Originals

Tomasz Ferenc[1], Andrzej Lewiński[2], Dariusz Lange[3], Hanna Niewiadomska[4], Jacek Syguť[5], Stanisław Sporny[6], Barbara Jarząb[3], Elżbieta Sałacińska-Łoś[5], Andrzej Kulig[8], Jan Włoch[3]

Analysis of P53 and P21WAF1 Proteins Expression in Follicular Thyroid Tumors*

[1]Department of Biology and Genetics, Medical University, Łódź,
[2]Department of Endocrinology and Isotope Therapy, Polish Mother's Memorial Hospital, Medical University, Łódź,
[3]Center of Oncology–MSC Memorial Institute, Gliwice,
[4]Chair of Oncology, Medical University, Łódź,
[5]Department of Surgical Pathology, Świętokrzyski Center of Oncology, Kielce,
[6]Department of Pathomorphology, Medical University, Łódź,
[7]Laboratory of Pathomorphology, Institute of Pediatrics, Medical University, Łódź,
[8]Department of Clinical Pathomorphology, Polish Mother's Memorial Hospital, Łódź

The expression of P53 and P21WAF1 proteins was analyzed immunohistochemically in archival material derived from 12 cases of follicular thyroid carcinoma, 57 cases of follicular adenoma and 17 cases of nodular goiter. In the follicular carcinoma group 6 out of 12 cases (50%) were positive for P53 protein and 4 out of 12 cases (33.3%) were positive for P21WAF1 protein. In the follicular adenoma group, 18 out of 57 cases (31.6%) were positive for P53 and 16 out of 57 cases (28.1%) were positive for P21WAF1 protein. No positive cases of P53 or P21WAF1 proteins presence were found in the nodular goiter group. Positive correlation between the expression of P53 and P21WAF1 proteins was found for follicular carcinoma and adenoma groups (p=0.034 and p=0.002, respectively). The obtained results demonstrate that simultaneous immunohistochemical detection of P53 and P21WAF1 proteins expression may be useful in determining functional status of P53 protein, helping to interpret expression of P53 protein in thyroid follicular carcinoma cells.

Introduction

The latest reports demonstrate that the process of carcinogenesis is inseparably linked to disturbances of the cell cycle. The transition of cell through consecutive cell cycle checkpoints is regulated by several proteins, among them P53 and P21WAF1 [5, 8, 12, 21, 23].

One of the basic functions of P53 protein in normal cells is to assure genome stability by regulating the cell cycle at the level of gene transcription (including p21WAF1) [19, 23, 28]. The product of non-mutated *p53* gene (locus 17p13.1) is a suppressor factor. However, a mutation of this gene may confer to P53 protein oncogenic properties [11]. The second important role, played by correct (wild) form of P53 protein, is DNA damage control. After an initial impact of DNA damaging agent, an accumulation of wild P53 protein takes place along with prolongation of G1 phase of the cell cycle in order to allow the cellular machinery to repair DNA damage before cell entry into S phase of the cycle [23, 28, 33]. The correct P53 protein is also an important regulator of apoptosis. In this process, P53 protein regulates expression of genes involved in the apoptotic pathway, among them *bax* and *bcl-2*, by either their activation or inhibition [23]. An important role in the self-regulation of *p53* gene expression is played by *mdm2* gene, located in the 12q13-14 region [28]. Mutations in the *p53* suppressor gene have been the most frequently observed molecular changes in human neoplasms and they are detected mainly at the progression stage [13]. It needs to be stressing that correct P53 protein may loose its suppressor function as a result of attaching to viral oncoproteins, inclu-

* The study was supported by the grant No. P05B 037 10 from the National Committee for Scientific Research

ding large T antigen of SV40 virus, human E1B adenoviruses or E6 papilloma viruses [8, 11].

p21WAF1/CIP1 gene encoding P21WAF1 protein is located in the 6p21.1 chromosome region. This protein plays a crucial role in cell cycle regulation as an inhibitor of cellular proliferation. In normal mammalian cells P21WAF1 protein takes part in the formation of four-element complexes, including besides one of cyclin-dependent cdk kinases, one of A, B, D or E cyclins and PCNA protein [5, 8, 12, 21]. The transcription of *p21WAF1/CIP1* gene is enhanced by non-mutated (wild) P53 protein, especially following cell exposure to genotoxic factors. The expression of *p21WAF1* gene may also be increased independently of P53 protein, mainly during differentiation and maturation of cells [8, 23]. Somatic point mutations of *p21WAF1/CIP1* gene in neoplasms rather rarely have been described. More often, an increased (compared to normal cells) *p21WAF1/CIP1* gene expression has been observed in various cancers, either at mRNA and/or at P21WAF1 protein level [4, 6, 22].

Material and Methods

Material

Paraffin embedded archival tissues from 12 thyroid follicular carcinomas, 57 thyroid follicular adenomas and 17 nodular goiters were studied. All the sections were examined by two pathologists (J.S. and S.S.) using a conference microscope and were histopathologically classified, as suggested by the WHO committee [14].

Immunohistochemical staining

Representative paraffin blocks, containing tumor material from each case were sectioned at 4μm, affixed to silanized slides and dried overnight at 56.7°C. Antigen retrieval was performed with citrate buffer (0.01M, pH 6.0) in a standard microwave unit. The sections for immunohistochemistry were stained, using the avidin-biotin (ABC) method according to Hsu et al. [16]. Deparaffinized sections were treated with 3% hydrogen peroxide (H_2O_2) in methanol for 5min to block endogenous peroxidase activity. Nonspecific antibody binding was reduced by incubation of the sections for 20min with normal goat serum. The slides were incubated with a 1:50 dilution of the primary mouse monoclonal anti-P53 antibody (clone: D0-7, Novocastra, UK) and a 1:20 dilution of the primary mouse monoclonal anti-P21WAF1 antibody (clone: 4D10, Novocastra, UK). In the negative control reaction the primary antibody was omitted. The reaction products were demonstrated using the Novostain Super ABC kit (NCL/ABCm) from Novocastra. 3,3'-diaminobenzidine (DAB) was used as chromogen, and the sections were counterstained with Mayers hematoxylin, dehydrated and mounted. The paraffin-embedded sections from ductal breast carcinoma were used as positive control. The immunohistochemical staining of cells was estimated by means of the half-quantitative method using Hogg's net. The results were expressed as the percentage of positive cells per 1000 thyroid follicular cells, counted in 10HPF (objective magnification x40). Only cells with an evidence of nuclear staining were considered positive. To score the P53 and P21WAF1 staining patterns, we used the criteria proposed by Cordon-Cardo et al. [7] (<20% as negative and >20% as positive). The relative number of immunoreactive cells was graded as follows: (–) – less than 20% of tumor cells stained positively; (+) – 20–50% of tumor cells stained positively; (++) – more than 50% of tumor cells stained positively. Lesions scored as (++) were considered as showing high expression (overexpression) of the proteins [31].

Statistical procedure

All the parameters presented as means were compared using Mann-Whitney's test, where $p<0.05$ was considered significant. Associations between the categorical variables and P53 or P21WAF1 expression were assessed using Fisher's exact test. The relationships (Spearman correlations) between P53 and P21WAF1 expression levels were analyzed.

Results

The average percentage of cells showing nuclear location of P53 protein (Fig. 1) was 23.3 (SD±21.2) in the follicular thyroid carcinoma group, 15.2 (SD±13.7) in the follicular adenoma group and 0.8 (SD±1.5) in the nodular goiter group. Within the follicular adenoma group the percentage of cells positive for P53 protein was 18.9 (SD±14.9) for microfollicular adenomas, 11.9 (SD±12.1) for normo- and macrofollicular adenomas and 8.6 (SD±8.1) for adenomas derived from oxyphilic cells (Table 1). A comparison of the average numbers of cells positive for P53 protein in the both follicular carcinoma and adenoma groups did not reveal any significant differences. The average percentage of cells positive for P53 protein in the follicular carcinoma and adenoma groups was statistically significantly higher, compared to that in the nodular goiter group ($p<0.001$ and $p<0.001$, respectively).

In the follicular carcinoma group 6 cases (50.0%) positive for P53 protein were found vs. 18 (31.6%) in the follicular adenoma group. In the nodular goiter group no cases positive for P53 protein were noted. Within the follicular adenoma group the number of cases positive for P53 protein was 14 (45.2%) for microfollicular adenomas, 3 (16.7%) for

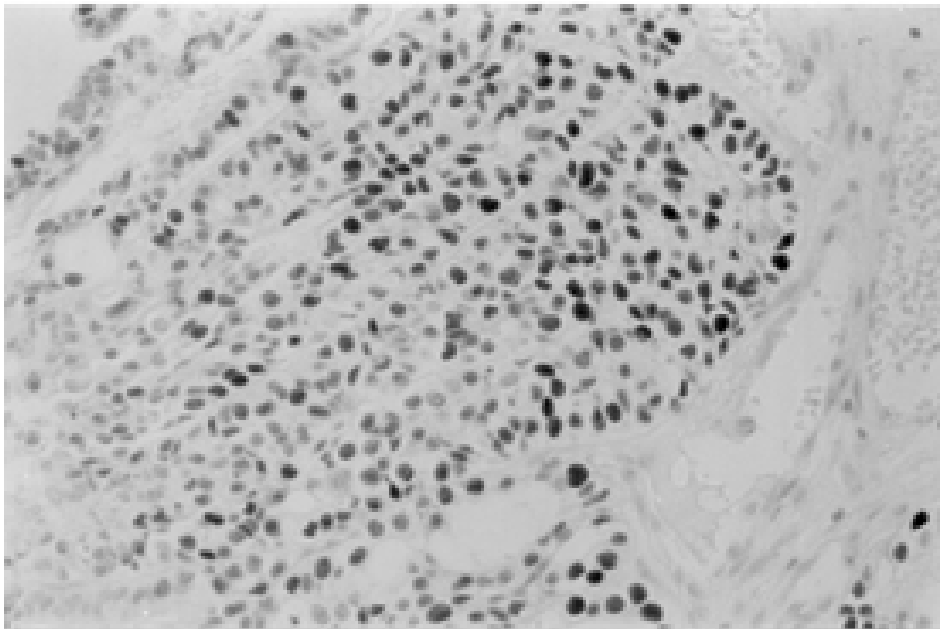

Fig. 1. Expression of P53 in thyroid follicular carcinoma.

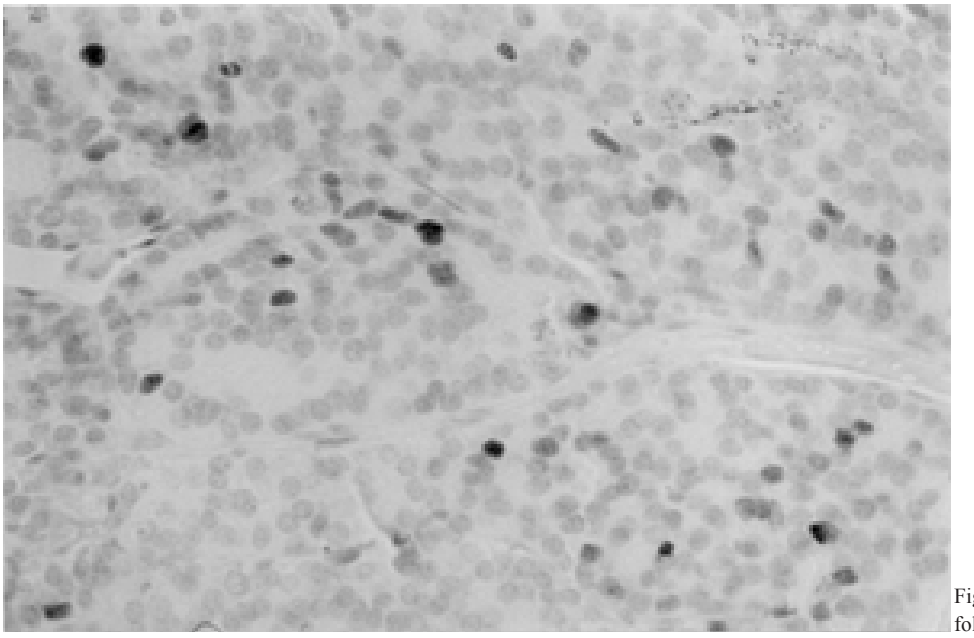

Fig. 2. Expression of P21WAF1 in thyroid follicular carcinoma.

TABLE 1
The average per cent of P53 protein-positive cells in the study groups

Group	N	M	SD	Min	Max
Follicular carcinoma	12	23.3	21.2	0.0	58.4
Follicular adenoma	57	15.2	13.7	0.0	47.8
including:					
– microfollicular	31	18.9	14.9	0.0	46.8
– normo- and macrofollicular	18	11.9	12.1	0.0	42.4
– from oxyphilic (Hürthle) cells	8	8.6	8.1	0.0	23.5
Nodular goiter	17	0.8	1.5	0.0	4.6

N – number of cases; M – arithmetic mean; SD – standard deviation; Min - minimum value; Max – maximum value

normo- and macrofollicular adenomas and 1 (12.5%) for adenomas derived from oxyphilic cells (Table 2).

The average percentage of cells showing nuclear location of P21WAF1 protein was 14.5% (SD±17.4) for the follicular thyroid carcinoma group (Fig. 2), 14.2% (SD±17.4) for the follicular adenoma group (Fig. 3) and 0.5% (SD±1.0) for nodular goiter group. Within the follicular adenoma group the percentage of cells positive for P21WAF1 protein was 16.4% (SD±11.4) for microfollicular adenomas, 10.7% (SD±7.7) for normo- and macrofollicular adenomas and 13.5 (SD±12.0) for adenomas derived from oxyphilic cells (Table 3). A comparison of the average numbers of cells positive for P21WAF1 protein in

TABLE 2
The number of P53 protein-positive and negative cases in the study groups

Group	N	N (%)		
		++	+	–
Follicular carcinoma	12	1 (8.3)	5 (41.7)	6 (50.0)
Follicular adenoma including:	57	0 (0.0)	18 (31.6)	39 (68.4)
– microfollicular	31	0 (0.0)	14 (45.2)	17 (54.8)
– normo- and macrofollicular	18	0 (0.0)	3 (16.7)	15 (83.3)
– from oxyphilic (Hürthle) cells	8	0 (0.0)	1 (12.5)	7 (87.5)
Nodular goiter	17	0 (0.0)	0 (0.0)	17 (100.0)

(++) – >50%; (+) – 20-50%; (–) – <20% positive cells; N – number of the cases studied; n (%) – number (per cent) of positive or negative cases

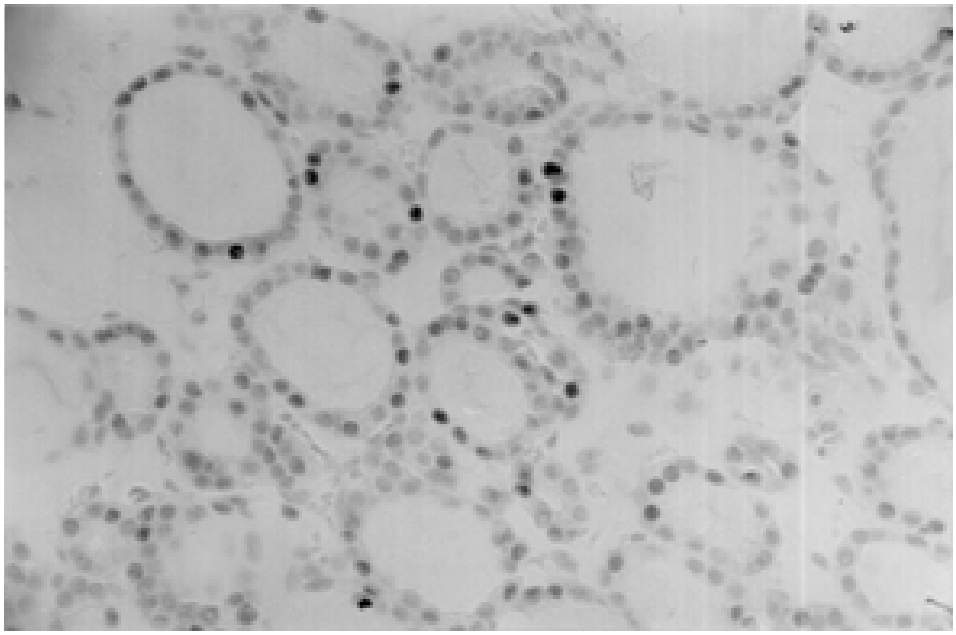

Fig. 3. Expression of P21WAF1 in thyroid follicular adenoma.

TABLE 3
The average per cent of P21WAF1 protein-positive cells in the study groups

Group	N	M	SD	Min	Max
Follicular carcinoma	12	14.5	17.4	0.0	61.6
Follicular adenoma including:	57	14.2	11.4	0.0	43.2
– microfollicular	31	16.4	12.8	0.0	43.2
– normo- and macrofollicular	18	10.7	7.7	0.3	28.7
– from oxyphilic (Hürthle) cells	8	13.5	12.0	0.4	36.2
Nodular goiter	17	0.5	1.0	0.0	3.2

N – number of cases; M – arithmetic mean; SD – standard deviation; Min – minimum value; Max – maximum value

both the follicular carcinoma and adenoma groups did not reveal significant differences. The average percentage of cells positive for P21WAF1 protein in the follicular carcinoma or adenoma groups was significantly higher, compared to the nodular goiter group ($p<0.001$ and $p<0.001$, respectively).

In the follicular carcinoma group 4 cases (33.3%) positive for P21WAF1 protein were found vs. 16 (28.1%) in the follicular adenoma group. In the nodular goiter group, no cases positive for P21WAF1 protein were noted. Within the follicular adenoma group, the number of cases positive for P21WAF1 protein was 11 (35.5%) for microfollicular adenomas, 2 (11.1%) for normo- and macrofollicular adenomas and 3 (37.5%) for adenomas derived from oxyphilic cells (Table 4).

In the follicular carcinoma group, a positive correlation was revealed between P53 and P21WAF1 proteins expression ($Rs=0.622$; $p=0.031$). Likewise, a positive correlation

TABLE 4
The number of P21WAF1 protein-positive and negative cases in the study groups

Group	N	n (%)		
		++	+	–
Follicular carcinoma	12	1 (8.3)	3 (25.0)	8 (66.7)
Follicular adenoma including:	57	0 (0.0)	16 (28.1)	41 (71.9)
– microfollicular	31	0 (0.0)	11 (35.5)	20 (64.5)
– normo- and macrofollicular	18	0 (0.0)	2 (11.1)	16 (88.9)
– from oxyphilic (Hürthle) cells	8	0 (0.0)	3 (37.5)	5 (62.5)
Nodular goiter	17	0 (0.0)	0 (0.0)	17 (100.0)

(++) – >50%; (+) – 20–50%; (–) – <20% positive cells; N – number of the cases studied; n (%) – number (per cent) of positive or negative cases

was found between the expression of P53 and P21WAF1 proteins in the group of follicular adenomas (Rs=0.400; p=0.002). Within the follicular adenoma group, a positive correlation between P53 and P21WAF1 proteins expression was found among adenomas derived from oxyphilic cells (Rs=0.711; p=0.049). In the microfollicular adenoma group, a positive correlation noted between P53 and P21WAF1 expression was borderline significant (Rs=0.339; p=0.069). In the group of normo- and macrofollicular adenomas, no significant correlation was found between P53 and P21WAF1 proteins expression (Rs=0.339; p=0.169).

A positive correlation was found between P53 and P21WAF1 proteins expression in the follicular carcinoma group (Rs=0.622; p=0.031). Likewise, a positive correlation was revealed between P53 and P21WAF1 proteins expression in the follicular adenoma group (Rs=0.400; p=0.002).

In the follicular carcinoma group, a positive reaction was found for both P53 and P21WAF1 proteins in 4 out of 12 cases (33.3%). Two out of 12 cases (16.7%) were positive for P53 protein and negative for P21WAF1. In the remaining 6 out of 12 follicular carcinomas (50.0%), no positive nuclear reaction was found for either P53 or P21WAF1 proteins (Table 5). In the microfollicular adenoma group 7 out of 31 cases (22.6%) showed positive nuclear staining for both P53 and P21WAF1 proteins. Seven out of 31 cases (22.6%) were positive for P53 protein and negative for P21WAF1. Four out of 31 cases (12.9%) showed positive nuclear reaction for P21WAF1 and negative for P53. In the remaining 13 out of 31 follicular adenomas (41.9%), no positive reaction was found for either P53 or P21WAF1 (Table 6). In the normo- and macrofollicular adenoma group 3 out of 18 cases (16.7%) showed positive reaction for P53 and negative for P21WAF1 protein. Two out of 18 cases (11.1%) were positive for P21WAF1 protein and negative for P53. In the remaining 13 out 18 normo- and macrofollicular adenoma cases (72.2%), no positive reaction was found for either P53 or P21WAF1 protein (Table 7). In the group of adenomas derived from oxyphilic cells one out of 8 cases (12.5%) showed a positive nuclear reaction for P53 and negative for P21WAF1. Three out of 8 cases (37.5%) were positive for P21WAF1 protein and negative for P53. In the remaining 4 out of 8 oxyphilic cell-derived adenomas (50%), no positive reaction for either P53 or P21WAF1 proteins was found (Table 8).

TABLE 5
Immunohistochemical reaction patterns for P53 and P21WAF1 proteins in the follicular carcinoma group

Patient (No.)	P53/P21WAF1
1.	+/+
2.	–/–
3.	+/–
4.	–/–
5.	–/–
6.	–/–
7.	+/+
8.	–/–
9.	+/+
10.	–/–
11.	–/–
12.	+/+

(+) – positive immunohistochemical reaction; (–) – no immunohistochemical reaction

Discussion

An analysis of P53 protein expression in thyroid tumors using immunohistochemical approach has been the subject of numerous studies [3, 4, 9, 10, 15, 24–27, 29, 31, 34]. Immunohistochemical detection of P53 protein can depend on numerous experimental variables, thus methodological studies were undertaken to assess the impact of fixation procedures, antigen retrieval techniques in various tissues, including thyroid tumors, and type of anti-P53 antibody clone used [2, 20, 30, 32].

Literature data pertaining to analysis of P53 protein expression in selected thyroid tumors are presented in Table

TABLE 6
Immunohistochemical reaction patterns for P53 and P21WAF1 proteins in the microfollicular adenoma group

Patient (No.)	P53/P21WAF1
1.	+/+
2.	–/–
3.	+/+
4.	–/+
5.	–/–
6.	–/–
7.	–/–
8.	–/–
9.	–/–
10	–/–
11.	–/–
12.	+/–
13.	+/+
14.	+/+
15.	+/–
16.	+/+
17.	+/–
18.	+/–
19.	–/+
20.	+/+
21.	–/–
22.	–/–
23.	–/+
24.	+/+
25.	–/+
26.	–/–
27.	–/–
28	–/–
29.	+/–
30.	+/–
31.	+/–

(+) – positive immunohistochemical reaction; (–) – no immunohistochemical reaction

9. Noteworthy is the wide percentage range of cells (from 5 to 30) judged as P53-positive.

In our study, a case was classified as positive if the percentage of cells with nuclear staining for P53 protein was 20 or more. In the follicular carcinoma group 6 out of 12 cases (50%) were positive. In only one out of 12 follicular carcinomas (8.3%), an overexpression of P53 protein was noted. In the group of adenomas, mainly microfollicular ones, 18 out of 57 cases (31.6%) were positive for P53 protein.

TABLE 7
Immunohistochemical reaction patterns for P53 and P21WAF1 proteins in the normo- and macrofollicular adenoma group

Patient (No.)	P53/P21WAF1
1.	–/–
2.	–/–
3.	–/–
4.	–/+
5.	–/–
6.	–/–
7.	–/–
8.	–/–
9.	–/–
10	+/–
11.	–/–
12.	–/–
13.	–/+
14.	+/–
15.	+/–
16.	–/–
17.	–/–
18.	–/–

(+) – positive immunohistochemical reaction, (–) – no immunohistochemical reaction

TABLE 8
Immunohistochemical reaction patterns for P53 and P21WAF1 proteins in the oxyphilic adenoma group

Patient (No.)	P53/P21WAF1
1.	–/–
2.	–/–
3.	–/+
4.	–/+
5.	–/–
6.	–/+
7.	–/–
8.	+/–

(+) – positive immunohistochemical reaction, (–) – no immunohistochemical reaction

Other investigators have stressed that positive nuclear reaction for P53 protein presence in malignant thyroid tumor cells, detected immunohistochemically, is not always linked to mutations in the *p53* gene [10, 15, 34]. However, as the grade of thyroid tumor malignancy increases, a positive correlation has been observed between the presence of mutations within *p53* gene and positive reaction for overexpression of P53 protein [10, 18]. Dobashi et al.

TABLE 9
P53 protein immunohistochemical detection in selected thyroid neoplasms

References	Follicular adenoma	Carcinoma		
		follicular	oxyphilic	undifferentiated
Dobashi et al. [10][a]		2/14 (14.3%)		7/11 (63.6%)
Czyż et al. [9]	2/49 (4%)	3/11 (27%)		
Pilotti et al. [26]				12/22 (54.5%)
Soares et al. [31][b]		2/10 (20%)		10/12 (83%)
Sapi et al. [29][c]		5/11 (45.5%) M+ 1/14 (7.1%) M-		
Ho et al. [15][d]	0/2 (0%)	5/9 (55.6)*	4/6 (66.7)*	2/4 (50%)* 1/4 (25%)
Papotti et al. [25]			4/20 (20%)	
Pollina et al. [27][e]				15/24 (62.5%)
Zedenius et al. [34][f]	5/104 (5%)	1/4 (25%)		4/4 (100%)
Chen et al. [4][g]		1/7 (14%)		8/10 (80%)
Moore et al. [24][h]	0/5 (0.0%)	1/6 (16.6%)		

*cytoplasmic reaction; [a] – positive cells >5%; [b] – positive cells >10%; M+ – follicular carcinoma with a metastasis; [c] – positive cells >30%; [d] – positive cells <5%; M– – follicular carcinoma without metastases; [e] – positive cells >5%; [f] – positive cells >10%; g – positive cells >10%; [h] – positive cells >25%

[10] reported that among poorly differentiated thyroid carcinomas, which revealed an overexpression of P53 protein (40.9%), point mutations in exons 7 and 8 were found in 2 out of 6 cases. Among undifferentiated carcinomas, which had revealed such overexpression (63.3%), 4 out of 6 cases showed point mutations in exons 5-8. In turn, Zedenius et al. [34] identified mutations in exons 5-8 of the *p53* gene only in 4 out of 21 cases of malignant thyroid tumors.

In the group of 93 malignant thyroid tumors, examined by Ito et al. [17] 30% of cases were positive for P21WAF1protein. P21WAF1-positive cases accounted for 31% of papillary carcinomas, 30% of follicular carcinomas and 26% of anaplastic carcinomas. Similarly, the percentage of P21WAF1-positive cells among positive cases in these groups was approximately the same and lower than 30%. These investigators did not report any correlation between P21WAF1 expression and clinical or pathological parameters of thyroid carcinoma cases studied. In our study 4 out of 12 follicular carcinomas (33.3%) were positive for P21WAF1 protein.

As mentioned previously, induction of *p21WAF1/CIP1* expression may occur independently of or depending on wild P53 protein [8, 23]. The expression of *p21WAF1* gene is induced by wild P53 protein, following cell exposure to genotoxic agents. As a result, P21WAF1 protein being a negative inhibitor of cyclin-dependent kinases, plays a significant role in arresting the cell in G1 phase of the cell cycle [23].

In turn, immunohistochemical detection of P53 protein may reflect an expression of the mutated P53 protein but it may also reflect the overexpression or discontinued degradation of wild P53 protein [34]. In case of thyroid tumors, overexpression of P53 protein may also be caused by patients' preoperative treatment, including neck area irradiation that may induce *p53* gene by damaging DNA [34].

Given that *p21WAF1* gene expression occurs only *via* stimulation by wild P53 protein, a simultaneous immunohistochemical detection of P53 and P21WAF1 proteins allows to assume that cases of thyroid tumors with P53(+)/P21WAF1(+) and P53(–)/p21WAF1(+) expression patterns involve an expression or excessive accumulation of wild P53 protein, while those with p53(+)/p21WAF1(-) expression pattern involve an expression or accumulation of the mutated form of P53 protein [17, 34]. According to Ito et al. [17], a positive nuclear reaction for P53 and P21WAF1 protein – p53(+)/p21WAF1(+) pattern – especially if seen in the tissue in dispersed form, may reflect *p53* gene mutation, while positive P21WAF1 protein reaction results in these cases from induced gene expression by factors other than the wild form of P53 protein. According to Ito et al. [17], this hypothesis is generally acceptable, but it has to be reexamined with the aid of immunohistochemical methods in tandem with molecular analyses of *p53* and *p21WAF1* gene expression in tissues derived from various tumors.

Simultaneous immunohistochemical analyses of P53 and P21WAF1 proteins in benign and malignant thyroid neoplasms were reported in papers of Ito et al. [17] and Zedenius et al. [34]. In the study of undifferentiated thyroid neoplasms performed by Ito et al. [17], 4 out of 5 cases (80%) were positive for P53 and P21WAF1 proteins;

among poorly differentiated neoplasms 2 out of 7 cases (28.6%) were positive. In turn, among undifferentiated and poorly differentiated thyroid carcinomas, the occurrence of cases positive for P21WAF1 protein and negative for P53 was very low (12.5% and 23.8%, respectively). Zedenius et al. [34] reported expression of P21WAF1 protein in 16 out of 33 cases (48.5%) of thyroid carcinoma. That expression was accompanied by a presence of wild *p53* gene, which was attested on molecular level.

Cases of thyroid carcinoma with positive immunohistochemical reaction for P53 protein and negative for P21WAF1, i.e. having p53(+)/p21WAF1(-) expression pattern, appear interesting. In our study, such configuration was found in 2 out of 12 follicular carcinomas (16.7%), in 7 out of 31 microfollicular adenomas (22.6%), in 3 out of 18 normo- and macrofollicular adenomas (16.7%), and in 1 out of 8 oxyphilic adenomas (12.5%). The study of Anttila et al. [1] demonstrated, that in case of ovarian carcinoma lack or a low expression of P21WAF1 protein, together with a positive reaction for P53 protein is associated with high risk of disease recurrence.

The results of our study suggest that simultaneous immunohistochemical analysis of P53 and P21WAF1 protein expression may be useful in determining the functional status of P53 protein helping to interpret the expression of this protein in follicular neoplasms of the thyroid.

On the one hand, limited clinical material as presented in our study does not allow us to draw conclusions, regarding potential diagnostic significance of simultaneous immunohistochemical analysis of P53 and P21WAF1 protein expression in follicular cancers of the thyroid. On the other hand, this indicates a need of further testing the hypothesis, according to which the expression pattern – p53(+)/p21WAF1(-) signals high risk of follicular thyroid carcinoma differentiation and unfavorable clinical outcome, due to metastases.

References

1. *Anttila MA, Kosma V-M, Hongxiu J, Puolakka J, Juhola M, Saarikoski S, Syrjanen K:* p21WAF1 expression as related to p53, cell proliferation and prognosis in epithelial ovarian cancer. Br J Cancer 1999, 79, 1870–1878.
2. *Bassarova AV, Popov A:* Immunohistochemical detection of p53 – effect of fixation and methods of antigen retrieval. Fol Histochem Cytobiol 1998, 36, 127–132.
3. *Caldes T, Iniesta P, Vega FJ, de Juan C, Lopez JA, Diaz-Rubio E, Fernandez C, Cerdan J, Balibrea JL, Benito M:* Comparative survival analysis of p53 gene mutations and protein accumulation in colorectal cancer. Oncology 1998, 55, 249–257.
4. *Chen R-X, Masuda T, Fujimori K, Naganuma H, Takaya K, Mori Y, Nagura H:* High-risk group of differentiated thyroid carcinoma with p53 overexpression and local recurrence. Thyroidol Clin Exp 1998, 10, 125–130.
5. *Chen X, Bargonetti J, Prives C:* p53, through p21 (WAF1/CIP1), induces cyclin D1 synthesis. Cancer Res 1998, 55, 4257–4263.
6. *Clasen S, Schulz WA, Gerharz C-D, Grimm M-O, Christoph F, Schmitz-Drager BJ:* Frequent and heterogeneous expression of cyclin-dependent kinase inhibitor WAF/p21 protein and mRNA in urothelial carcinoma. Br J Cancer 1998, 77, 515–521.
7. *Cordon-Cardo C, Zhang Z-F, Dalbangi G, Drobnjak M, Charytonowicz E, Hu S-X, Xu H-J, Reuter VE, Benedict WF:* Cooperative effects of p53 and pRB alterations in primary superficial bladder tumors. Cancer Res 1997, 57, 1217–1221.
8. *Cordon-Cardo C:* Mutation of cell cycle regulators. Am J Pathol 1995, 147, 545–560.
9. *Czyz W, Joensuu H, Pylkkanen L, Klemi PJ:* p53 protein, PCNA staining, and DNA content in follicular neoplasms of the thyroid gland. J Pathol 1994, 174, 1–8.
10. *Dobashi Y, Sugimura H, Sakamoto A, Mernyei M, Mori M, Oyama T, Macinami R:* Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. Diagn Mol Pathol 1994, 3, 9–14.
11. *Donehower LA, Bradley A:* The tumor suppressor p53. Biochim Biophys Acta 1993, 1155, 181–205.
12. *Gillett CM, Barnes DM:* Cell cycle. J Clin Pathol Mol Pathol 1998, 51, 310–316.
13. *Harris CC:* p53 tumor suppressor gene: from the basic research laboratory to the clinican abridged historical perspective. Carcinogenesis 1996, 17, 1187–1198.
14. *Hedinger CE, Williams ED, Sobin LH:* Histological Typing of Thyroid Tumors. The WHO International Histological Classification of Tumours. 2nd rev ed. Springer-Verlag, Berlin 1988.
15. *Ho Y-S, Tseng S-C, Cin T-Y, Hsieh L-L, Lin J-D:* p53 gene mutation in thyroid carcinoma. Cancer Lett 1996, 103, 57–63.
16. *Hsu SM, Raine L, Fanger H:* Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29, 577–580.
17. *Ito Y, Kobayashi T, Takeda et al;* Expression of p21 (WAF1/CIP1) protein in clinical thyroid tissues. Br J Cancer 1996, 74, 1269–1274.
18. *Ito T, Seyama T, Mizuno T et al:* Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res 1992, 52, 1369–1371.
19. *Kaelin WG:* The emerging p53 gene family. J Natl Cancer Inst 1999, 91, 594–598.
20. *Kraggerud SM, Jacobsen KD, Berner A et al:* A comparison of different modes for the detection of p53 protein accumulation. Pathol Res Pract 1997, 193, 471–478.
21. *Lloyd RV, Erickson LA, Jin L, Kulig E, Qian X, Cheville JC, Scheithauer BW:* $p27^{kip1}$: a multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. Am J Pathol 1999, 154, 313–323.
22. *Lu X, Toki T, Konishi I, Nikaido T, Fujii S:* Expression of $p21^{WAF1/CIP1}$ in adenocarcinoma of the uterine cervix. Cancer 1998, 82, 2409–2417.
23. *Lundberg AS, Weinberg RA:* Control of the cell cycle and apoptosis. Eur J Cancer 1999, 35, 531-539.
24. *Moore D, Ohene-Fianko D, Garcia B, Chakrabarti S:* Apoptosis in thyroid neoplasms: relationship with p53 and bcl-2 expression. Histopathology 1998, 32, 35–42.
25. *Papotti M, Torchio B, Grassi L, Favero A, Bussolati G:* Poorly differentiated oxyphilic (Hürthle cell) carcinomas of the thyroid. Am J Surg Pathol 1996, 20, 686–694.
26. *Pilotti S, Collini P, Del Bo R, Cattoretti G, Pierotti MA, Rilke F:* A novel panel of antibodies that segregates immunocytochemically poorly differentiated carcinoma from undifferentiated carcinoma of thyroid gland. Am J Surg Pathol 1994, 18, 1054–1064.

27. *Pollina L, Pacini F, Fontanini G, Viganti S, Bevilacqua G, Basolo F:* bcl-2, p53 and proliferating cell nuclear antigen expression is related to the degree of differentiation in thyroid carcinomas. Br J Cancer 1996, 73, 139–143.
28. *Prives C, Hall PA:* The p53 pathway. J Pathol 1999, 187, 112–126.
29. *Sapi Z, Lukacs G, Sztan M, Papp J, Olah E:* Contribution of p53 gene alterations to development of metastatic forms of follicular thyroid carcinoma. Diagn Mol Pathol 1995, 4, 256–260.
30. *Save V, Nylander K, Hall PA:* Why is p53 protein stabilized in neoplasia. Some answers but many more questions. J Pathol 1998, 184, 348–350.
31. *Soares P, Cameselle-Teijeiro J, Sobrinho-Simoes M:* Immunohistochemical detection of p53 in differentiated, poorly differentiated and undifferentiated carcinomas of the thyroid. Histopathology 1994, 24, 205–210.
32. *Werner M, von Wasilewski R, Komminoth P:* Antigen retrieval, signal amplification and intensification in immunohistochemistry. Histochem Cell Biol 1996, 105, 253–260.
33. *Wynford-Thomas D:* Cellular senescence and cancer. J Pathol 1999, 187, 100–111.
34. *Zedenius J, Larrson C, Wallin G:* Alterations of p53 and expression of WAF1/p21 in human thyroid tumors. Thyroid 1996, 6, 1–9.

Address for correspondence and reprint requests to:
Tomasz Ferenc M.D., Ph.D
Department of Biology and Genetics
Pl. Hallera1, 90-647 Łódź
tel: 042 6330594

Ogłoszenie

Redakcja **Polish Journal of Pathology** informuje, że posiada w sprzedaży następujące pozycje:

- **„Atlas patomorfologiczny diagnostyki różnicowej chorób skóry ze szczególnym uwzględnieniem nowotworów" cz. I i II**, łącznie 6 zeszytów; cena zeszytu – 10 zł, cena kompletu – 60 zł;
- **„Immunohistotechnika"** pod red. prof. J. Stachury – cena 10 zł;
- **„Zasady postępowania diagnostycznego w raku sutka"** rekomendowane przez PTP – cena 5 zł;
- **„Zarys zasad histomorfometrii w badaniach patomorfologicznych"** prof. K.W. Zielińskiego – cena 10 zł;
- **„Choroby zwyrodnieniowe układu nerwowego: nowotwory białek (protein cancers)"** pod redakcją prof. P.P. Liberskiego – 20 zł;
- **„Organizacja i wyposażenie pracowni patomorfologicznej oraz zasady postępowania z materiałami do badań histopatologicznych i cytologicznych"** pod redakcją prof. A. Kuliga, prof. M. Danilewicza i dr S. Łukaszka – cena 15 zł;
- **„Nowotwory mózgu u dzieci"** pod redakcją prof. P.P. Liberskiego – cena 30 zł;
- **„Choroby infekcyjne układu nerwowego"** cz. I i II, pod redakcją prof. P.P. Liberskiego; cena cz. I – 50 zł, cz. II – 30 zł.

Należność przy zamówieniu płatna na konto:

Polskie Towarzystwo Patologów, Oddział w Krakowie

I Oddz. PKO BP Kraków 37 1020 2892 0000 5802 0015 6828

z adnotacją z wyszczególnieniem zamawianych tytułów.