CMLS, Cell. Mol. Life Sci. 62 (2005) 53–64
1420-682X/05/010053-12
DOI 10.1007/s00018-004-4329-z

CMLS Cellular and Molecular Life Sciences

Research Article

Cisplatin-induced genes as potential markers for thyroid cancer

G. Lapouge[a], R. Millon[b], D. Muller[b], J. Abecassis[b], M. Eber[c], J. P. Bergerat[a] and C. Klein-Soyer[a,*]

[a] Laboratoire de Cancérologie Expérimentale et de Radiobiologie, Institut de Recherche contre les Cancers de l'Appareil Digestif, Hôpitaux Universitaires de Strasbourg, B.P. 426, 67091 Strasbourg (France), Fax: +33 3 88 11 90 97, e-mail: claudine.soyer@ircad.u-strasbg.fr
[b] Laboratoire de Biologie Tumorale, Centre Paul Strauss, B.P. 42, 67065 Strasbourg (France)
[c] Laboratoire d'Immunoanalyse, Centre Paul Strauss, B.P. 42, 67065 Strasbourg (France)

Received 26 July 2004; received after revision 14 September 2004; accepted 26 October 2004

Abstract. Despite the uncontested role of p53 in cycle arrest/cell death after cisplatin treatment, to date the question whether wild-type p53 confers a resistant or sensitive status on the cell is still a matter of debate. Isogenic and isophenotypic human thyroid papillary carcinoma cell line variants for p53 differently expressed cycle genes after cisplatin treatment. Seven genes (*CDC6*-related protein, *CCNC*, *GAS1*, *TFDP2*, *MAPK10/JNK3*, *WEE1*, *RPA1*) selected after expression on an Atlas human cell cycle array were analyzed by quantitative real-time PCR. While cisplatin treatment increased their expression in p53 wild-type cells it decreased it in cells with inactivated p53 and had no or less effect on cells with mutated p53. These results show that in a well-defined system, different alterations of p53 can lead to a different regulation of genes and hence to either resistance or sensitivity to cisplatin. Moreover for the first time, *MAPK10/JNK3* was identified in human thyroid cells and tissue. Four of the genes (*CDC6-related protein, CCNC, GAS1* and *TFDP2*) were decreased in human papillary carcinoma tissues. Relevance of these genes (especially a decrease in *GAS1* in thyroid papillary carcinoma) in various malignant pathologies has already been shown. These genes may be explored as new markers in advanced thyroid cancer such as metastatic and anaplastic forms displaying p53 alterations.

Key words. Thyroid; K1 cell; p53 status; cDNA expression array; quantitative real-time PCR.

Cisplatin (CDDP) is among the most potent antitumor agents due to its ability to cross-link to DNA and thus create severe lesions, especially in highly replicating cells such as tumor cells, leading them to programmed cell death [1]. Nevertheless, the efficacy of the treatment may be impaired by intrinsic or acquired resistance to the drug.

The tumor suppressor gene p53 stands at the junction of highly connected pathways [2]. It has recently been linked to physiologic functions in the cell such as density-dependent contact inhibition and cell motility [3] but is essentially known for its critical role in cell cycle control, DNA repair and apoptosis [4]. It is altered in over 50% of tumors and p53 has been presumed to be implicated in both the sensitivity and resistance to CDDP [1]. Generally, cancer cells with mutated p53 are more resistant to CDDP than are wild-type cells although inactivation of p53 in some cancer cells expressing wild-type p53 sensitizes them to CDDP [5, 6]. But altered p53 function does not necessarily allow one to anticipate the subsequent sensitivity or resistance of the cells to genotoxic damage [7, 8]. Thus, some tumors retaining wild-type p53 are constitutively chemo- or radioresistant. Attempts to correlate the chemo/radiosensitivity or resistance of tumors

* Corresponding author.

to the status of p53 have shown that the therapeutic response not only depends on the type of p53 alteration (mutation, deletion, inactivation) and the type of treatment (drugs, radiation) but also on the experiments designed to evaluate the therapeutic response (proliferation, apoptosis, clonogenic survival) and it is largely cell/tissue specific [9].

In our laboratory, to elucidate some of the mechanisms involved in the sensitivity/resistance to CDDP in a well-defined system we used the phenotypically and karyotypically stable K1 cell line derived from a human papillary thyroid carcinoma [10] and displaying a wild-type p53 status, and its isogenic and isophenotypic variants [11] with either mutated or abrogated p53 functions. These cell lines present as a model of progression from the well-differentiated (wild-type p53) to the undifferentiated forms of thyroid cancer (altered functions of p53). CDDP has been used for a long time in combination with other drugs for the treatment of advanced thyroid carcinoma [12–16]. In this cancer, the prevalence of p53 mutations can reach from 40 to 75% [17, 18]. We recently examined the proliferation, apoptosis induction and clonogenic survival response to CDDP treatment of the K1 cell lines [19]. We showed that CDDP treatment identically affected the proliferation rate of each cell line but on the whole, the cells appeared rather resistant to apoptosis induction since a 4-h treatment with a high concentration of CDDP was necessary to trigger apoptosis in the cell lines expressing wild-type p53. The rate of apoptotic nuclei reached 60% by 72 h after treatment, while no apoptosis was detected in cells with altered p53. Interestingly, in these conditions, the cell line with mutated p53 displayed a one log better survival than the other cell lines including the cell line with inactivated p53 functions.

In the present paper we were interested in examining whether such a difference between cell lines with different p53 alterations would be detected during early events such as the cell cycle. As a first approach, effects of CDDP on cell cycle kinetics were examined. Then, differences in gene expression were analyzed by a cDNA expression array in untreated cell lines and after treatment with CDDP. Some genes whose expression appeared to be different between samples after cDNA array analysis were selected and their expression was further analyzed by quantitative real-time PCR (QRT-PCR). Subsequently, to assess the relevance of the selected genes in thyroid cancer we investigated their expression in human normal thyroid tissue and papillary carcinoma.

Materials and methods

Materials

Cell culture media, Dulbecco's modified Eagle's medium with sodium pyruvate and pyridoxine, Ham's F12 and MCDB 104, Hank's balanced salt solution (HBSS), phosphate-buffered saline (PBS) Dulbecco's formulation, trypsin-EDTA, antibiotics (penicillin and streptomycin) fetal calf serum (FCS) (endotoxin, virus and mycoplasma screened) and Superscript RNase H$^-$ reverse transcriptase were from Gibco/InVitrogen Life Technologies (Paisley, UK). Culture dishes were from Falcon, Becton-Dickinson Company (Lincoln Park, N. J., USA). Cis-dichlorodiammine platinum (cisplatin, CDDP) was from Merck génériques (Lyon, France). The total RNA extraction kit, RNeasy Midi and RNase-free DNase set were from Qiagen (Courtaboeuf, France). The Atlas nylon human cell cycle cDNA array and AtlasImage software were from Clontech (Ozyme, Saint-Quentin Yvelines, France). Alpha-^{32}P-dATP (≈3000 Ci/mmol, 250 µCi), Kodak BioMax MS film and BioMax MS intensifying screen were from Amersham Biosciences (Orsay, France). Recombinant ribonuclease inhibitor was from Promega (Charbonnières, France) and random priming hexamers were from Boehringer (Meylan, France). Gene-specific primers were synthesized by Proligo (Paris, France) while the LC FastStart DNA SYBR green I kit and LC capillaries were purchased from Roche Diagnostics (Meylan, France). Additional chemicals were of analytical grade from Sigma (St-Quentin-Fallavier, France) or Merck (Darmstadt, Germany).

Cell cultures, CDDP treatment and cell cycle analysis

The wild-type human thyroid carcinoma cell line K1 and its derivates have been previously described [11, 19, 20]. Briefly, the parental wild-type line K1wt, and its derivates K1µt and K1E6 (K1 SCX3 transfected with the plasmid pc53-SCX3 expressing a dominant-negative human mutant p53 143^{ala} and K1E6.4 transfected with the retroviral vector PA317–16E6 and expressing the E6 gene from human papilloma virus type 16) were grown in a mixture of Dulbecco's modified Eagle's medium, Ham's F12 and MCDB 104, (2:1:1, v/v/v) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% FCS.

Cells from growth-arrested cultures were seeded at a density of 9×10^3 cells/cm^2 and grown in triplicate in 100/15-mm culture dishes in medium containing 10% FCS. After 24 h, when about 30% of the cells were in the S phase, representative dishes were exposed to 5, 10 or 20 µg/ml (respectively 17, 33 or 67 µM) CDDP for 1 h. At the end of the incubation period, the cells were rinsed three times with HBSS and further incubated in complete medium. This point constituted the time zero of the experiment. Cells from the different conditions were collected 16, 24 and 40 h after treatment and processed for cell cycle analysis as described previously [20]. Cell cycle analysis was performed by flow cytometry in a FACSCalibur cell sorter (Becton Dickinson, San Jose, Calif.) and the cell

distribution in the different phases of the cycle was calculated using Modfit 2.0 cell cycle analysis software.

RNA isolation from cultured cells

Total RNA was extracted at time point 16 h after CDDP treatment from five to six pooled Petri dishes representing 1 to 2×10^7 untreated or CDDP (10 μg/ml, 1 h) treated cells using the RNeasy kit and including DNase I treatment. The RNA concentration was measured in a GeneQuant II spectrophotometer (Pharmacia Biotech, Cambridge, UK) and the concentrations and the quality of RNA preparations were checked on standard 2% agarose gels which included RNA standards. This extraction method provided approximatively 100 μg total RNA for each condition and the preparations were stored at –70 °C.

Tissue collection and RNA extraction

Thyroid tissue was obtained, with informed consent, at the time of surgery from patients with preliminary diagnosis for thyroid disease. A 2-mm^3 fragment was dissected from the tumor tissue by a pathologist, immediatly frozen in liquid nitrogen and stored at –80 °C until use. The rest of the tumor was fixed in 6% buffered formaldehyde and embedded in paraffin for histological analysis. Similarly, histologically checked normal thyroid tissue was obtained from patients undergoing thyroidectomy for a solitary benign thyroid neoplasm. Total RNA from these tissues was extracted after disruption and homogenization in a Mixer Mill (Qiagen) using the RNeasy kit as above. Total RNA from 15 individual normal human thyroid tissues and from 17 thyroid papillary carcinomas was prepared and stored at –80 °C. The concentration and the quality of the RNAs were checked as described above. Equal amounts from three individual normal human thyroid and thyroid papillary carcinoma RNA samples were pooled for preliminary experiments.

Hybridization to Atlas nylon human cell cycle cDNA expression array

Atla nylon human cell cycle cDNA expression arrays contain the cDNAs from nine housekeeping genes and 111 genes involved in cell cycle regulation (gene list accessible at www.clontech.com, PT3549-3, includes gene/protein classification). Five micrograms of total RNA was converted into ^{32}P-labeled first-strand cDNA by incorporation of [α-^{32}P]-dATP using the specific cDNA synthesis primer mix provided by the manufacturer. Each probe was hybridized once to the nylon membrane as indicated in the user manual. For each cell line, probes from untreated and CDDP-treated cells were hybridized in the same experiment. Following hybridization, the membranes were washed in successive buffers with increasing stringency and then exposed to BioMax films at –80 °C and developed at several time points. Autoradiograms displaying hybridization signals with similar intensity were scanned using the Bio Rad Gel Doc 2000 analyzer with image acquisition software Quantity One (version 4.2) (Hercules, Calif.) in order to compare gene expression profiles between the different samples, and were saved as Tiff files for further quantification. Quantitative analysis was performed using the AtlasImage 2.01 software program with the global normalization mode to compare the different arrays. Results representing the expression of every gene for each condition were compared pairwise and exported in a tabular report.

Supervised gene selection

Normalized, log2-transformed data were compared using the Significance Analysis of Microarrays add-in to Microsoft Excel [21] (http://www-stat.stanford.edu/~tibs/SAM/index.html). The genes were selected with the SAM default parameters and no minimal fold change to cover the largest significant set of differentially expressed genes. Comparison of untreated cell lines with cell lines with a different p53 status resulted in 34 significant genes and paired comparison of CDDP-treated cells to corresponding untreated cells in 29 significant genes.

Quantitative real-time PCR

QRT-PCR was used to analyze several genes differentially expressed on arrays from different samples. First-strand cDNA was prepared starting with 1 μg of total RNA which was reverse transcribed using Superscript II reverse transcriptase and random-priming hexamers according to the manufacturer's recommendations. All RNA samples were reverse transcribed simultaneously and three separate reverse transcriptions were performed. cDNA primers specific for the selected genes were designed using Primer3 software (table 1). For each target gene, the primers are located in two different exons except for *GAS1*, the gene of which spans only one exon. PCR amplification and analysis was achieved using a LightCycler instrument and software (version 5.3.2) (Roche, Mannheim, Germany). The PCR master mix was prepared using the FastStart DNA SYBR Green I kit according to protocols provided by the manufacturer (http://www.roche-applied-science.com/lightcycler-online). Optimal PCR conditions were set up for each primer set using standard cDNA prepared from total RNA extracted from exponentially growing K1wt cells (table 1). Efficiencies were calculated from standard curves constructed using standard cDNA dilutions ranging from 1/10 to 1/1000 which are equivalent to 10 to 0.1 ng of initial total RNA. With the standard curve, for each gene, a relative initial template copy number was estimated applying the formula $N_0 = N/E^n$ where N_0 = initial copy number; E = efficiency of PCR reaction = $10^{-1/p}$; p = slope of the curve; n = number of cycles at the intercept and $N = 10^{10}$ copies assuming that it is the number of PCR

Table 1. Primer sequences and optimal PCR conditions.

Gene	Sequence	Amplicon size (bp)	GenBank Accession No. for cDNA	Magnesium concentration (mM)	Annealing temperature (°C)	Annealing time (s)	Efficiency $E = 10^{-1/p}$
CDC6	F GCAGTTTGTTCAGGGGCTTG R GCCCGAGACAGCTTCCTTTT	244	U77949	4	60	5	5.67
CCNC	F AGATGCCAGGCAATGGTTTG R TTGGACCCTGCTCTCCTTCA	185	M74091	3.5	60	5	22.14
TFDP2	F TAAAGCGGATGGGAATGTCG R TAAGGTGGCACCAGTGGTCA	203	U18422	4	63	5	0.01
*GAS1**	F CGGAGCTTGACTTCTTGGAC R CCCAACCCTTCAAATTGCTA	198	L13698	3.5	60	5	0.29
*MAPK10***	F CTTCCCAGCGGACTCCGAGCACAA R TGTCAGATGCCAGGGTCTGGTCGG	395	U34819	4	65	2	0.06
RPA1	F TTCTCGAAAGGCACCCTGAA R AGCTCTTGCAGATCCCGATG	208	M63488	4	60	5	11.17
WEE1	F ACCTCGGATACCACAAGTGC R TTGCCATCTGTGCTTTCTTG	221	U10564	5	60	5	20.17

p = Slope of standard curve.
* Sequence communicated by Dr Huang [25].
** Sequence communicated by Clontech.

product copies necessary to give a signal [22]. Unknown sample expression levels were evaluated with these standard-curve values. cDNAs from samples were tested diluted at 1/50, 1/100 and 1/200. Each experiment was repeated twice using each time cDNA from separate reverse transcriptions. The generated data were averaged and expressed as relative units of copy number. For *GAS1* and *MAPK10* genes, additional experiments were performed on cDNAs prepared from 1 µg total RNA as described above from 12 normal thyroid and 14 thyroid papillary carcinoma tissue samples.

Results

Effects of CDDP on cell cycle kinetics of human thyroid papillary carcinoma K1 cell lines displaying different p53 status

Exponentially growing K1 cell lines have a mean doubling time of about 24 h whatever their p53 status [20]. In these conditions, the distribution of untreated cells in the different cycle phases was as follows G0/G1 48%, S 27%, G2/M 25% and progressively changed to G0/G1 81%, S 12% and G2/M 7% when reaching confluence after 40 h of culture (fig. 1). Treatment of K1wt cells expressing wild-type p53 with increasing concentrations of CDDP (5, 10 and 20 µg/ml) for 1 h led to a transient slowing down of S phase traverse concomitant with the persistence of cells in the G0/G1 phase and with a progressive accumulation of cells in the G2/M phase. The time course of these events depended on the concentration of CDDP, with the accumulation of cells in the G2/M phase attaining 33% and 63% 24 h after treatment with 5 and 10 µg/ml CDDP, respectively. It reached 80% at time 40 h after the cells had first accumulated in S phase (80% at time 24 h) when treated with 20 µg/ml while at the lowest concentration cell proliferation resumed at time 40 h. In all these conditions a G1 peak persisted, confirming as expected the existence of the G1/S checkpoint after damage, for cells expressing wild-type p53.

K1 cells with altered p53 treated with CDDP underwent the same time- and CDDP concentration-dependent events affecting the S and G2/M phase but in addition showed a progressive emptying of the G1 phase. This latter event is a hallmark for the loss of functional p53. In K1E6 cells displaying inactivated p53, the emptying of the G1 phase was complete by 40 h after treatment with 20 µg/ml CDDP while the persistence of a minor fraction of cells in G1 was observed in the K1µt cell line with mutated p53. Such a difference in G1 exit between these two cell lines has been previously observed after γ irradiation [20] or after bleomycin treatment (which results in the complete loss of p21 expression in K1E6 but not K1µt cells) [11].

Expression of cell cycle genes in exponentially growing K1 cell lines: effect of CDDP treatment

The clearest event reflected by cell cycle analysis after cell damage is the accumulation of cells in the G2/M phase. A consistent G2/M peak was already observed at

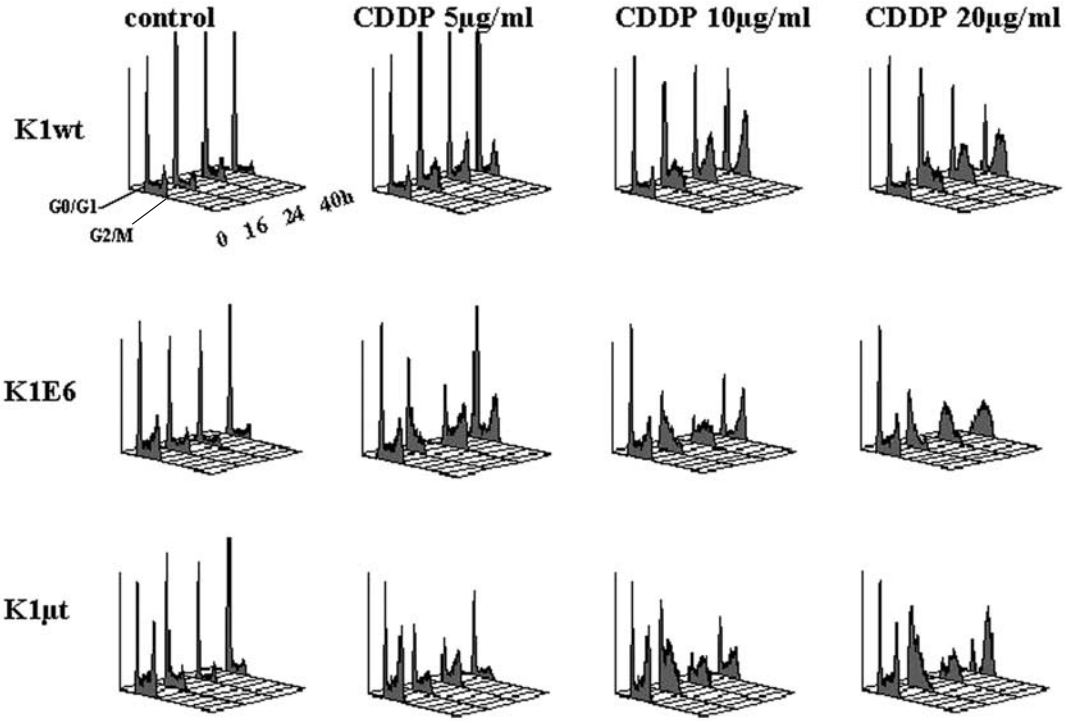

Figure 1. Effect of CDDP on cell cycle kinetics of exponentially growing K1 cell lines. Experiments were performed as described in Materials and methods and the cell phase distribution is presented as a function of time. The results for each cell line are representative data from two to three separate flow cytometry experiments.

24 h in each cell line after a 1-h treatment with 10 µg/ml CDDP. Hence, since mRNA expression precedes protein expression by 6–8 h, differences in cell cycle gene expression between the different variants of the K1 cell line were examined in exponentially growing cells, 16 h after treatment with 10 µg/ml CDDP. An Atlas nylon human cell cycle cDNA expression array was used for this purpose. Data acquisition was performed using AtlasImage software and applying the global normalization mode to compare the different arrays. This method was the most suitable for our experimental conditions (cells with different p53 status, cycling cells and CDDP treatment which induces growth arrest) as it is now evident that the expression levels of the most commonly used housekeeping genes such as glyceraldehyde-3-phosphatedehydrogenase or β-actin can vary widely according to individuals, tissues or experimental conditions [23, 24].

Significant analysis of microarrays (SAM) was used to compare K1 cell lines with altered p53 to the K1 cell line with wild-type p53, and CDDP-treated K1 cell lines to the corresponding untreated cell lines. This resulted in 30 positive and 4 negative selected genes in the first case (table 2), and in 28 positive and 1 negative selected genes in the case of CCDP-treated cells (table 3).

Seven genes for which the expression levels differed at least in one condition by over twofold were selected: *CDC6-related protein* (*CDC6*), *TFDP2* and cyclin C/*CCNC* which display specific effects during the G1 phase, *WEE1*, intervening at the G2/M transition point, and *RPA1* which is implicated in DNA replication and repair. *GAS1* was selected as it has recently been shown to be diminished in thyroid papillary carcinoma [25] and *MAPK10/JNK3* as it has up to now mainly been localized in the neural tissue, with a little in the heart and the testis but has never been found in the thyroid gland. Very little is known concerning the effects of CDDP on these selected genes. The genes were located in the cell cycle at their potential intervention site(s) (fig. 2). Details and literature concerning the specific functions of these genes are available on-line (http://atlasinfo.clontech.com/atlasinfo).

Analysis of the expression of selected genes by QRT-PCR

The expression of five genes (*CDC6, CCNC, GAS1, WEE1* and *RPA1*) was similar in K1wt with wild-type p53 and K1µt with mutated p53 but significantly higher in K1E6, in which p53 is altered by inactivation (fig. 3). QRT-PCR results confirmed the higher expression of *CCNC* and *GAS1* observed on arrays in K1E6 compared to K1wt but this differential expression was not confirmed in K1µt. Moreover, the expression of *MAPK10* was highest in the K1wt cell line in contrast to data from array experiments. The protocol differences concerning

Table 2. Comparison of untreated cell lines by SAM.

Gene name	Accession	Gene ID	Ratio K1E6/K1wt	Ratio K1μt/K1wt
Thirty positive significant genes				
120 (TFDP2)	*U18422*	*transcriptin factor DP2 (Humdp2); E2F dimerization partner 2*	*2.42*	*2.51*
4D (CCNC)	*M74091*	*G1/S specific cyclin C*	*1.57*	*2.14*
6C	U34051	cyclin-dependent kinase 5 activator isoform p391 precursor (CDK5 activator; NC5AI)	3.24	2.71
6N	X85753	cell division protein kinase 8 (CDK8); protein kinase K35	2.32	2.13
18F (MAPK10/JNK3)	*U34819*	*C-jun N-terminal kinase 3 alpha2 (JNK3A2); PRKM10 + MAP kinase p493F12*	*1.61*	*2.06*
10D	M87339	activator 1 37-kDa subunit; replication factor C 37-kDa subunit (RFC37); RFC4	1.66	1.59
14B (GAS1)	*L13698*	*growth-arrest-specific protein 1 (GAS1)*	*5.29*	*3.26*
6B	U40343	cyclin-dependent kinase 4 inhibitor D (CDKN2D); p19-INK4D)	16.72	16.43
10G	L22005	ubiquitin-conjugating enzyme E2 32-kDa complementing protein; ubiquitin-protein ligase; uniquitin carrier protein; CDC34	2.54	1.93
16B	U25265	dual specificity nitogen-activated protein kinase 5 (MAP kinase kinase 5; MAPKK 5)	2.57	1.99
10N	Y10479	E2F-3	1.21	1.53
14B	S40706	growth arrest & DNA-damage-inducible protein 153 (GADD153); DNA-damage-inducible transcript 3 (DDIT3); C/EBP homologous protein (CHOP)	1.57	1.36
6J	M14505	cell division protein kinase 4; cyclin-dependent kinase 4 (CDK4); PSK-J3	1.17	1.34
10F	M87338	activator 1 40-kDa subunit; replication factor C 40-kDa subunit (RFC40); RFC2	2	1.31
6I	M23410	junction plakoglobin (JUP); desmoplakin III (DP3)	1.08	1.41
16G	U25278	extracellular signal-regulated kinase 5 (ERK5); BMK1 kinase	2.84	1.55
22D	M74524	ubiquitin-conjugating enzyme E2 17-kDa (UBE2A); ubiquitin-protein ligase; ubiquitin carrier protein; HR6A	1.04	1.34
4L	D50310	cyclin I	1.05	1.3
4E	X59798	G1/S-specific cyclin D1 (CCND1); cyclin PRAD1; bcl-1 oncogene	1.04	1.32
6D	X80343	cyclin-dependent kinase 5 activator precursor (CDK5 activator); tau protein kinase II 23-kDa subunit; TPKII regulatory sbunit	2.36	1.06
14I	L05624	dual-specificity mitogen-activated protein kinase kinase 1 (MAP kinase kinase 1; MAPKK 1; MKK1); extracellular signal-regulated kinase 1; ERK activator kinase 1	1.04	1.29
14H	D89667	c-myc binding protein MM-1	1.01	1.32
22I	D21235	HHR23A; UV excision repair protein RAD23A	1.04	1.26
20H	L40027	glycogen synthase kinase 3 alpha (GSK3 alpha)	1	1.31
22E	D63878	NEDD5 protein homolog; DIFF6; KIAA0158	1.19	1.09
16H	X79483	extracellular signal-regulated kinase 6 (ERK6); stress-activated protein kinase-3; mitogen-activated protein kinase p38 gamma; (MAP kinase p38 gamma)	2.51	1.06
18C	U53442	mitogen-activated protein kinase P38 beta (MAP kinase P38 beta); stress-activated protein kinse 2 (SAPK2)	1.86	1.39
4C	M25753	G2/mitotic-specific cyclin B1 (CCNB1)	1.06	1.12
8F	L25676	cell division protein kinase 9 (CDK9); serine/threonine protein kinase PITALRE	1.56	1
6G	X05360	cell division protein 2 homolog (CDC2); p34 protein kinase; cyclin-dependent kinase 1 (CDK1)	1.74	1.21
Four negative significant genes				
12L	U66469	p53-dependent cell groowth regulator CGR19	0.29	0.45
12M	Z12020	p53-associated mdm2 protein	0.44	0.53
18I	X03484	c-raf poto-oncogene	0.43	0.56
6L	X66365	cell division protein kinase 6 (CDK6); serine/theonine protein kinase PLSTIRE	0.39	0.58

Selected genes are italicized. Ratio between cell lines are those determined by AtlasImage software.

Table 3. Comparison of CDDP-treated to corresponding untreated cell lines by SAM.

Gene name	Accession	Gene ID	Ratio TK1wt/K1wt	Ratio TK1E6/K1E6	Ratio TK1μt/K1μt
Twenty-eight positive significant genes					
6E	U09579	cyclin-dependent kinase inhibitor 1 (CDKN1A); melanoma differentiation-associated protein 6 (MDA6); CDK-interacting protein 1 (CIP1); WAF1	1.19	1.34	1.58
6G	X05360	cell division control protein 2 homolog (CDC2); p34 protein kinase; cyclin-dependent kinase 1 (CDK1)	2.13	1.61	1.9
10I	S72008	CDC10 protein homolog	1.31	3.06	1.89
12M	Z12020	p53-associated mdm2 protein	1.15	1.58	1.59
14C	M60974	growth arrest & DNA-damage-inducible protein (GADD45); DNA-damage-inducible transcript 1 (DDIT1)	1.29	2.05	1.33
10H (CDC6)	*U77949*	*CDC6-related protein*	*1.45*	*2.54*	*1.4*
12I	X85133	RBQ1 retinoplastoma binding protein	1.21	2.74	1.67
4I	U47413	G2/mitotic-specific cyclin G1 (CCNG1; CYCG1)	1.05	1.78	1.46
12B	L23959	E2F dimerization partner 1; DRTF1-polypeptide 1 (DP1)	1.92	1.5	2.33
8O	M81934	CDC25B; CDC25HU2; M-phase inducer phosphatase 2	1.12	1.06	1.14
4B	X51688	G2/mitotic-specific cyclin A (CCNA; CCN1)	0.99	1.69	1.35
6L	X66365	cell division protein kinase 6 (CDK6); serine/theonine protein kinase PLSTIRE	1.04	1.25	1.37
22F (RPA1)	*M63488*	*replication protein A 70-kDa subunit (RPA70; REPA1; RF-A); single stranded DNA-binding protein*	*0.94*	*2.38*	*1.5*
10B	M34065	CDC25C; M-phase inducer phosphatase 3	1.01	1.39	1.9
18I	X03484	c-raf proto-oncogene	1.05	1.93	1.34
8M	M80629	CDC2-related protein kinase CHED	1.05	3.01	1.62
12E	X74594	retinoblastoma-like protein 2 (RBL2; RB2); 130-kDa retinoblastoma-associated protein	1.21	1.68	1.53
6O (WEE1)	*U10564*	*wee1Hu CDK tyrosine 15-kinase wee-1-like protein kinase*	*1.17*	*2.02*	*0.94*
12D	M15400	retinoblastoma-associated protein (RB1); PP110; P105-RB	0.99	2.05	2.89
22G	K00065	cytosolic superoxide dismutase 1 (SOD1)	1.02	1.03	1.2
20F	M29870	ras-related C3 botulinum toxin substrate 1; p21-rac1; ras-like protein TC25	1.01	1.02	1.12
20D	M35543	CDC42 homolog; G25K GTP-binding protein (brain isoform + placental isoform)	0.97	1.11	1.61
12F	X74262	chromatin assembly factor 1 p48 subunit (CAF1 p48 subunit); retinoblastoma-binding protein 4 (RBBP4); RBAP48; msi1 protein homolog	1.03	1.44	0.84
10E	L07541	replication factor C 38-kDa subunit (RFC38); activator 1 38-kDa subunit	1.09	1.04	1.21
12L	U66469	p53-dependent cell groowth regulator CGR19	0.96	2.17	1.31
8H	U24152	p21-activated kinase alpha (PAK-alpha, PAK1)	1.07	1.11	1.55
12J	X85134	RBQ-3	0.96	2.71	1.23
10J	U18291	CDC16HS	1	1.9	1.05
One negative significant gene					
12C(TFDP2)	*U18422*	*transcription factor DP2 (Humdp2); E2F dimerization partner 2*	*0.58*	*0.9*	*0.78*

Selected genes are italicized. Ratio between treated (TK1) and untreated (K1) cells are those determined using AtlasImage software.

reverse transcription of mRNA to cDNA (specific primer sets but unique annealing temperature in array experiments, vs random hexamers in QRT-PCR experiments) between the two technologies used, as well as the unique hybridization condition in array experiments but specific PCR conditions for each gene in QRT-PCR experiments (table1) may explain these discrepancies. The QRT-PCR method chosen to corroborate the array results may be more specific than array experiments.

In K1wt expressing wild-type p53, CDDP treatment systematically, although at different rates, enhanced the expression of the selected genes except for *MAPK10* which remained unchanged. CDDP treatment of K1E6 cells with inactivated p53 tended to decrease gene expression while it did not significantly affect their expression in K1μt with mutated p53 with the exception of *MAPK10* which it tended to decrease (fig. 4).

Expression of selected genes in normal or tumoral thyroid tissue

Expression of the selected genes was investigated in a pooled normal human thyroid tissue (NT) and thyroid

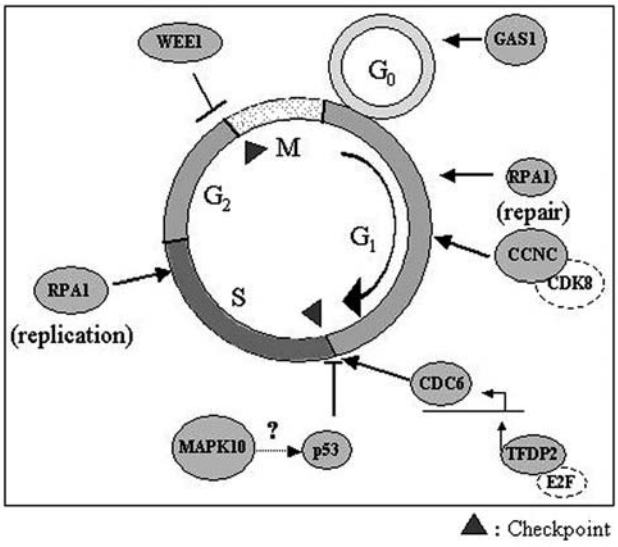

Figure 2. Visualization of selected genes [*CDC6, CCNC, GAS1, TFDP2, MAPK10/JNK3, WEE1, RPA1,* and possible partner(s)] in the cell cycle at their potential intervention sites.

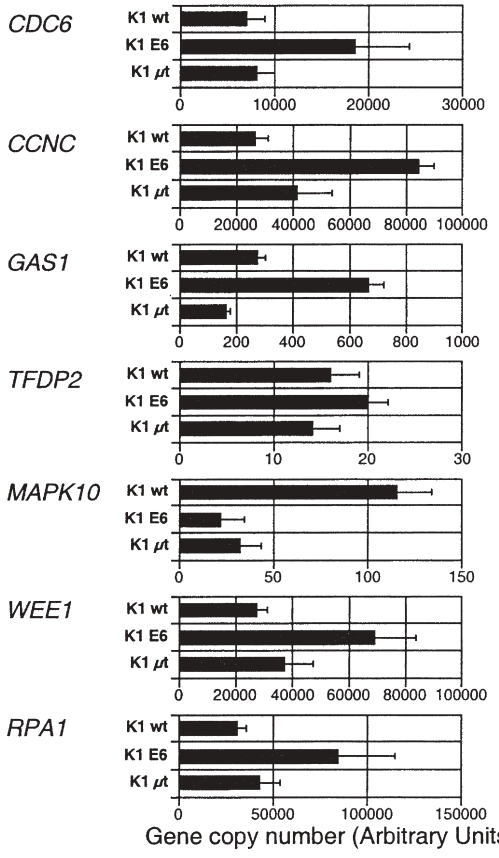

Figure 3. QRT-PCR analysis of selected genes in untreated K1 cell lines. QRT-PCR experiments were performed as described in Materials and methods. Values on the abscissa represent the relative copy number of each gene expressed as arbitrary fluorescence units. Results for each gene are the mean of two to three independent QRT-PCRs including 2 to 3 dilutions per conditions using cDNAs from two to three independent reverse transcriptions (mean ± SEM).

SEM: standard error of the mean = $\frac{SE}{\sqrt{n}}$
n = number of samples.

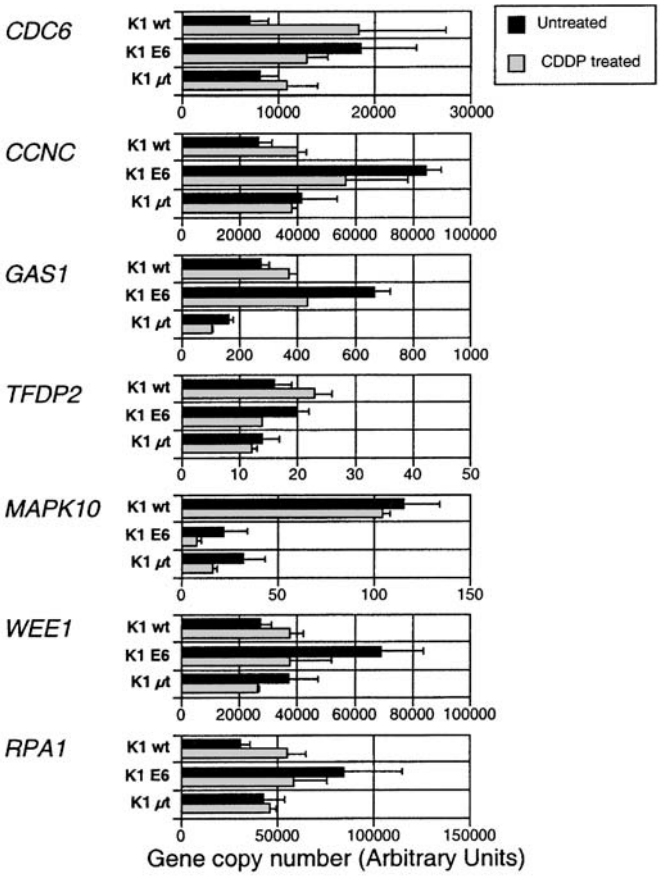

Figure 4. QRT-PCR analysis of the effects of CDDP on the expression of selected genes in K1 cell lines. QRT-PCR experiments were performed as described in Materials and methods. Results were obtained as reported in figure 3.

papillary carcinoma tissue (PTC) (fig. 5). We observed that the expression of each gene, except for *MAPK10* and *WEE1,* was always lower in PTC compared to their expression in NT. Hence the expression of *GAS1* in the PTC pool was decreased by about sixfold. Such a decrease has previously been described in PTC [18]. In addition we detected the presence of the *MAPK10* gene in NT and PCT. To confirm these results, we further analyzed the expression of *GAS1* and *MAPK10* in 12 individual NT and 14 individual PTC and compared crossing points for cDNAs diluted to 1/100. The crossing points for *GAS1* were 30.95 ± 0.34 and 32.43 ± 0.62 cycles and for *MAPK10* 33.29 ± 0.32 and 33.48 ± 0.34 cycles for NT and PTC, respectively (mean ± SEM, n = 12 and 14, two separate experiments). In addition, in 9 out of 14 PTC, GAS1 expression was decreased by at least fivefold compared to mean expression in NT samples. The presence of *MAPK10* in NT and PTC samples was confirmed but its expression did not appear to differ between normal and tumoral tissue.

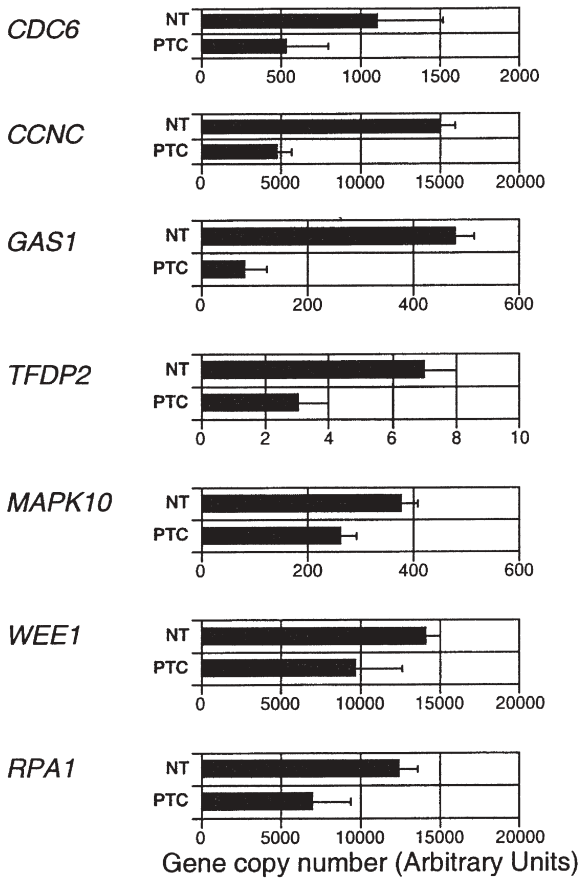

Figure 5. Comparison by QRT-PCR of the expression of selected genes in normal and tumoral thyroid tissue. Total RNA from normal human thyroid tissue and thyroid papillary carcinoma tissue was obtained as described in Material and methods. QRT-PCR was performed as previously. Results were obtained as reported in figure 3 (mean ± SEM, two to three independent experiments).

Discussion

A cDNA expression array was used to investigate cell cycle gene expression in human thyroid papillary carcinoma cell lines, differing in their p53 status, either untreated or early after exposure to CDDP when cells slowly exit the G1 phase and are retarded in S phase before accumulating in the G2/M phase. Among the differently expressed genes, seven (*CDC6, CCNC, GAS1, TFDP2, MAPK10/JNK3, WEE1* and *RPA1*) were selected and their expression further examined using QRT-PCR. These analyses reproduced cDNA expression differences of *CCNC* and *GAS1* between K1wt and K1E6 observed in array experiments; in addition, among the seven genes examined, five (*CDC6, CCNC, GAS1, WEE1* and *RPA1*) were overexpressed in untreated K1E6 as compared to K1wt or K1μt expressing mutated p53. These results confirm that not only alteration of p53 is important for cellular responses but also the way in which it is altered [7–9]. Several expression differences from array assays were not confirmed by QRT-PCR experiments, particularly for *MAPK10* for which the primers used in QRT-PCR corresponded to those used in array experiments (primer sequences communicated by Clontech). These discrepancies may be due to sequence-dependent hybridization characteristics and variations inherent in the hybridization reaction. In the case of *MAPK10*, the high homology between *JNK1*, *JNK2* and *JNK3* may also partially explain the expression discrepancies between array and QRT-PCR experiments for *MAPK10*/*JNK3*. Considering the large selection set for SAM analysis and the low threshold (2–3) that we chose, our results are within the range of studies published using similar criteria [26–28]. To our knowledge, to date, nothing is known concerning the effects of CDDP on *CDC6, CCNC, TFDP2* and *MAPK10* and very little about its effects on *GAS1, WEE1* and *RPA1*. CDDP treatment increased the expression of *CDC6* in K1wt but did not significantly affect its expression in K1 cells with altered p53. This could reflect an accumulation due to cycle arrest at the G1 checkpoint induced by CDDP in p53 wild-type cells since *CDC6* intervenes in the early G1 phase. This checkpoint is lost in cells with altered p53 as shown by the progressive emptying of the G1 phase after CDDP treatment. CDC6 has been shown to be overexpressed in cervix carcinoma [29]. This proliferation marker improves the detection of cancer cells in cervical smears. In addition, the histological distribution of CDC6 immunoreactive cells is similar to that of cells with a strong signal for HPV DNA [30]. This is in agreement with enhanced cell proliferation of cancer cells and correlates well with increased expression in cells which have lost their G1/S checkpoint due to p53 inactivation by the E6 protein of HPV16 as is the case for K1E6 cells. Paradoxically, down-regulation of *CDC6,* probably resulting from altered interactions with transcription factors, was observed in prostate cancer where a progressive decrease is correlated with the transition from benign hyperplasia to metastatic cancer [31]. We observed that *CDC6* tended to be decreased in the PTC pool, yet further investigations are necessary to determine a potential role for CDC6 as a marker of 'malignancy stage' in thyroid cancer.

CCNC expression was slightly increased after CDDP treatment in K1wt but not in K1E6 and K1μt cells, which may indicate a different effect of the drug depending on p53 status. *CCNC* has recently been implicated in various pathologies. *CCNC* has been associated with the pathogenesis of Alzheimer disease, its overexpression being correlated with dedifferentiation and degeneration processes [32]. *CCNC* is located on chromosome 6q21 and deletions on chromosome 6 in the region coding for this gene have been reported in human lymphoid malignancies [33–35]. Chromosomal abnormalities including a progressive loss of 6q21 from poorly differentiated to

anaplastic carcinoma have also been identified in thyroid cancer [36]. Interestingly, we found a significant decrease in *CCNC* expression in the PTC pool. Thus *CCNC* may serve as a potential marker in thyroid cancer pathology.

TFDP2 locates to a region of the genome which is frequently altered in lymphoproliferative diseases [37]. No effect of CDDP on this gene has been described up to now. Our results with K1 cell lines could suggest that CDDP may increase TFDP2 expression in wild-type cells and repress it in cells with altered p53 functions although through yet unknown mechanisms. *TFDP2* was significantly decreased in the PTC pool. Analysis at the protein level may shed more light on its effects.

Suppression of *WEE1* has been shown in colon carcinoma cells and histologically confirmed in human tissue, suggesting a potential role as a tumor suppressor [38]. A decrease of *WEE1* may be crucial in the case of DNA damage, allowing transition of cells from the G2 to M phase without damage repair thus leading to accumulation of genetic alterations. Effects of CDDP on WEE1 expression in *Schizosaccaromyces pombe* has shown that strains defective in WEE1 function failed to arrest in G2 following CDDP treatment and that all strains failing to arrest in G2 were hypersensitive to CDDP [39]. On the other hand, analysis of cycle regulation of human lung adenocarcinoma PC-9 cells in which p53 is mutated concluded that WEE1 was not affected by CDDP treatment [40]. In our experiments, *WEE1* was only moderately affected by exposure of cells to CDDP: it was slightly increased in K1wt cells consistent with a G2/M block but paradoxically tended to be decreased in K1E6 and K1μt despite the presence of a protracted G2/M block. No significant difference in W*EE1* expression was observed between normal and tumoral thyroid tissues.

RPA1 expression was increased in K1E6 control cells, suggesting that these cells would have better replication/repair capacities. It was also increased after CDDP treatment in K1 cells expressing wild-type p53, consistent with the induction of repair mechanisms, but this was not the case in K1 cells in which p53 was altered and thus calls into question the implication of p53 in the regulation of *RPA1* expression. *RPA1* was moderately decreased in thyroid tumoral tissue. If this is confirmed, it could mean that repair mechanisms involving *RPA1* may be impaired in thyroid papillary carcinoma.

GAS1 is localized on chromosome 9q21.3-q22, a region frequently deleted in myeloid malignancies [41]. Its expression is associated with the quiescent state in normal cells and its growth suppressive effects are mediated by p53 through transactivation-independent functions [42]. Thus *GAS1* no longer abolishes cell proliferation in SV40-transformed cells [43]. While such an effect has not yet been described in E6-transfected cells, it may explain the paradoxical overexpression of *GAS1* observed in exponentially growing K1E6 cells. In the literature, toxicity tests performed in two human cell lines expressing wild-type p53 led to the conclusion that CDDP does not alter *GAS1* expression [44]. Consistent with an expected growth arrest induction, in our experiments, *GAS1* was moderately increased in K1wt cells after exposure to CDDP but not in the cell lines in which p53 was altered. *GAS1* was significantly decreased in the PTC pool and further comparison between normal individual thyroid samples and individual PTC samples confirmed a significant decrease in over 60% of our PTC samples. Thus our results confirm the recently described decrease of *GAS1* expression in PTC by Huang et al. [25] using Affymetrix oligonucleotide arrays.

MAPK10/JNK3 is considered as a candidate tumor suppressor gene since mutations were identified in human brain tumors [reviewed in ref 45] while its expression is increased in lymphocytes from radiation-exposed individuals [46]. Up to now, this gene has only been localized in neural tissue [47] and at least in mouse, little in the heart and the testis [48]. We report for the first time expression of this gene in human thyroid carcinoma cell lines. *MAPK10* expression although lower than expected according to array results was nevertheless confirmed in QRT-PCR experiments especially in thyroid tissue where it was identically expressed in NT and PTC samples. The high homology between JNK1, JNK2 and JNK3 may complicate an assessment of the specific expression of MAPK10/JNK3 protein in tissues. In the mouse, activated JNK3 (similar to the widely distributed members of the same family JNK1 and JNK2) phosphorylates p53 on serine 34 (equivalent to serine 33 in human p53) [49]. We observed a significant decrease (between 3.5- to 5-fold) of *MAPK10/JNK3* in K1 cells in which p53 is altered. Furthermore, CDDP treatment diminished *MAPK10* expression by at least 2-fold in K1 cells with altered p53 while it did not affect its expression in K1 cells retaining wild-type p53.

To summarize, we confirm that cells with altered p53 can differently express genes depending on the type of p53 alteration. Hence, in a well-defined system, sensitivity or resistance to CDDP implies a fine-tuned regulation of the numerous pathways/downstream genes connected to the p53 network. On the whole, CDDP treatment enhanced expression of the selected genes regulating the cycle in cells expressing wild-type p53, possibly reflecting blockage at different proliferation steps or checkpoints, while it rather seemed to decrease these genes in cells with altered p53 and often at different rates depending on whether p53 was inactivated or mutated. Whether these differences translate to protein functions remains an open investigation field.

Thyroid cancers are rare, rendering randomized trials hypothetical. Although they generally have a good prognosis, survival rates have not improved over several decades [50] while an increased incidence of thyroid cancer, at

least in France, has been observed over the same period [51, 52].

Histological studies of anaplastic cancers show the existence of differentiated thyroid carcinoma together with areas of dedifferentiation and this transition has often been associated with expression of mutated p53, strongly suggesting that p53 alteration may be a key event in the transition from differentiated to anaplastic thyroid carcinoma [53–56]. Furthermore, treatments of metastatic and anaplastic forms remain of little benefit and deserve investigations for new therapeutic guidelines [50]. The present model provides a tool to dissect cell cycle events as a function of p53 status after treatment of thyroid cancer. We have identified four genes (*CDC6*, *CCNC, GAS1* and *TFDP2*) which are decreased in thyroid papillary carcinomas. The relevance of these genes in various malignant pathologies has already been shown. If our results are further confirmed, these genes could be used as new markers in thyroid cancer and may be explored in advanced thyroid cancer such as metastatic and anaplastic forms. As such, the decrease in *GAS1* in thyroid papillary carcinoma deserves to be further investigated in thyroid cancer. Finally, we detected for the first time expression of *MAPK10/JNK3* in human cell lines and in thyroid tissue. Since this gene has up to now essentially been localized to the brain where it may act as a tumor suppressor gene, we think that it is worth a thorough exploration in normal and pathological thyroid tissue.

Acknowledgements. The authors would like to thank Prof. M. Lessard for access to AtlasImage software, Mrs S. Krugel, C. Macabre and F. Noël for excellent technical assistance, Dr D. Winford-Thomas for providing K1µt and K1E6 cell lines, the Department of Pathology of the Comprehensive Cancer Center Paul Strauss of Strasbourg and Dr O. Schneeghans for helpful discussions. Part of this work was supported by the Association pour la Thérapie Génique des Cancers (ATGC).

References

1 Siddik Z. H. (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene **22:** 7265–7279
2 Vogelstein B., Lane D. and Levine A. J. (2000) Surfing the p53 network. Nature **408:** 307–310
3 Meerson A., Milyavsky M. and Rotter V. (2004) p53 mediates density-dependent growth arrest. FEBS Lett. **559:** 152–158
4 El-Deiry W. S. (2003) The role of p53 in chemosensitivity and radiosensitivity. Oncogene **22:** 7486–7495
5 Kartalou M. and Essigmann J. M. (2001) Mechanism of resistance to cisplatin. Mutat Res. **478:** 23–43
6 Nieder H., Christen R., Lin X., Kondo A. and Howell S. B. (2001) Identification of genes that mediate sensitivity to cisplatin. Mol. Pharmacol. **60:** 1153–1160
7 Pirollo K. F., Bouker K. B. and Chang E. H. (2000) Does p53 status influence tumor response to anticancer therapies? Anti-Cancer Drugs **11:** 419–432
8 Soussi T. and Béroud C. (2001) Assessing TP53 status in human tumors to evaluate clinical outcome. Nat. Rev. Cancer. **1:** 233–240
9 Weller M. (1998) Predicting response to cancer chemotherapy: the role of p53. Cell Tissue Res. **292:** 435–445
10 Wyllie F. S., Haughton M. F., Blaydes J. P., Schlumberger M. and Wynford-Thomas D. (1995) Evasion of p53-mediated growth control occurs by three alternative mechanisms in transformed thyroid epithelial cells. Oncogene **10**: 49–59
11 Wyllie F. S., Haughton M. F., Rowson J. M. and Wynford-Thomas D. (1999) Human thyroid cancer cells as a source of iso-genic, iso-phenotypic cell lines with or without functional p53. Br. J. Cancer **79:** 1111–1120
12 Droz J. P., Rougier P., Goddefroy V., Schlumberger M., Gardet P. and Parmentier C. (1984) Chemotherapy for medullar cancer of the thyroid: phase II trials with adriamycin and cis-platinum administered as monochemotherapy. Bull. Cancer **71:** 195–199
13 Schimaoka K., Schoenfeld D. A., DeWys W. D., Creech R. H. and DeConti R. (1985) A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. Cancer **56:** 2155–2160
14 Kober F., Heiss A., Keminger K. and Depisch D. (1990) Chemotherapy of highly malignant thyroid tumors. Wien. Klin. Wochenschr. **102:** 274–276
15 De Besi P., Busnardo B., Toso S., Girelli M. E., Nacamulli D., Simioni N. et al. (1991) Combined chemotherapy with bleomycin, adriamycin, and platinum in advanced thyroid cancer. J. Endocrinol. Invest. **14:** 475–480
16 Asakawa H., Kobayashi T., Komoike Y., Maruyama H., Nakano Y., Tamaki Y. et al. (1997) Chemosensitivity of anaplastic thyroid carcinoma and poorly differentiated thyroid carcinoma. Anticancer Res. **17:** 2757–2762
17 Salabè G. B. (2001) Pathogenesis of thyroid nodules: histological classification? Biomed. Pharmacother. **55**: 39–53
18 Learoyd D. L., Messina M., Zedenius J. and Robinson B. G. (2000) Molecular genetics of thyroid tumors and surgical decision-making. World J. Surg. **24:** 923–933
19 Céraline J., Deplanque G., Noël F., Natarajan-Amé S., Bergerat J. P. and Klein-Soyer C. (2003) Sensitivity to cisplatin treatment of human K1 thyroid carcinoma cell lines with altered p53 function. Cancer Chemother. Pharmacol. **51:** 91–95
20 Deplanque G., Céraline J., Mah-Becherel M. C. M., Cazenave J. P., Bergerat J. P. and Klein-Soyer C. (2001) Caffeine and the G2/M block override: a concept resulting from a misleading cell kinetic delay, independent of functional p53. Int. J. Cancer **94:** 363–369
21 Tusher V. G., Tibshirani R. and Chu G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA **98:** 5116–5121
22 Rasmussen R. (2001) Quantification on the Lightcycler. In: Rapid Cycle Real-Time PCR: Methods and Applications, pp. 21–34, Meuer S., Wittwer C., Nakagawara K. (eds), Springer, Berlin
23 Bustin S. A. (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J. Mol. Endocrinol. **25:** 169–193
24 Schmittgen T. D. and Zakrajsek B. A. (2000) Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. J. Biochem. Biophys. Methods **46:** 69–81
25 Huang Y., Prased M., Lemaon W. J., Hample H., Wright F. A., Kornacker K. et al. (2001) Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc. Natl. Acad. Sci. USA **98:** 15044–15049
26 Duan Z., Feller A. J., Penson R. T., Chabner A. and Seiden M. V. (1999) Discovery of diffentially expressed genes associated with paclitaxel resistance using cDNA array technology: analysis of interleukin (IL) 6, IL-8, and monocyte chemotactic protein 1 in the paclitaxel-resistant phenotype. Clin. Cancer Res. **5:** 3445–3453
27 Rajeevan M. S., Ranamukhaarachchi D. G., Vernon S. D. and Unger E. R. (2001) Use of real-time quantitative PCR to vali-

date the results of cDNA array and differential display PCR technologies. Methods **25:** 443–451
28 Chuaqui R. F., Bonner R. F., Best C. J. M., Gillespie J. W., Flaig M. J. and Hewitt S. M. (2002) Post-analysis follow-up and validation of microarray experiments. Nat. Genetics **32:** 509–514
29 Williams G. H., Romanowski P., Morris L., Madine M., Mills A. D. and Stoeber K. (1998) Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc. Natl. Acad. Sci. USA **95:** 14932–14937
30 Bonds L., Baker P., Gup C. and Shroyer K. R. (2002) Immunohistochemical localization of Cdc6 in squamous and glandular neoplasia of the uterine cervix. Arch. Pathol. Lab. Med. **126:** 1163–1168
31 Robles L. D., Frost A. R., Davila M., Hutson A. D., Grizzle W. E. and Chakrabarti R. (2002) Down-regulation of *Cdc6*, a cell cycle regulatory gene, in prostate cancer. J. Biol. Chem. **27:** 25431–25438
32 Ueberham U., Hessel A. and Arendt T. (2003) Cyclin C is involved in the pathogenesis of Alzheimer's disease. Neurobiol. Aging **24:** 427–435
33 Li H., Lahti J. M. and Kidd V. J. (1996a) Alternatively spliced cyclin C mRNA is widely expressed, cell cycle regulated, and encodes a truncated cyclin box. Oncogene **13:** 705–712
34 Li H., Lahti J. M., Valentine M., Saito M., Reed S. I., Look A. T. et al. (1996b) Molecular cloning and chromosomal localization of the human cyclin C (CCNC) and cyclin E (CCNE) genes: deletion of the CCNC gene in human tumors. Genomics **32:** 253–259
35 Jackson A., Carrara P., Duke V., Sinclair P., Papaioannou M., Harrison C. J. et al. (2000) Deletion of 6q16-q21 in human lymphoid malignancies: mapping and deletion analysis. Cancer Res. **60:** 2775–2779
36 Wreesmann V. B., Ghossein R. A., Patel S. G., Harris C. P., Schnaser E. A., Shaha A. R. et al. (2002) Genome-wide appraisal of thyroid cancer progression. Am. J. Pathol. **161:** 1549–1556
37 Korz C., Pscherer A., Benner A., Mertens D., Schaffner C., Leupolt E. et al. (2002) Evidence for distinct pathomechanisms in B-cell chonic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell cycle and apoptosis-associated genes. Blood **99:** 4554–4561
38 Backert S., Gelos M., Kobalz U., Hanski M. L., Böhm C., Mann B. et al. (1999) Differential gene expression in colon carcinoma cells and tissues detected with a cDNA array. Int. J. Cancer **82:** 868–874
39 Thiebaut F., Enns R. and Howell S. B. (1994) Cisplatin sensitivity correlates with its ability to cause cell cycle arrest via wee1 kinase-dependent pathway in *Schizosaccharomyces pombe*. J. Cell. Physiol. **159:** 506–514
40 Nishio K., Fujiwara Y., Miyahara Y., Takeda Y., Ohira T., Kubota N. et al. (1993) Cis-diamminedichloroplatinum(II) inhibits p34cdc2 protein kinase in human lung cancer cells. Int. J. Cancer **55:** 616–622
41 Evdokiou A., Webb G. C., Peters G. B., Dobrovic A., O'Keefe D. S., Forbes I. J. et al. (1993) Localization of the human growth-arrest specific gene (GAS1) to chromosome bands 9q21. 3-q22, a region frequently deleted in myeloid malignancies. Genomics **18:** 731–733
42 Del Sal G., Ruaro E. M., Utreta R., Cole C. N., Levine A. J. and Schneider C. (1995) Gas–1 induced growth suppression requires a transactivation-independent p53 function. Mol. Cell. Biol. **15:** 7152–7160
43 Del Sal G., Ruaro M. A., Philipson L. and Schneider C. (1992) The growth arrest-specific gene, GAS1, is involved in growth suppression. Cell **70:** 595–607
44 Vikhanskaya F., D'Incalci M. and Broggini M. (1993) Effects of DNA damaging agents on gene expression in two human cancer cell lines. Cell. Mol. Biol; **39:** 855–862
45 Manning A. M. and Davis R. J. (2003) Targeting JNK for therapeutic benefit: from junk to gold? Nat. Drug Disc. **2:** 554–565
46 Sakamoto-Hojo E. T., Mello S. S., Pereira E., Fachin A. L., Cardoso R. S., Junta C. M. et al. (2003) Gene expression profiles in human cells submitted to genotoxic stress. Mutat. Res. **544:** 403–413
47 Mohit A. A., Martin J. H. and Miller C. A. (1995) $p49^{3F12}$ kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. Neuron **14:** 67–78
48 Martin J. H., Mohit A. A. and Miller C. A. (1996) Developmental expression in the mouse nervous system of the $p49^{3F12}$ SAP kinase. Mol. Brain Res. **35:** 47–57
49 Hu M. C. T., Qiu W. R. and Wang Y. P. (1997) JNK1, JNK2 and JNK3 are p53 N-terminal serine 34 kinases. Oncogene **15:** 2277–2287
50 Sherman S. I. (2003) Thyroid carcinoma. Lancet **361:** 501–511
51 Colonna M., Grosclaude P., Remontet L., Schvartz C., Mace-Leseche J., Velten M. et al. (2002) Incidence of thyroid cancer in adults recorded by French cancer registries (1978–1997). Eur. J. Cancer **38:** 1762–1768
52 Verger P., Catelinois O., Tirmarche M., Cherie-Challine L., Pirard P., Colonna M. et al. (2003) Thyroid cancers in France and the Tchernobyl accident: risk assessment and recommendations for improving epidemiological knowledge. Health Phys. **85:** 323–329
53 Fagin J. A., Matsuo K., Karmakar A., Chen D. L., Tang S. H. and Koeffler H. P. (1993) High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J. Clin. Invest. **91:** 179–184
54 Hadar T., Mor C., Shvero J., Levy R. and Segal K. (1993) Anaplastic carcinoma of the thyroid. Eur. J. Surg. Oncol. **19:** 511–516
55 Dobashi Y., Sugimura H., Sakamoto A., Mernyei M., Mori M., Oyama T. et al. (1994) Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. Diagn. Mol. Pathol. **3:** 9–14
56 Suarez H. G. (2000) Molecular basis of epithelial thyroid tumorigenesis. C. R. Acad. Sci. Life Sci. **323:** 519–528