spots of different samples is not possible. To measure the quantitative variability of our analysis, we ran two gels for each sample, each on a separate day. About 300 matching spots between gels of the same sample were identified. For each spot, the normalized intensity value obtained in the first gel was divided by the intensity value obtained in the second gel. The log value of each ratio was then obtained (LR). LR mean and median values measured for the same sample were calculated, as shown in Table 1.

LR mean and median values were clustered around the 0 value, as expected if the mean error of our analysis was random and followed a normal distribution. We used the Kolmogorov–Smirnov (KS) test (performed by Instat 3.0 software, Graphpad San Diego, CA, USA) to determine whether the LR values of each sample followed a normal distribution. In this case, the KS value should approach the 0 value. Indeed, the KS parameter indicated that these values showed a Gaussian

distribution (Table 1). Therefore, the S.D. of these distributions defined the variability of our analysis. In our experiments, the average LR S.D. was 0.191. Therefore, a LR value above 0.573 (over 3 S.D.) was set as the cut-off point to evaluate protein species differentially expressed between distinct samples. From a statistical point of view, in fact, values over 3 S.D. from the mean have less than 1% probability of representing non-differentially expressed protein species. No significant variability was detectable when analysing the same sample in different 2-D PAGE performed on different days (mean matching rate of 98%, data not shown). The analysis described above cannot be applied to the evaluation of qualitative difference, which was detected by visual inspection.

This analytic procedure identified spots over- and underexpressed in V143A and S392A mutant cell lines, with respect to wild-type cells. To show examples of the differentially expressed spots, cropped images of gels appear in Fig. 2. This figure shows spots of protein

Figure 2 Images from 2-D PAGE focusing on areas containing representative under- or overexpressed spots corresponding to the indicated protein species. Under- or overexpressed proteins are indicated by arrows.

Table 2 Number of modified spots (%) in mutant cells lines

	In V143A alone	In S392A alone	In both mutants
Modifications with respect to wild-type cells (300 matches)			
▲ Overexpression	29 (9.7%)	10 (3.3%)	14 (4.7%)
▼ Underexpression	17 (5.7%)	7 (2.3%)	11 (3.7%)
Total	46 (15.3%)	17 (5.7%)	25 (8.3%)

species whose expression modification was subsequently investigated by Western blot (see below). In Table 2, the number and percentage of spots over- or underexpressed in mutant cell lines with respect to wild-type cells are shown. Most of the observed variations occurred in the V143A mutant alone (15.3%) and in both mutants (8.3%). However, a significant fraction of modification (5.7%) was also present only in the S392A mutant, indicating that molecular modifications induced by this p53 allele were not only a subset of those induced by the V143A allele. Several differentially expressed spots were subjected to mass spectrometry analysis for protein identification. Spots were analysed by combined peptide mass fingerprint and tandem mass spectrometry experiments. In Table 3, the identified proteins are listed together with the quantification of expression in mutant cells with respect to wild-type cells. We used two approaches to test the reproducibility of our methodology. First, cell extracts from a further set of cells preparation were analysed by 2-D PAGE. All matching spots were quantitated as above. By this independent measure, the expression modification in mutant cells, with respect to wild-type ones, was never significantly different than values listed in Table 3 (data not shown). Moreover, Western blot analysis of some of the identified proteins was performed to confirm quantitative changes deriving from the 2-D PAGE analysis by a different procedure. Galectin-1, vimentin, Hsp-90 beta and calreticulin displayed differential expression between the three cell types, as detected by differential 2-D PAGE (Table 3). With specific antibodies, Western blot analysis of these protein species was used as internal control to verify the data set obtained. These experiments showed an expression pattern in strong agreement with that observed by 2-D PAGE for each of the four protein species (Fig. 3). This finding demonstrated that the entire evaluation of protein expression modification by 2-D PAGE analysis was correct.

Discussion

The proteomic analysis reported in this paper demonstrates that PC Cl3 cell transformation by the V143A mutant p53 allele modifies the expression of 23.6% of

total protein species detected, as compared with wild-type cells. On the other hand, PC Cl3 S392A mutant cells showed changes in 14.0% of protein components. This difference was expected, because the biological effects induced by the V143A mutant (loss of TSH dependence for growth and loss of the thyroid differentiated phenotype) are more pronounced than those induced by the S392A mutant (loss of TSH dependence for growth only). These results demonstrate for the first time that the expression of a significant proportion of protein species is modified by both p53 mutations. Roughly speaking, about 10 000 genes are expressed in each cell type, and these encode about 100 000 different protein species (taking into account post-transcriptional and post-translational modifications). Thus, based on the percentages indicated in Table 2, in the V142A mutant cell line, for example, about 15 000 protein species would be differentially expressed with respect to wild-type and S392A mutant cell lines.

A question arising from these conclusions is whether the protein components visualized by 2-D PAGE analysis are representative of the whole PC Cl3 cell protein content. Thus, we adopted a procedure for protein extraction commonly used in proteomic investigations of various cell lines. The only bias should be related to the detection of poorly abundant species or membrane components. In fact, only the most soluble abundant proteins are visualized by 2-D PAGE analysis of extracts from entire cellular lysates, without any organelle purification. However, there is no reason to believe that less abundant proteins behave differently from highly expressed proteins, at least in quantitative terms. The V143A mutant leads to loss of differentiation markers as well as loss of TSH dependence for growth. Only this latter function is lost in cells transformed by the S392A mutant. Although most protein species modified by the S392A mutant are identical to those modified by the V143A mutant, there are protein species whose expression is specifically modified in the S392A mutant, and not in the V143A mutant. These molecular findings suggest that the S392A mutation induces some biological effects that are not induced by the V143A mutation. Thus, we could hypothesize that cells transformed by the S392A mutant should have phenotypic changes, with respect to the wild-type cells,

Table 3 Identified proteins and relative expression in the V143A and S392A mutants with respect to the wild-type cells (▲: overexpression; ▼: underexpression)

No.	Protein (Swiss-Prot accession number)	QL	QT	V143A	S392A	Exp MW	Exp pl	Est'd Z score	Matched peptides	Biological function
1	T-complex protein epsilon subunit (P80316)		+	83*	22 ▼	60	5.8	2.34	16	Molecular chaperone
2	26S protease regulatory subunit 4 (P62193)		+	23 ▼	72	49	5.0	2.37	12	Degradation of ubiquitinated proteins
3	Ornithine aminotransferase (P04182)		+	23 ▼	21 ▼	48	6.5	2.36	12	Mitochondrial enzyme
4	Phosphoglycerate kinase (P16617)		+	553 ▲	130	37	6.3	1.82	6	Glycolytic enzyme
5	Alpha enolase (frag) (P04764)		+	666 ▲	443 ▲	28	5.6	2.16	7	Glycolytic enzyme
6	Protein disulfide isomerase (frag) (P04785)		+	429 ▲	396 ▲	34	4.8	2.36	9	ER Molecular chaperone
7	Heat shock protein HSP-90 beta (frag) (P34058)		+	489 ▲	547 ▲	29	4.8	1.87	5	Molecular chaperone
8	Vimentin (P31000)		+	451 ▲	95	55	4.8	2.42	19	Intermediate filaments protein
9	Galectin-1 (P11762)		+	827 ▲	122	15	5.7	2.39	9	Beta galactoside binding lectin
10	Calreticulin (P18418)		+	24 ▼	109	50	4.3	2.32	19	ER Molecular chaperone
11	6 kDa heat-shock protein (P63039)	+		▲	–	47	6.9	2.30	10	Molecular chaperone
12	T-complex protein beta subunit (P80314)	+		▼	▼	53	6.6	1.82	8	Molecular chaperone
13	Glucose-regulated protein (GRP78) (P06761)	+		▼	–	70	5.1	2.30	24	ER Molecular chaperone
14	Tubulin beta-5 chain (P05218)	+		▼	▼	49	6.3	2.40	12	Cytoskeletal protein
15	Aminoacylase 1 (Q99JW2)	+		▼	–	44	6.6	2.27	13	Unknown
16	UNR-interacting protein (Q9Z1Z2)	+		▼	–	40	5.2	2.36	8	Unknown
17	Aldose reductase (P07943)	+		▲	▲	36	6.5	2.09	10	Aldehyde reductase enzyme
18	APC-binding protein EB1 homolog (Q61166)	+		▲	–	35	5.4	2.20	8	Unknown
19	Triosephosphate isomerase (P48500)	+		▲	–	28	5.8	2.39	10	Metabolic pathways

QL: Qualitative difference; QT: Quantitative difference; Exp: experimental; Est'd: estimated.

*In the case of quantitative differences; numbers in V143A and S392A columns indicate the expression in respective mutant cells, considering arbitrarily as 100 the expression of the corresponding protein in wild-type cells.

not present in cells transformed by the V143A mutant. Accordingly, S392A cells have been shown to present basal cAMP levels higher than parental and V143A cells (Casamassimi *et al.* 1998).

Among molecular species whose expression is affected by p53 mutants, several proteins having chaperone or chaperone-like activities were found to be significantly altered. Particularly, two members of the heat-shock protein family, Hsp90-beta and Hsp60, were found to be significantly increased. Hsp90-beta upregulation is very important for p53 mutant stabilization (Peng *et al.* 2001) and is largely associated with mammalian cell proliferating status (Helmbrecht *et al.* 2000). Interestingly, due to its biological roles and overexpression in cancer cells,

a number of pharmacological strategies have been developed in order to inhibit Hsp90 molecular chaperone function (Blank *et al.* 2003, Workman 2003, 2004). Hsp60 also seems to be associated with the carcinogenic process associated with p53 mutations (Hsu & Hsu 1998), therefore strengthening our findings.

We show here that the expression of the T-complex protein beta subunit is reduced in both p53 mutants. On the other hand, the expression of the T-complex protein epsilon subunit is reduced only in mutant S392A. T-complex proteins are subunits of a cytoplasmic chaperon machinery (Kubota *et al.* 1995). By the use of H-1 parvovirus as a selective agent, cells with suppressed malignant phenotype were derived from malignant cell

Figure 3 Western blot analysis of calreticulin, vimentin, Hsp90-beta and galectin-1 expression in PC Cl3, PC Cl3 V143A and PC Cl3 S392A cell lines. The indicated proteins were identified by using the antibodies described in the Materials and methods section. The anti-Hsp90 antibody recognizes both the alpha and beta subunits of the protein. Below each band is the quantification of the signal (obtained by densitometric scanning), considering as 100 the value obtained in the PC Cl3 wild-type cells.

lines (Roperch *et al.* 1999). Suppressed cells showed overexpression of a T-complex protein with respect to malignant cells. Very recently, Berns *et al.* (2004) have demonstrated that RNA silencing of the T-complex protein beta subunit prevents p53-dependent growth arrest in primary human BJ fibroblast cells. Thus, both loss-of-function experiments (Berns *et al.* 2004) and differential protein expression upon introduction of p53 mutants (our data) indicate that T-complex proteins are effectors of the p53 function. The differential effects of the V143A and S392A mutants on the beta and epsilon subunits of the T-complex chaperon suggest that this multiprotein machinery is finely tuned by p53, an effect that would regulate expression of the distinct subunits by different mechanisms.

A protein that we found to be significantly upregulated by both p53 mutants is aldose reductase, for which no evident association has been described up to now. This is an interesting enzyme, whose expression has been correlated with the transformation process

possibly associated with its roles in mitogenic signalling, as demonstrated by several recent papers (Lee *et al.* 2001, Zeindl-Eberhart *et al.* 2001, 2004, Ramana *et al.* 2002, 2003).

Inhibition of galectin-1 (Gal-1) gene expression has been closely studied in normal PC Cl3 cells, which do not normally express Gal-1 due to a promotorial inhibition by methylation at the CpG island (Benvenuto *et al.* 1996). Its expression levels well correlate with the degree of thyroid tumour malignancy and the proliferative state of thyroid tumour cells (Chiariotti *et al.* 1995). The differential presence of Gal-1 expression in the V143A mutant suggests a specific involvement of p53 in Gal-1 expression rather than an epiphenomenon linked to the proliferation state. In fact, the mutant S392A can actively proliferate, but no effect on Gal-1 expression is evident. These data suggest a causal role of p53 specific mutations in Gal-1 gene expression.

Similarly, aminoacylase 1 (Acyl1) was underexpressed in the V143A mutant. This finding well complements recent proteomic investigations that demonstrated a decreased protein expression in early transformed human bronchial epithelial cell lines (Ying *et al.* 2003) and in dissected malignant kidney areas from patients with clear-cell type renal carcinoma (Balabanov *et al.* 2001). The Acyl1 gene has been assigned to chromosome 3p21.1; this region is reduced to homozygosity in small-cell lung and renal cancer (Miller *et al.* 1989). Accordingly, aminoacylase 1 has been reported to exhibit reduced or absent expression levels in SCLC cell lines and various tumours (Jones *et al.* 1991, Scaloni *et al.* 1992). These findings should support the hypothetical role of p53 in Acyl1 gene expression control.

Finally, calreticulin downregulation has been recently reported to be associated with tumour metastasis (Ding *et al.* 2004). Interestingly, a role for calreticulin has also been demonstrated in regulating p53 function by affecting its rate of degradation and nuclear localization (Mesaeli & Phillipson 2004). Our data, demonstrating a downregulation of calreticulin in the V143A mutant, point to the presence of an autoregulatory loop between wild-type p53 and calreticulin expression itself.

In conclusion, our study identified a series of protein species associated with cell transformation induced by mutant p53 alleles. The correspondence between proteins identified by us and by other groups in the study of malignancy or the proliferative state of other tumour cells indicates the validity of our approach. Future investigations evaluating the expression of p53 in human tissues will definitively test the value of identified proteins in the management of thyroid tumours.

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