ORIGINAL ARTICLE

Immunoexpression analysis of selected JAK/STAT pathway molecules in patients with non–small-cell lung cancer

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KEY WORDS

cyclooxygenase 2, non–small-cell lung cancer, PIAS3, SOCS3, STAT

ABSTRACT

INTRODUCTION Signal transducer and activator of transcription (STAT) proteins are critically involved in tumorigenesis in various cancers, including lung cancer.

OBJECTIVES The aim of the study was to analyze the immunoexpression levels of 3 STAT proteins: STAT3, STAT5, and STAT6 in their phosphorylated forms (pSTATs), STAT inhibitors PIAS3 and SOCS3, and additionally cyclooxygenase 2 (COX-2), as potential diagnostic and prognostic markers in lung cancer.

PATIENTS AND METHODS The study included 71 patients diagnosed with non–small-cell lung cancer (NSCLC). The immunoexpression levels of the proteins were assessed in lung tissue samples, using an enzyme-linked immunosorbent assay. Tumors were staged using the postoperative TNM classification.

RESULTS All studied STATs were overexpressed in 54% to 55% of NSCLC specimens. Significantly higher STAT3 and STAT6 immunoexpression levels were observed in squamous cell carcinoma. Significant differences between NSCLC samples and controls were found for STAT5. Significantly higher STAT5 levels were observed in pT2 tumors. The COX-2 overexpression was observed in 55% of NSCLC specimens and was significantly higher in T2 tumors. STAT inhibitors were underexpressed in 56% to 58% of NSCLC specimens. The PIAS3 immunoexpression was significantly lower in non–squamous cell carcinoma. The SOCS3 level was significantly lower in smaller tumors (pT1). Negative correlations between STAT5 and PIAS3 levels, as well as between STAT6 and SOCS levels, and a positive correlation between STAT5 and COX-2 levels were observed.

CONCLUSIONS The deregulated expression of the studied pSTATs and their inhibitors may be involved in the development and progression of lung cancer. The observed differences between the histotypes suggest the potential usefulness of STAT proteins as diagnostic markers. Our results may contribute to the search for targets in lung cancer therapy.

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INTRODUCTION Signal transducers and activators of transcription (STAT) proteins comprise a family of transcription factors latent in the cytoplasm, with 7 different members: STAT1, -2, -3, -4, -5A, -5B, and -6. They are activated by various extracellular signaling proteins (cytokines, growth factors, and hormones) that bind to specific cell-surface receptors, thus allowing the recruitment of Janus kinases (JAKs). JAKs cross-phosphorylate each other and the receptor on key tyrosine residues. STAT molecules bind to these phosphorylated docking sites, and are in turn phosphorylated, dimerized, and enter the nucleus. STATs can be also activated independently of JAKs, most notably by c-Src kinases. After activation, the resulting signal transduction pathways permit STATs to play different roles in normal physiological cell processes, such as differentiation, proliferation, apoptosis, and angiogenesis.[1]

There are 3 families of natural inhibitors of STAT proteins: the protein inhibitors of activated STAT (PIAS), the suppressors of cytokine signaling (SOCS), and the Src homology 2 containing phosphatase (SHP). All 3 inhibitors are known to participate in the negative regulation of the STAT signal transduction pathway.[2] The proteins of the PIAS family (PIAS1, PIAS3, PIASxα, PIASxβ, and PIASy) bind to activated STATs upon cytokine stimulation and inhibit DNA-binding ability without directly dephosphorylating the STAT proteins.[3] SOCS family members (SOCS1–SOCS7 and cytokine-inducible SH2-domain) modulate signaling by several mechanisms, such as inactivation of JAKs or blocking access of STATs to receptor binding sites, and also may target signaling complexes for ubiquitination and degradation.[4]

Constitutive activation of several STATs has been observed in a wide number of human cancer cell lines and primary tumors. The most comprehensive studies focused on STAT3, the elevated level of which was found in blood malignancies and several solid neoplasms; the activation of STAT5 was found particularly in leukemias and lymphomas; and the activation of STAT6, which for a long time was studied for its role in immune function, tumor immunosurveillance, and lymphomagenesis, was also observed in solid tumors.[5-7]

The role of STAT inhibitors was also evaluated in solid tumors, although to a limited extent. Regarding their involvement in lung tumorigenesis, they activate apoptotic genes, decrease lung cancer growth, and increase the antitumor effects of epidermal growth factor receptor inhibitors.[3,8] In several cancer types, a decrease or loss of the PIAS3 expression was confirmed, indicating it as a protein with putative tumor suppressor function.[9] Similarly, SOCS3 deficiency was documented in some types of cancer, indicating its gene promoter methylation as the reason.[10] Restoration of the SOCS3 expression in lung cancer cells resulted in the downregulation of active STAT3, induction of apoptosis, and growth suppression.[11,13]

Cyclooxygenase 2 (COX-2), a member of the COX family (COX-1, COX-2, and COX-3), is the key enzyme in the biosynthesis of prostaglandins; it can affect cell proliferation and alter the response of the immune system to malignant cells. Genetic studies support a cause–effect relationship between COX-2 and tumorigenesis. COX-2 is overexpressed in most solid tumors.[10,12,14] In lung cancer cell lines, some associations between the immunoexpression levels of COX-2 and STAT3 or STAT5 were observed.[15,16]

In our previous reports,[17,18] we demonstrated elevated levels of *STAT3*, *STAT5A*, *STAT5B*, and *COX-2* genes and a decreased expression of the *PIAS3* gene on the mRNA level. We also confirmed high expression of the STAT5B protein. However, the gene's mRNA level may not predict its protein level, due to alternative splicing and posttranslational modifications. Therefore, in the present study, we continued our research and evaluated the immunoexpression levels of phosphorylated STAT3 (pSTAT3), pSTAT5, and pSTAT6, as well as PIAS, SOCS3, and COX-2 in non–small-cell lung cancer (NSCLC) tissue. The aim of the study was also to investigate the relationship between the studied protein levels and lung tumor parameters as well as the characteristics of patients.

PATIENTS AND METHODS

Non–small-cell lung cancer tissue and characteristics of patients

Lung tissue samples were obtained from 84 patients treated by lobectomy or pneumonectomy in the Department of Thoracic Surgery, General and Oncologic Surgery at the Medical University of Lodz, Łódź, in the years 2010–2012.

After tumor resection, cancerous tissue samples (100–150 mg) from the primary lesion and some macroscopically unchanged tissue samples (100 mg; 10 cm distant from the primary lesion; control group, n = 5) were placed in a stabilization buffer (RNAlater®; Qiagen, Mainz, Germany) and frozen at −80°C.

A postoperative histopathological evaluation and classification according to the American Joint Committee on Cancer and postoperative TNM (pTNM) staging systems was performed. Based on the results, 13 patients were excluded owing to the presence of concomitant malignancy, the suspected metastasis to the lung, or the lack of full clinical information. The final study group included 71 patients with confirmed NSCLC. Histopathological features of tumor specimens and demographic characteristics of the patients are presented in **TABLE 1**.

Tissue homogenization

Lung tissue samples (30–50 mg) were rinsed in ice-cold phosphate buffer saline (PBS; 0.01 mol/l; pH, 7.0–7.2) and homogenized in 5 ml of PBS. The suspension was subjected to 2 cycles of freezing and thawing. Then, the homogenates were centrifuged for 5 minutes at 5000 × *g*, the supernatant was removed, and the suspension was aliquoted and stored at −80°C until further analysis.

Protein immunoexpression was assessed using the following enzyme-linked immunosorbent assay (ELISA) kits: Custom Human Phosphotyrosine STAT3 ELISA (RayBiotech, Norcross, Georgia, United States), Custom Human Phosphotyrosine STAT5 ELISA (RayBiotech), Custom Human Phosphotyrosine STAT6 ELISA (RayBiotech), Enzyme-linked Immunosorbent Assay Kit For Protein Inhibitor Of Activated STAT3 (USCN Life, Wuhan, China), Enzyme-linked Immunosorbent Assay Kit For Cyclooxygenase-2 (USCN Life), Enzyme-linked Immunosorbent Assay Kit For Suppressor of Cytokine Signaling 3 (USCN Life), according to the manufacturers' instructions. The intensity of the final colorimetric reaction, in proportion to the amount of protein bound,

TABLE 1 Clinical characteristics of patients with non–small-cell lung cancer ($n = 71$)

Parameter		Value
Sex, n (%)	Women	25 (35)
	Men	46 (65)
Age, y, mean (SD)	Women	63 (8.72)
	Men	65 (8.23)
Age, y, n (%)	≤60	20 (30)
	61–70	35 (50)
	>70	16 (20)
Smoking status, n (%)		
Smokers, total		66 (93)
Smokers[a]	<40 pack-years	30 (45)
	≥40 pack-years	36 (55)
Nonsmokers		5 (7)
NSCLC histotypes, n (%)		
SCC		41 (58)
NSCC		30 (42)
Adenocarcinoma		23 (32)
LCC		7 (10)
AJCC classification[b], n (%)		
IA		14 (20)
IB		11 (16)
IIA		13 (18)
IIB		10 (14)
Tumor size according to the pTNM classification[c], n (%)		
T1a+T1b		19 (27)
T2a+T2b		33 (46)
T3+T4		19 (27)

[a] Pack-years were calculated according to the NCI Dictionary of Cancer Terms: 1 pack-year is equal to 20 cigarettes smoked per day for 1 year (http://www.cancer.gov/dictionary?CdrID=306 510).

[b] The AJCC classification according to the International Association for the Study of Lung Cancer staging project, 7th ed. (2010)

[c] pTNM: postoperative tumor–node–metastasis classification according to the World Health Organization histological typing of lung tumor

Abbreviations: AJCC, American Joint Committee on Cancer Staging; LCC, large-cell carcinoma; NSCC, non–squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma

was measured in a plate reader (ELx800, BioTek, Winooski,Vermont, United States) at 450 nm. The obtained results were compared to the standard solution of known concentrations (specific for individual ELISA kits).

Ethical approval The study was approved by the Ethics Committee of the Medical University of Lodz (no. RNN/64/11/KE). Informed consent was obtained from each study participant.

Statistical analysis The levels of protein immunoexpression between the NSCLC subtypes were compared using the analysis of variance (ANOVA) Kruskal–Wallis or Mann–Whitney test (depending on the number of groups) and the Neuman–Keuls' multiple comparison test. The Spearman rank correlation coefficient, Mann–Whitney test, and ANOVA Kruskal–Wallis test were performed to evaluate the relationship between the protein immunoexpression levels and clinical characteristics of the patients (age, sex, smoking status, and tumor stages according to the pTNM classification). The accepted level of statistical significance was a P value of less than 0.05. Statistical analysis was performed using Statistica for Windows 10.0 (StatSoft, Kraków, Poland).

RESULTS Immunoexpression analyses revealed the increased levels of pSTAT3 in 55% of lung tissue samples, pSTAT5 in 54% of NSCLC samples, pSTAT6 in 54% of NSCLC samples, and COX-2 in 55% of NSCLC samples. The immunoexpression levels of PIAS3 and SOCS3 were decreased in 56% and 58% of NSCLC samples, respectively. A much higher percentage of samples with increased or decreased protein levels were observed in squamous cell carcinoma (SCC; 71%–93%) as compared with non–squamous cell carcinoma (NSCC; 10%–29%). The percentage values of the obtained results, for the whole group and for the NSCLC subtypes, are shown in Supplementary material (*Table S1*), and concentration values (ng/ml) for each protein are presented in **TABLE 2**.

All STAT proteins were simultaneously overexpressed in 68% of NSCLC samples, and there was no significant difference between SCC and NSCC histotypes (71% and 63%, respectively). *Table S2* in Supplementary material shows the results for paired proteins: the frequencies of NSCLC samples with increased immunoexpression of both paired proteins or samples with an increased protein level and a simultaneous decrease in the protein inhibitor level.

Apart from the comparison between SCC and NSCC (**TABLE 2**; Supplementary material, *Table S1*), we also looked for potential differences between SCC, adenocarcinoma, and large-cell carcinoma (LCC) subgroups. We found higher immunoexpression levels of pSTAT3 in SCC vs adenocarcinoma ($P = 0.047$), higher immunoexpression levels of pSTAT6 in SCC vs LCC ($P = 0.041$), and lower immunoexpression levels of PIAS3 in LCC vs adenocarcinoma ($P = 0.029$).

The Kruskal–Wallis test revealed significant differences in relation to the tumor size (pTNM) and immunoexpression levels of pSTAT5, SOCS3, and COX-2. The differences between the individual tumor size groups (Neuman–Keuls' multiple comparison test) are shown in **FIGURE 1**, and the relevant percentage values are presented in Supplementary material (*Table S1*).

Spearman's rank correlation revealed negative correlations between STAT5 and PIAS3 levels ($r = -0.65$, $P = 0.016$) and between STAT6 and SOCS3 levels ($r = -0.27$, $P = 0.042$). On the other hand, STAT5 was positively correlated with COX-2 ($r = 0.88$, $P = 0.032$).

No significant associations were found between the immunoexpression levels of the studied proteins and the patient's age, sex, history of smoking, or tumor stage (pTNM).

TABLE 2 Protein levels in the study groups

Protein, ng/ml	Control (n = 5)	NSCLC (n = 71)	*P* value[a]	NSCLC subtypes		*P* value[b]
				SCC (n = 41)	NSCC (n = 30)	
pSTAT3	20.18 (0.9)	57.14 (1.2)	>0.05	69.98 (0.5)	51.56 (0.7)	0.03
pSTAT5	35.17 (1.0)	154.31 (1.3)	0.014	172.98 (0.6)	120.92 (0.9)	>0.05
pSTAT6	21.83 (0.3)	93.39 (0.7)	>0.05	93.39 (0.9)	74.76 (0.8)	0.004
COX-2	5.73 (0.5)	24.27 (0.8)	>0.05	24.27 (1.1)	15.88 (0.7)	>0.05
SOCS3	53.52 (0.6)	9.52 (0.9)	>0.05	9.52 (1.0)	7.99 (0.7)	>0.05
PIAS3	35.72 (0.5)	7.87 (0.8)	>0.05	7.87 (0.8)	5.32 (1.2)	0.03

Data are presented as mean (SEM).

a Mann–Whitney test; b Kruskal–Wallis test

Abbreviations: COX-2, cyclooxygenase 2; PIAS3, protein inhibitors of activated STAT; SOCS3, suppressor of cytokine signaling 3; pSTAT3, phosphorylated signal transducer and activator of transcription 3; pSTAT5, phosphorylated signal transducer and activator of transcription 5; pSTAT6, phosphorylated signal transducer and activator of transcription 6; others, see **TABLE 1**

DISCUSSION

The JAK/STAT pathway is essential in normal human physiology and development; however, it also regulates oncogenic signaling in many different tumor types.[1,19] Among the STAT proteins, STAT3 has been most comprehensively studied and its role in carcinogenesis seems to be established.[20] The oncogenic roles have been also assigned to STAT5[21] and STAT6.[22]

In our study, we assessed the levels of selected STAT proteins and their inhibitors using the quantitative ELISA in NSCLC tissue samples. This method provides enhanced specificity and generates numerical results amenable to an objective and consistent interpretation. We were interested in protein concentrations, and not its localization, as we chose ELISA kits dedicated to phosphorylated proteins. We looked for JAK/STAT pathway molecules whose modified immunoexpression could have diagnostic or prognostic value in lung cancer and would support clinicians in their daily practice.[23,24] Our results confirmed the increased levels of STATs, namely pSTAT3, pSTAT5, and pSTAT6, in NSCLC specimens and suggested their importance in lung carcinogenesis. The simultaneous overexpression of proteins was observed in nearly 70% of tumor samples and was similarly high, regardless of the NSCLC histotype (SCC or NSCC).

STAT3 was reported to be activated in 38% to 55% of NSCLC primary tumors and lung cancer-derived cell lines,[25-28] with a significantly higher frequency in adenocarcinoma than in SCC.[28] However, other investigators did not observe such correlation.[25,26] We found significantly higher pSTAT3 immunoexpression in SCC than in NSCC samples, and also in SCC than in adenocarcinoma samples. The possible explanation of the differences between our results and those of other investigators could be the method of analysis (immunostaining scores or protein concentrations). However, the highest STAT3 protein level in the SCC subtype is in line with our previous analysis of the *STAT3* mRNA level in patients with NSCLC.[18]

The prognostic role of pSTAT3 in NSCLC remains controversial. Its expression was observed more frequently in smaller tumors,[27] and no relationship with overall survival was found.[26,28] However, the results of a recent meta-analysis have suggested that high STAT3 mRNA and protein levels have poor prognostic significance in NSCLC, associated with poorly differentiated carcinoma, stage III–IV cancer, and lymph node metastasis.[29] In our study, the highest frequency of the increased pSTAT3 expression was found in pT3+pT4 tumor samples (95% of NSCLC samples) and in samples with lymph node metastasis (42% in the N1 group and 56% in the N2 group, according to the pTNM classification). However, the results were not significant, confirming the role of pSTAT3 in more advanced lung tumors.

On the other hand, it is also recognized that STAT3 promotes cancer development via the induction of proproliferative and antiapoptotic genes.[1] Similar functions are displayed by STAT5. Our results confirmed the high frequency of increased STAT5 immunoexpression levels, especially in SCC samples, which was even higher than that observed by other investigators.[25] Again, similarly to pSTAT3, the overexpression of pSTAT5 prevailed in the largest tumors (pT3+pT4). Although a significantly higher immunoexpression level was observed in pT2 vs pT3/pT4 tumors, this could be due to the smaller number of samples in the latter group. Still, the obtained results could suggest the role of pSTAT5 in NSCLC progression and poor prognosis. This is in line with the results of Sanchez-Ceja et al,[25] who suggested that STAT5 may play a role in advanced stages of lung cancer, promoting survival through apoptosis blockade. A significant difference in pSTAT5 levels between patients with NSCLC and controls, as observed in our study, could suggest the diagnostic importance of pSTAT5 and its potential role as a therapeutic target. Although we are aware that the small size of the control group is a limitation of the study, our results warrant further investigation.

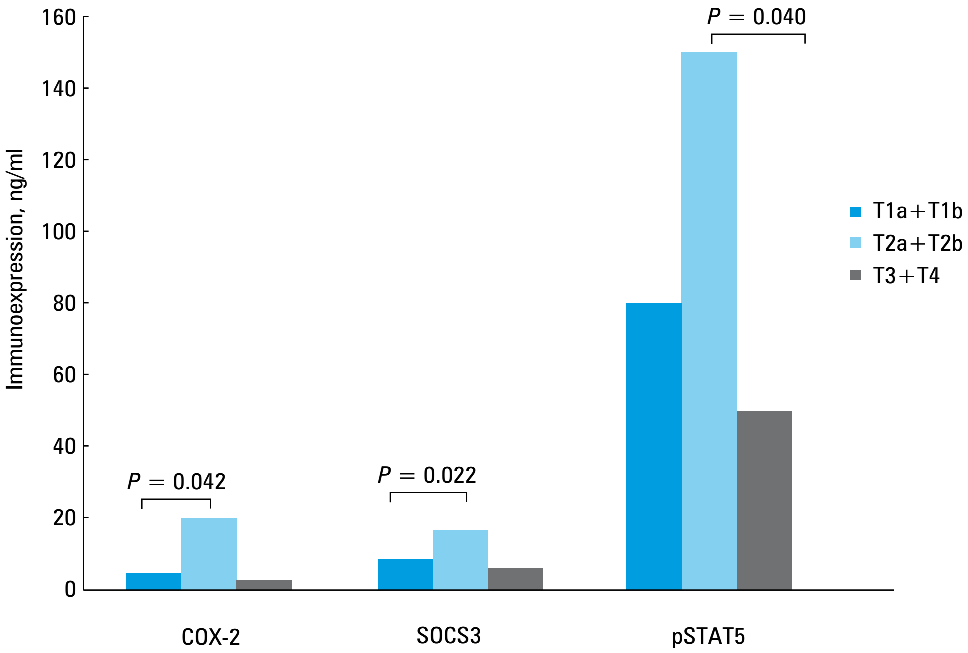

FIGURE 1 Differences in pSTAT5, SOCS3, and COX-2 immunoexpression levels in the study groups depending on the tumor size (pTNM classification; Neuman–Keuls' multiple comparison test) Abbreviations: see **TABLE 2**

We observed the simultaneous overexpression of STAT3 and STAT5 in more than 70% of NSCLC samples. In breast cancer, the concurrent activation of STAT5 and STAT3 was associated with longer patient survival.[30] The relative contribution of these 2 STATs to lung carcinogenesis is unknown. Such knowledge would be of great value, as it could be a decisive factor regarding the use of potential inhibitors of STAT3 and STAT5 in lung cancer therapy. On the other hand, since STAT3 and STAT5 share the overlapping regulatory pathways, the specific inhibitors could be useful in cells that contain activated STAT3 or STAT5, or both.[31]

The third analyzed STAT protein, STAT6, was also upregulated in a similar percentage of NSCLC samples as pSTAT3 and pSTAT5. In our previous research, we observed the increased expression of the *STAT6* mRNA level (unpublished results), and in the present study, we confirmed its increased protein level in patients with NSCLC. As reported by Dubey et al,[32] *STAT6* silencing (via STAT6-specific siRNA) induces apoptosis in cancer, including also lung cancer cell lines. However, there have been no reports describing the level of pSTAT6 immunoexpression in patients with NSCLC, and, to the best of our knowledge, we are the first to observe significant differences between NSCLC histotypes. This may be valuable, and pSTAT6 may gain importance as a differentiating marker of NSCLC. It is all the more worth emphasizing because specific subtypes of NSCLC display varying responses to different chemotherapeutic agents, and new molecular markers for lung cancer are being sought.[33]

Our results confirmed the high levels of all 3 STAT proteins in their active forms in NSCLC tissue, which clearly suggests their role in lung cancer development. Simultaneously, we observed differences between the histopathological subtypes of NSCLC. Significant differences between SCC and NSCC observed for pSTAT3 and pSTAT6 indicate the possibility of using these genes as markers differentiating the NSCLC subtypes. Additionally, the immunoexpression level of pSTAT3 differentiated SCC and adenocarcinoma, and in the case of pSTAT6, it differentiated SCC and LCC. The results of the present study confirmed our previous findings (both published and unpublished) concerning the increased expression of STATs on the mRNA level.[17,18]

Experimental and population studies, as well as preclinical and clinical trials, clearly indicate that all essential features of carcinogenesis (mutagenesis, mitogenesis, angiogenesis, reduced apoptosis, metastasis, and immunosuppression) are linked to COX-2-driven prostaglandins.[34] Indeed, the overexpression of COX-2 has been suggested to contribute to the progression of solid tumors, including lung cancer[35-37] and its precursor lesions.[10] We observed increased COX-2 immunoexpresion in the majority of NSCLC samples, with a higher frequency and level in SCC than in NSCC samples. This is in contrast to the results of other investigators[36,38]; however, our findings were not significant (possibly because of the small sample size).

The COX-2 overexpression was reported to be associated with poor prognosis and short survival of patients with NSCLC,[39] although further studies did not confirm this correlation.[38] We found the association between COX-2 activity and malignant progression of lung tumors; in our study, COX-2 protein levels were significantly higher in larger tumors, classified as pT2. This supports our previous findings regarding the *COX-2* mRNA level.[17]

Interestingly, we found a positive correlation between the immunoexpression levels of COX-2 and pSTAT5. A similar correlation was also reported by Cao et al[15] in human lung adenocarcinoma cell line, who observed that STAT5 mediated and regulated the activation of COX-2 via the epidermal growth factor stimulation. Moreover, an association between an increased pSTAT3 expression and COX-2 overexpression in NSCLC cells was observed,[16] suggesting the survivin-dependent apoptosis resistance. In our study, no relationship between COX-2 and pSTAT3 or pSTAT6 immunoexpression was found, although all those proteins showed elevated levels, and the simultaneous overexpression frequencies of COX-2 and pSTAT3, as well as COX-2 and pSTAT6, exceeded 60% for both pairs.

Regarding the role of COX-2 in carcinogenesis, possibly via its inflammatory actions, and its increased expression also in lung cancer,[17,38-40] it seems reasonable that selective COX-2 blockade (eg, via small-molecule antagonists) might represent a novel antitumor therapy.[41,42]

We also examined 2 STAT inhibitors: PIAS3 and SOCS3. PIAS3, a protein inhibitor of activated STAT3, is a specific inhibitor of the STAT3 signaling pathway, negatively regulating STAT3 transcriptional activity and cell growth in a concentration-dependent manner.[43] It was also reported that PIAS3 inhibits STAT5 activity.[44] Indeed, we observed a negative correlation between the pSTAT5 level and PIAS3 immunoexpression. To the best of our knowledge, it is the first such finding reported for lung cancer. In our opinion, this is important and could have potential therapeutic utility, as STAT5 activity might be modulated via the PIAS3 mechanism. The inhibitory effect of PIAS3 on constitutively active STAT3 and the associated potential therapeutic effect in lung cancer were reported.[45]

Generally, we observed underexpression of PIAS3 in most samples, with a significantly lower level in NSCC than in SCC. This confirms our previous findings regarding the *PIAS3* mRNA level.[17] Low PIAS3 immunoexpression levels in lung cancer were observed by other investigators,[3,45] who reported different PIAS3 expression levels among NSCLC histotypes, with significantly lower PIAS3 immunoexpression in SCC than in adenocarcinoma. These opposite results could be due to the different methods used. However, considering the percentage of cells with a decreased expression of PIAS3 in our study, we similarly observed a higher rate of PIAS3 underexpression in SCC samples as compared with NSCC specimens, but without significance.

The other negative regulators of the JAK/STAT signaling cascade are SOCS proteins. SOCS can inhibit the JAK/STAT pathway activity via a feedback loop, binding to both a cytokine receptor and JAK itself, and the failure of feedback suppression by SOCS proteins may lead to carcinogenesis.[46,47] We observed a negative correlation between SOCS3 and pSTAT6 expression, which is consistent with our previous findings concerning the level of the *SOCS3* gene and its inverse correlation with the overexpression of *STAT6* gene (unpublished results). It confirms a functional relationship between the SOCS3 and STAT6 signaling pathway and underlines the role of STAT6 in lung carcinogenesis. Additionally, the increased pSTAT3 expression was accompanied by decreased SOCS3 immunoexpression in more than 60% of the samples, and in the case of pSTAT5, it was observed in more than 70% of NSCLC specimens. It could suggest the role of SOCS3 in NSCLC therapy by inhibiting STAT proteins: STAT3, STAT5, or STAT6.

Generally, we observed a decreased immunoexpression of SOCS3 in most tumor samples, especially in the SCC subtype. As reported by other authors, the restoration of the SOCS3 expression increased the apoptosis and decreased cell invasiveness.[11,13,47,48] Additionally, in NSCLC cells, the induced overexpression of SOCS3 resulted in enhanced cell radiosensitivity,[48] thus suggesting another potential use of the *SOCS3* gene in lung cancer therapy. We observed a significantly lower SOCS3 immunoexpression level in T1 than in T2 tumors. This could suggest the role of SOCS3 downregulation in the early stage of lung carcinogenesis. There have been no similar studies regarding lung cancer and the prognostic role of SOCS3. The results concerning other tumor types are divergent. In breast and prostate cancers, significantly downregulated SOCS3 protein expression was correlated with more aggressive phenotypes and poor prognosis[49,50]; however, in head and neck SCC, the loss of SOCS3 was an early event, correlated with a histological grade of dysplasia.[51]

Conclusions Summing up, in many cancers, including lung cancers, constitutively activated STAT proteins are associated with uncontrolled growth signaling and cell proliferation. This confirms their critical role in carcinogenesis. In this respect, the blockade of STATs—either on the mRNA or protein level or acting via their inhibitors—seems to be an attractive target for cancer therapy by focusing on tumor growth inhibition or enhancing tumor-specific immunity. We hope that our findings will encourage future research on the use of these molecules as diagnostic markers or targets in lung cancer therapy.

Supplementary material Supplementary material is available with the article at www.pamw.pl.

Contribution statement EB-L conceived the idea for the study. DP-L and DD-S contributed to the design of the research. JK provided the study material. EN, DD-S, and JMK conducted the experiments. KHC and MM-S were involved in data analysis and statistical analysis. DP-L and AA drafted the manuscript. All authors approved the final version of the manuscript.

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