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IMMUNOHISTOCHEMICAL EVALUATION OF P53 EXPRESSION IN LUNG CANCER OF PATIENTS WITH PARANEOPLASTIC RHEUMATIC SYNDROME

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***Aim*: To study p53 expression in the tumor tissue of lung cancer (LC) patients with paraneoplastic rheumatic syndrome (PNRS). *Materials and Methods*: There have been used either biopsy or surgically resected tumor samples of 140 LC patients (83 patients without PNRS, 57 patients with PNRS). For evaluation of p53 expression in LC samples, immunohistochemical analysis was performed. *Results*: It has been shown that p53 expression in tumor samples from LC patients with PNRS was significantly higher compared to that in LC patients without PNRS. It has been shown that p53 expression is more frequently registered in patients with lung adenocarcinoma with PNRS than in patients with lung adenocarcinoma without this syndrome. *Conclusion*: The presence of PNRS in LC patients with p53 expression is associated with higher aggressiveness of tumor.**

***Key Words*: p53 expression, oncoprotein, lung cancer, paraneoplastic rheumatic syndrome.**

It is well known that oncoproteins can influence tumor growth, ability for invasion, metastasis, and formation of resistance to anticancer means [1, 2]. One of the main oncoproteins is p53 — a protein which is a tumor suppressor encoded by TP53 gene in humans. If a mutation occurs in this gene, the person may be more susceptible to the development of cancer [3, 4]. Oncoprotein p53 regulates the cell cycle and can serve as an anti-oncogene which can prevent cancer development. Due to this, p53 is sometimes called the "Protector of the genome", "Guardian angel gene" or "senior caretaker", referring to its function to maintain stability, preventing genome mutation [5, 6].

Anti-tumor functions of p53 are mediated by: 1) inhibition of abnormal cell growth, since it recognizes damaged DNA and may induce temporary cessation of cell division in the so called control points (checkpoint) of the cell cycle [7, 8]; 2) activation of protein coding genes, correcting the DNA damage; 3) inhibition of angiogenesis.

The most universal molecular change in the various human neoplasms is the inactivation of the p53 function. In more than half of all human tumors (50–60% of all neoplasms which are over 50 different types) the mutation in p53 gene has been detected [9–12]. In contrast to other tumor suppressor genes, which are characterized by mutations ceasing protein synthesis (deletions, formation of stop codons coding frame shift, violations in mRNA splicing), the vast majority (over 90%) of p53 mutations is a missense mutation leading to the replacement of one of the amino acids in a protein molecule to another. Another feature of the mutations of p53 in tumor cells is that they, in contrast to mutations in other tumor suppressor genes, are often heterozygous, i.e., affect only one of the two alleles of the gene. These mutations which lead to dysfunction of p53 result in the replication of cells with DNA damage and the accumulation of other oncogenic mutations, contributing further to unregulated cell growth and the development of tumor cells [13].

It is known that the majority of patients with lung cancer (LC) (more than 50%) showed p53 expression [14, 15]. Moreover, LC is associated with paraneoplastic syndrome [16]. In clinical practice, very often this syndrome has a rheumatic presentation that has allowed to call it as paraneoplastic rheumatic syndrome (PNRS) [17, 18]. Taking into account the relevant clinical importance both of p53 expression in tumor and PNRS the current study was aimed to evaluate the correlation of p53 expression in LC of patients with and without PNRS.

MATERIALS AND METHODS

Patients. 140 LC patients were enrolled into the study. They were treated at the Vinnitsa Regional Clinical Oncology Center during 2011–2012. The first group (control) included 83 LC patients without PNRS, the second group (experimental) — 57 LC patients with PNRS. The patient age ranged from 40 to 78 years, there were 122 (87%) men and 18 (13%) women. The author has obtained the consent of Ethical Committee of Pirogov National Medical University (Vinnitsa, Ukraine) to perform this study.

Immunohistochemistry (IHC). Tumor material is first treated with 10.0% neutral buffered formalin solution, and then embedded in paraffin. Histological examination was performed on sections of 4–5 microns thick and stained with hematoxylin-eosin stain (H&E Stain).

Determination of the expression of p53 protein was performed on sections where paraffin was removed by preliminary unmasking of antigen by citrate buffer (pH 6.0) in water for 30 min. Evaluation of the IHC staining was performed and photographed using a light microscope (magnification x100 and x400). As primary antibodies, monoclonal antibody (clone DO-7) to the

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Abbreviations used: IHC – immunohistochemistry; LC – lung cancer; NSCLC – non-small cell lung cancer; PNRS – paraneoplastic rheumatic syndrome; SCLC – small cell lung cancer.

p53 Protein (p53) from "DAKO Cytomation" (Denmark) was used. To visualize the products of the reaction, EnVision+ system (DAKO) and chromogen DAB+ (DAKO) were used.

IHC reaction was evaluated by distinct nuclear staining. Then, the number of cells positive for the reaction was counted in the area and they were in greater quantity. Level of expression of p53 was determined by the ratio of colored chromogen DAB+ nuclei to unstained nuclei in 10 fields of view and expressed in percentage. Thus, we counted the number of positively stained cells, which came to around 800–1000 tumor cells. The absence of cells staining was assessed as a negative reaction, staining of < 40% cells as a weak expression, and > 40% cells as a strong expression.

Statistics. Statistical processing of quantitative indicators was performed using the parametric criteria of Student or Student's t-test. To test the significance of differences of attribute values in the groups Fisher's exact test was used. Differences were considered statistically significant at $p < 0.05$. Computer software "Biostat" was used.

RESULTS

Among all patients, stage I of LC was diagnosed in 33 patients (23.6%), II stage — in 47 patients (33.6%) and stage III — in 60 patients (42.8%). By histological forms (Table 1) the tumors were classified as follows: small cell lung cancer (SCLC, 29 cases — 20.7%)) and non-small cell lung cancer (NSCLC, 111 cases — 79.3%, which includes adenocarcinoma (36 cases — 25.7%) and squamous cell carcinoma (75 cases — 53.6%)).

Table 1. Distribution of LC patients by histological forms of cancer

Groups of patients	SCLC	Adenocarcinoma	Squamous cell carcinoma
LC without PNRS, %	19.3	22.9	57.8
LC with PNRS, %	22.8	29.8	47.4
Total, %	20.7	25.7	53.6

In the first group of patients with lung squamous cell carcinoma was detected in 57.8% of cases, with adenocarcinoma — 22.9% and with SCLC — 19.3% (Table 1). It should also be noted that in the second group of patients, NSCLC were diagnosed in 44 cases (77.2% in relation to all patients with PNRS), and SCLC — in 13 cases (22.8%). Among the variations of NSCLC squamous cell carcinoma (27 patients, (47.4%) and adenocarcinoma (17 patients, 29.8%) were predominant.

It was found that in SCLC samples, p53 expression (Table 2) was observed more frequently than in NSCLC: in 28 (96.6%) of 29 patients. Similarly, in NSCLC, p53 expression was observed in tumor cells of 80 patients (72.1% of cases in relation to all patients with NSCLC).

In general, the expression of p53 protein in tumor was observed in 71.1% of patients (59 cases) with LC without PNRS and 86.0% of patients (49 cases) with PNRS manifestations (Table 2). The difference between groups was statistically significant (Fisher's exact test, $p < 0.05$).

It should also be noted that more than 40% of tumor cells were evaluated as p53-positive in patients in both groups. In LC patients without PNRS (Fig. 1), the number of p53-positive cells was 50.2 ± 6.8%, while in LC patients with PNRS manifestations (Fig. 2) the average number of p53-positive cells was significantly higher, i.e., 72.3 ± 7.5% (Fisher's exact test, $p < 0.05$). It was observed the link between tumor histology and the level of p53 expression in tumor: it was higher in squamous cell carcinoma in comparison to SCLC in both groups.

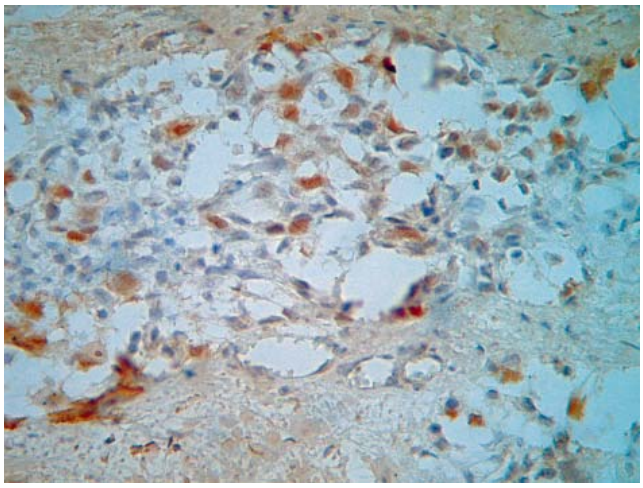

Fig. 1. Weak p53 expression in tumor cells (squamous cell LC, ***patient O.*** without PNRS), × 400

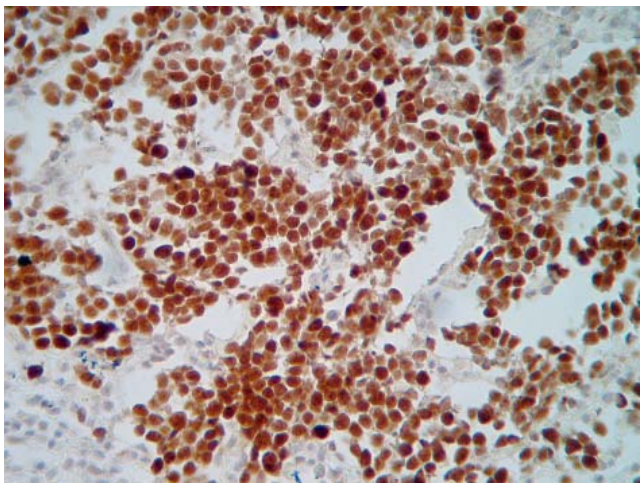

Fig. 2. High p53 expression in tumor cells (squamous cell LC, ***patient I.*** with PNRS), × 400

As can be seen from Table 2, in patients with SCLC without PNRS p53 expression was registered in 93.8% cases (15 out of 16), while in patients with PNRS (Fig. 3) it was 100%. At the same time the difference between the groups was not statistically significant ($p > 0.1$).

In all NSCLC patients without PNRS (44 patients, 53.0% among all patients in the first group) p53 expression was observed in 11 adenocarcinoma (13.3%) and 33 squamous cell carcinoma (39.8%) cases. Among NSCLC patients with PNRS the expression of p53 in tumor was observed in 36 patients (63.2% of all cases in the second group): 15 adenocarcinoma (26.3%) and 21 squamous cell carcinoma (36.8%). It should also be noted that p53 expression in NSCLC

was most often associated with adenocarcinoma, and more precisely in the presence of PNRS (Fig. 4, 5).

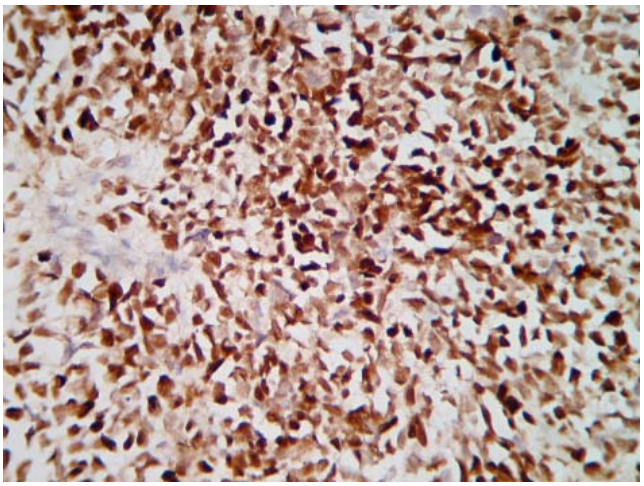

Fig. 3. High p53 expression in tumor cells (SCLC, ***patient N.*** with PNRS), ×400

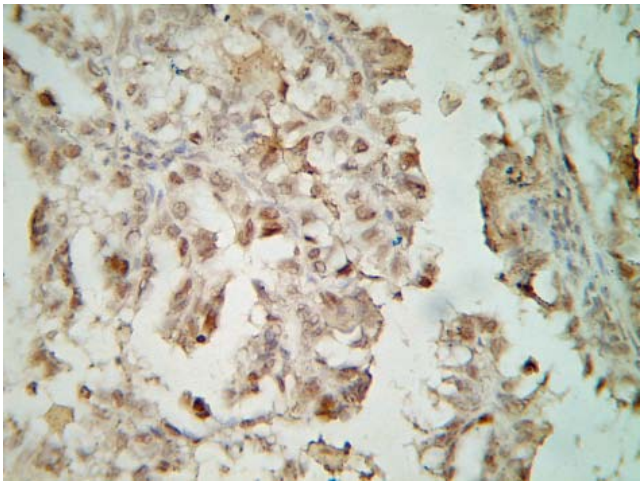

Fig. 4. p53 expression in tumor cells (lung adenocarcinoma, ***patient L.*** without PNRS), × 400

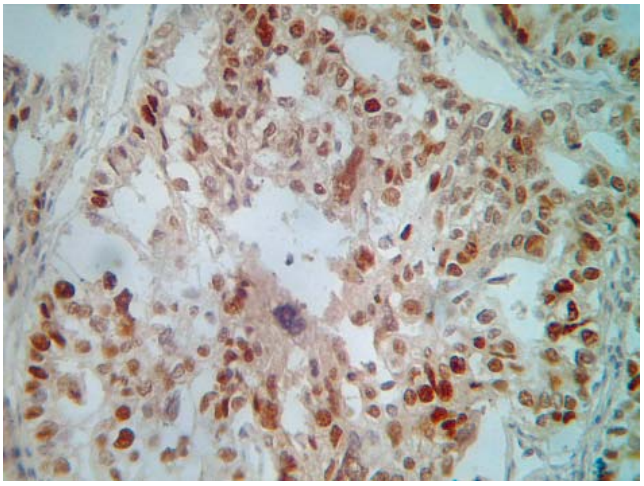

Fig. 5. p53 expression in tumor cells (lung adenocarcinoma, ***patient K.*** with PNRS), × 400

It was determined that the number of patients with adenocarcinoma and PNRS (15 cases) and p53-positive tumor in relation to all cases of adenocarcinoma in the second group (17 cases) was 88.2%, that is higher than the number of p53-positive adenocarcinoma (57.9%, 11 cases out of 19) in patients without PNRS (Fisher's exact test, $p < 0.05$) (Table 2).

In the cases of squamous cell carcinoma, the difference between p53 expression in tumor samples from the first and the second groups ($p > 0.1$) was statistically insignificant (Table 2). Moreover, the p53 expression in squamous cell carcinoma of patients without PNRS was observed in 68.8% of cases (33 of 48), while in patients with the similar histological type with manifestations of PNRS was observed in 77.8% of cases (21 of 27).

Table 2. p53-positive cells in LC

Histological form	p53-positive cells in LC			
	Patients without PNRS		Patients with PNRS	
	Number of patients	Number of positive samples, %	Number of patients	Number of positive samples, %
SCLC	15	93.8	13	100
Adenocarcinoma	11	57.9	15	88.2
Squamous	33	68.8	21	77.8
Total	59	71.1	49	86.0

DISCUSSION

Analyzing literature on this subject, it should be noted that other researchers did not study the p53 expression in LC patients with manifestations of PNRS. The general study of the expression of the p53 in LC and its relation to the degree of malignancy, the distribution process, prognosis and survival of patients was found [19, 20]. Some studies in recent years indicate that the expression of p53 in tumor is found in 45–80% of patients with LC [14]. In SCLC a high amount of p53-positive cells (70% of patients or more) was found when compared to NSCLC (45–65% of patients) [21, 22]. Also the correlation between high levels of p53-positive cells in tumor and the distribution of tumor process was noted (patients with metastases to regional lymph nodes) for both histological forms of LC (NSCLC and SCLC) [14, 23, 24]. There is also evidence of mutual positive correlation of p53 expression with expression of proliferation marker Ki-67 [14, 20]. It was demonstrated that p53 expression is the prognostic factor in LC, in particular its high expression was detected in patients with a poor outcomes (metastasis, tumor recurrence and death) [25]. Also, a high level of p53-positive cells in tumor demonstrates the negative impact on the survival of patients with LC, especially radically operated patients with stage III of NSCLC [23–26]. Among such patients, the number of p53-positive cells in tumor greater than 10% is also one of the 6 most important factors for prognosis as well as choice of treatment [15].

Our studies confirm that the expression of p53 in LC depends on the histological structure of the tumor, as well as on the clinical characteristics of the patient, in particular the presence of PNRS. Thus, in the group of LC patients with PNRS significantly higher level of p53-positive tumor cells in comparison to those in LC patients without PNRS has been shown that may indicate more aggressive tumor.

Similarly, in LC patients with PNRS a significantly higher number of p53-positive tumor cells were found in comparison with those in patients without PNRS. This fact indicates a higher malignant and

aggressive nature of tumors in LC patients with PNRS. This suggestion may be supported by the fact that high p53 expression is a negative prognostic factor for LC patients [14, 15, 20–22].

We observed that the frequency of high p53 expression in lung adenocarcinoma was higher in patients with PNRS than in patients with lung adenocarcinoma without PNRS. It should also be noted that the identification of PNRS among patients with LC leads to a more complete assessment of the prognosis, the degree of malignancy and helps to further improve the treatment of patients.

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