ORIGINAL RESEARCH ARTICLE

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The Expression Levels of IL-4/IL-13/STAT6 Signaling Pathway Genes and *SOCS3* Could Help to Differentiate the Histopathological Subtypes of Non-Small Cell Lung Carcinoma

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Abstract

Background The interleukin (IL)-4/IL-13/signal transducer and activator of transcription (STAT) 6 signaling pathway and the *SOCS3* gene, one of its main regulators, constitute an important link between the inflammation process in the epithelial cells and inflammatory-related tumorigenesis. The present study is the first to evaluate *IL-4, IL-13, STAT6*, and *SOCS3* mRNA expression in non-small cell lung carcinoma (NSCLC) histopathological subtypes.

Methods Gene expression levels were assessed using TaqMan® probes by quantitative reverse transcription PCR (qRT-PCR) in lung tumor samples and unchanged lung tissue samples.

Results Increased expression of *IL-4*, *IL-13*, and *STAT6* was observed in all histopathological NSCLC subtypes (squamous cell carcinoma [SCC], adenocarcinoma [AC], and large cell carcinoma [LCC]). Significantly higher expression of *IL-13* and *STAT6* ($p = 0.019$ and $p = 0.008$, respectively) was found in SCC than in LCC. No statistically significant differences were found for *IL-4*. Significantly higher *SOCS3* expression was found in LCC than in AC ($p = 0.027$). A negative correlation (rho $= -0.519$) was observed for the *STAT6* and *SOCS3* genes in SCC ($p = 0.005$). No associations were found between gene expression and tumor staging (post-operative Tumor Node Metastasis [pTNM], American Joint Committee on Cancer [AJCC]), patients' age, sex, or history of smoking.

Conclusions As the number of LCC cases in our study was quite low, the statistically significant results obtained should be confirmed in a larger group of patients, particularly as the relationships identified between increased *IL-4, IL-13,* and *STAT6 mRNA* expression and decreased *SOCS3* expression suggest that these genes may serve as potential diagnostic markers for differentiating between NSCLC histopathological subtypes.

Key Points

The analysis of *IL-13/STAT6/SOCS3* expression could be useful as a support tool in non-small cell lung carcinoma (NSCLC) histopathological examination.

The presence of a negative correlation between *STAT6* and *SOCS3* confirms that suppressors of cytokine signaling (SOCS) act as a negative regulator of signal transducer and activator of transcription (STAT).

An accurate subclassification based on molecular analysis could, in the future, offer more personalized therapies for NSCLC patients.

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1 Introduction

Interleukin (IL)-4, IL-13, and signal transducer and activator of transcription (STAT) 6 signaling pathways are known to exhibit a range of immunomodulatory functions, particularly IL-4 and IL-13 [1]. Both ILs are recognized as crucial cytokines that regulate the immune reactions taking place in the lung; these are characterized by the recruitment of inflammatory cells and lymphocytes and may be involved in lung remodeling [2]. Moreover, IL-4 and IL-13 are believed to play a key role in encouraging allergic airway inflammation by acting as the main stimulators of IgE production in B lymphocytes and T helper (Th) 2-type differentiation in T cells [3–5]. STAT6 seems to be the main target for IL-4 and IL-13 [6] and appears to be essential for Th1/Th2 balance during the autoimmunity response [7]. The results of animal studies suggest that IL-4, IL-13, or STAT6 deficiency may influence IgE synthesis and Th2-type reactions [8]. Additionally, it has been documented that STAT6 overexpression and secretion of IL-4 may significantly increase the level of cell apoptosis during cancer development [9]. In vitro studies have also demonstrated that IL-4 may directly inhibit the growth of transformed cells in human lung cancer, among other cancers [10, 11].

The negative regulation of Janus kinase (JAK)/STAT signaling is highly dependent on the action of suppressors of the cytokine signaling (SOCS) proteins SOCS1–SOCS7 and cytokine-inducible SH2 protein (CIS) [12, 13]. Overexpression of SOCS3 has been observed in human chronic inflammatory diseases [14, 15]; however, the deficiency of this protein has been recorded in some types of cancer, including lung cancer [16, 17]. Moreover, it has been confirmed that the main biochemical target for SOCS3, except STAT3, appears to also be STAT6 (via IL-4/IL-13/STAT6 signaling) [18]. STAT6 is an important protein known to be regulated by SOCS3, as two possible STAT-response elements (SREs) able to bind STAT6 have been identified within the *SOCS3* gene promoter [19, 20]. Hence, *SOCS3* deregulation in cancer development has been attributed to cross-talk between *STAT6* and *SOCS3* genes on both the protein and mRNA levels; however, this mechanism remains unclear.

Therefore, it is possible that molecular changes in the IL-4/IL-13/STAT6 signaling pathway on the transcriptional or translational level may have a significant influence on the development of human inflammatory diseases, as well as in the development of the inflammatory-related tumors, including lung cancer [8, 9, 21–23].

The present study evaluates the relationship between the expression of *STAT6* and *SOCS3* and their mRNA level in non-small cell lung carcinoma (NSCLC) histopathological subtypes to identify potential diagnostic molecular markers.

2 Materials and Methods

2.1 Clinical Characterization of the Studied Patients

Seventy-one patients with a confirmed diagnosis of NSCLC (25 women, mean age 63 ± 8.717 years; 46 men, mean age 65 ± 8.234 years) were enrolled into the study.

The smoking history was available for 71 patients: five patients were non-smokers and 66 were smokers or former smokers. They were divided into groups according to duration of tobacco addiction and number of cigarettes smoked; the latter was presented as pack years (PYs), and was calculated according to the NCI Dictionary of Cancer Terms [24] (1 PY is equal to 20 cigarettes smoked per day for 1 year) (see Table 1).

2.2 Characterization of the Non-Small Cell Lung Carcinoma Tissue Samples

Lung tissue samples (100–150 mg) were received from patients with preoperatively diagnosed lung cancer who had undergone pulmonectomy or lobectomy at the Department of Thoracic Surgery, General and Oncologic Surgery, Medical University of Lodz, Lodz, Poland between July 2010 and June 2012. Immediately after resection, the tissue samples were collected in RNAlater® buffer (ThermoFisher Scientific, Waltham, MA, USA) and frozen at –80 °C. For each patient, two kinds of tissue samples were collected: a lung tissue sample from the primary lesion and a sample of adjacent non-cancerous macroscopically unchanged lung tissue (10 cm distant from the primary lesion) to serve as a control.

Table 1 Characterization of the studied non-small cell lung cancer patients ($n = 71$) regarding their tobacco addiction and consumption (number of cigarettes smoked per day and pack years)

Tobacco addiction and consumption	n (%)
Smoking period	
Smokers	66 (93)
< 40 years	37 (52)
≥ 40 years	29 (41)
Non-smokers	5 (7)
Number of cigarettes smoked per day	
10–15	6 (8)
20 (1 pack)	43 (61)
30–40 (1.5–2 packs)	17 (24)
PYs	
< 40	30 (42)
≥ 40	36 (51)

PYs pack years

The diagnosis of lung cancer was made according to the 2015 World Health Organization (WHO) Classification of Lung Tumours diagnostic criteria [25]. Squamous cell carcinoma (SCC) was diagnosed when keratinization, pearl formation, and/or intercellular bridges were observed. Furthermore, in case of high-grade SCC, immunohistochemical staining for cytokeratin (CK) 5/6 and CK 34 beta E12 was performed. Diagnosis of large cell carcinoma (LCC) was made after ruling out the presence of a component of SCC, adenocarcinoma (AC), or small-cell carcinoma. In case of AC diagnosis, glandular differentiation and/or mucin production were confirmed. Additionally, for unambiguous verification of AC, thyroid transcription factor-1 (TTF-1) and CK 7, immunohistochemical expression was assessed.

The resected specimens were subjected to post-operative histopathological evaluation and classified according to the American Joint Committee on Cancer (AJCC) staging [26] and post-operative Tumor Node Metastasis (pTNM) classification.

The results of the histopathological verification of tumor specimens, based on pathomorphological reports, are summarized in Table 2.

2.3 RNA Extraction and Expression Analysis of *IL-4*, *IL-13*, *STAT6*, and *SOCS3*

RNA was extracted from tissue samples using a Universal RNA Purification Kit (Eurix, Gdańsk, Poland) according to the manufacturer's recommendations. Complementary DNA (cDNA) was transcribed from 100 ng of total RNA using a

Table 2 Histopathological verifications of non-small cell lung cancer samples

Histopathological type of NSCLC	n (%)
Squamous cell carcinoma	41 (58)
Non-squamous cell carcinoma	30 (42)
Adenocarcinoma	23 (32)
Large cell carcinoma	7 (10)
AJCC	
AJCC IA	14 (20)
AJCC IB	11 (16)
AJCC IIA	13 (18)
AJCC IIB	10 (14)
AJCC IIIA/IIIB	23 (32)
pTNM	
T1	19 (27)
T2	33 (47)
T3–4	19 (27)

AJCC American Joint Committee on Cancer Staging according to the IASLC Staging Project [26], *NSCLC* non-small cell lung cancer, *pTNM* post-operative Tumor Node Metastasis classification according to the World Health Organization Histological Typing of Lung Tumor

High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), in a total volume of 20 µL. Reverse transcription (RT) master mix contained the following: 10×RT buffer, 25×dNTP (deoxynucleotide) mix (100 mM), 10×RT random primers, MultiScribe™ reverse transcriptase, RNase inhibitor, and nuclease-free water. The RT reaction was performed in a Personal Thermocycler (Eppendorf, Hamburg, Germany) in the following conditions: 10 min at 25 °C, followed by 120 min at 37 °C, then the samples were heated to 85 °C for 5 s, and hold at 4 °C.

The relative expression (RQ) of *IL-4*, *IL-13*, *STAT6*, and *SOCS3* was assessed using TaqMan® probes (Applied Biosystems) for the studied genes (Hs00932431_m1, Hs00174379_m1, Hs00598625_m1, and Hs02330328_m1, respectively), with *ACTB* (Hs99999903_m1) as the reference gene. The procedure was performed in an Applied Biosystems 7900HT Fast Real-Time PCR System, for 39 cycles. The PCR mixture was as follows: cDNA (1–100 ng), 20×TaqMan® Gene Expression Assay, 2×TaqMan® Gene Expression Master Mix, and RNase-free water, in a total volume of 20 µL. The RQ of each sample was assessed using the Comparative $\Delta\Delta C_T$ method adjusted to *ACTB* (endogenous control) expression and presented as the RQ value.

The following formula was used to determine the $\Delta\Delta C_T$ value: $\Delta\Delta C_T = \Delta C_T$ test sample − ΔC_T calibrator sample. Higher $\Delta\Delta C_T$ values indicated lower expression of *IL-4*, *IL-13*, *STAT6*, or *SOCS3* in the specimen [27].

In relation to the expression level of the calibrator (macroscopically unchanged lung tissue), for which RQ = 1, the obtained results were compared between NSCLC patients in regard to histopathological NSCLC subtype, tumor staging (TNM, AJCC), patients' age, sex, and smoking history. For the studied samples, an increased expression value was recognized when the RQ value was > 1 and decreased expression was when the RQ value was < 1.

2.4 Statistical Analysis

The analysis of variance (ANOVA) Kruskal-Wallis test was used to compare the RQ of *IL-4*, *IL-13*, *STAT6*, and *SOCS3* between NSCLC subtypes (SCC, AC, and LCC). Neuman-Keuls' multiple comparison test was used to identify possible significant differences in RQ values between the individual NSCLC subtypes.

Spearman's rank correlation coefficient, the Mann-Whitney test, and the ANOVA Kruskal-Wallis test were performed to evaluate the relationships between the expression of the studied genes and the other examined parameters [i.e., patient age, sex, and tumor staging (pTNM, AJCC)]. Statistical significance was regarded as $p < 0.05$. Additionally, the statistical significance level for multiple analyses was adjusted to a *p*-value of 0.032 according to Bonferroni's correction. Statistica™ for Windows 10.0 (TIBCO, Palo Alto,

CA, USA) was used for calculations. The RQ values for the studied genes are presented as means ± standard error of the mean and means ± standard deviation.

3 Results

The results of the expression analysis were calculated using the $\Delta\Delta C_T$ method adjusted to *ACTB* expression (endogenous control) and in relation to the expression level of calibrator (macroscopically unchanged lung tissue), for which RQ = 1.

IL-4, *IL-13*, and *STAT6* demonstrated increased expression (RQ value > 1) in all histopathological NSCLC subtypes, being seen in 57–90% of samples, depending on histotype and gene. For all studied genes, the highest level of expression was observed in the SCC subtype, followed by AC, with the lowest level being observed for LCC. *SOCS3* was decreased (RQ value < 1) in 93–96% of SCC and AC samples, but increased (RQ value > 1) in 57% of LCC samples. The results are presented in Fig. 1.

Statistically significant differences in *IL-13*, *STAT6*, and *SOCS3* expression were observed between the histopathological NSCLC subtypes (p < 0.05, ANOVA Kruskal–Wallis test). The Neuman-Keuls' multiple comparison test found significant differences between the SCC and LCC histotypes with regard to *IL-13* (p = 0.019) and *STAT6* expression (p = 0.008), as given in Figs. 2 and 3. Significantly lower *SOCS3* expression was found in AC than in LCC (p = 0.027; Neuman-Keuls' multiple comparison test) (Fig. 4).

No significant differences were found between any of the NSCLC subtypes with regard to *IL-4* expression (p = 0.079; ANOVA Kruskal–Wallis test).

Spearman's rank correlation revealed a statistically significant negative correlation (rho = –0.519) between the RQ values of the *STAT6* and *SOCS3* genes in the SCC subtype (p = 0.005). While a negative correlation (rho = –0.538) was also observed between the expression of *STAT6* and *SOCS3* in the AC subtype, this relationship was not significant (p = 0.08; Spearman's rank correlation). A positive correlation (rho = 0.671) between *STAT6* and *SOCS3* was found in the LCC subtype, but, again, this was insignificant (p = 0.56; Spearman's rank correlation).

No statistically significant correlations were found between the RQ values of the studied genes and the clinical features of NSCLC patients, i.e., patient age, sex, or history of smoking assessed as PYs (p > 0.05; ANOVA Kruskal-Wallis test, Mann-Whitney test, followed by Spearman's rank correlation coefficient). Similarly, no associations
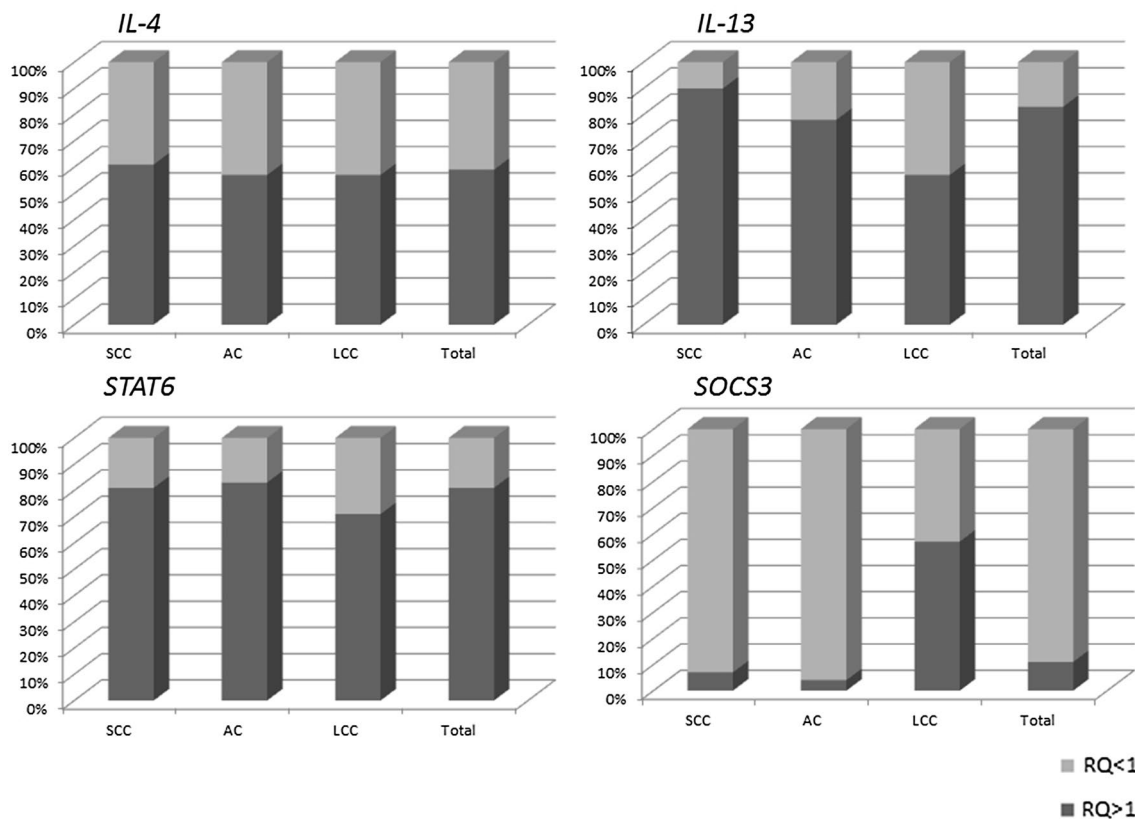

Fig. 1 Percentage of samples with increased (RQ > 1) and decreased (RQ < 1) expression of the examined genes in individual histopathological NSCLC subtypes. *NSCLC* non-small cell lung cancer, *RQ* relative expression
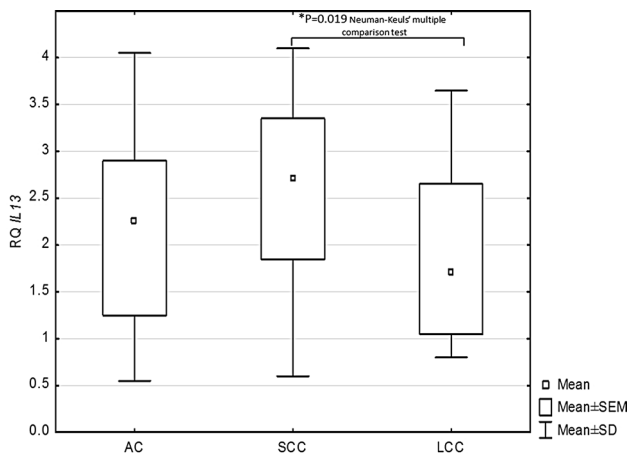
Fig. 2 Box-and-whisker plots representing mean *IL-13* expression in the studied NSCLC subtypes ($p = 0.019$; ANOVA Kruskal-Wallis test). *Indicates a statistically significant difference between SCC and LCC ($p = 0.019$; Neuman-Keuls' multiple comparison test). *ANOVA* analysis of variance, *LCC* large cell carcinoma, *NSCLC* non-small cell lung cancer, *SCC* squamous cell carcinoma
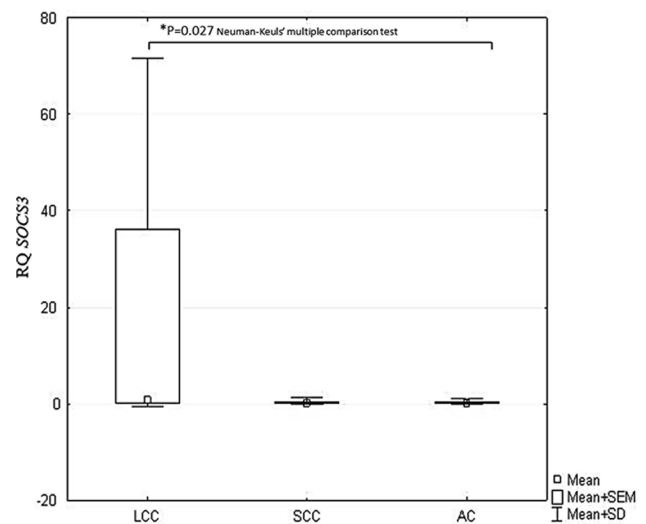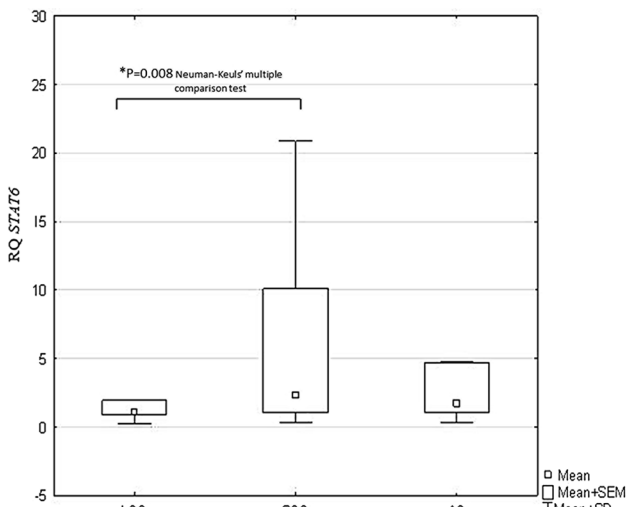

Fig. 3 Box-and-whisker plots representing mean *STAT6* expression in the studied NSCLC subtypes ($p = 0.037$; ANOVA Kruskal-Wallis test). *Indicates a statistically significant difference between LCC and SCC ($p = 0.008$; Neuman-Keuls' multiple comparison test). *ANOVA* analysis of variance, *LCC* large cell carcinoma, *NSCLC* non-small cell lung cancer, *SCC* squamous cell carcinoma

were found with tumor staging according to the pTNM and AJCC classifications ($p > 0.05$; ANOVA Kruskal-Wallis test, Mann-Whitney test).

No statistically significant mutual correlations were found between *IL-4*, *IL-13*, *STAT6*, and *SOCS3* gene expression and clinical features, i.e., patient age, sex, history of smoking, or tumor staging according to the pTNM or AJCC classifications.

Fig. 4 Box-and-whisker plots representing mean *SOCS3* expression in the studied NSCLC subtypes ($p = 0.029$; ANOVA Kruskal-Wallis test). *Indicates a statistically significant difference between LCC and AC ($p = 0.027$; Neuman-Keuls' multiple comparison test). *AC* adenocarcinoma, *ANOVA* analysis of variance, *LCC* large cell carcinoma, *NSCLC* non-small cell lung cancer

4 Study Limitations

The main limitation of our study is that it is based on only a small number of patients with diagnosed LCC, which can be attributed to the fact that it was a single-center study. The research material was obtained from one hospital, in which only a small number of cases were reported during the study period. However, it is important to bear in mind that genetic and environmental factors have a strong influence on lung cancer incidence and the contribution of its histopathological subtypes, especially in polluted areas such as large urban areas.

However, we believe that the results obtained indicating significant differences between NSCLC histopathological subtypes, including the LCC group, are nevertheless valuable and encourage further research aimed at supporting lung cancer diagnostics (NSCLC subtyping) with molecular methods.

5 Discussion

There is a growing need for molecular studies of the etiology of NSCLC, as lung cancer constitutes a leading cause of mortality worldwide. Hence, to identify informative molecular diagnostic markers in NSCLC the present study evaluates the mRNA expression of *IL-4*, *IL-13*, *STAT6*, and *SOCS3*, these being important factors in signal transduction and transcription regulation. Due to the high heterogeneity

of NSCLC cells, histological subtyping should be supplemented by molecular analysis of cancer cells. Therefore, the study analyzes the differences in the expressions of the studied genes between three different NSCLC histopathological subtypes: SCC, AC, and LCC.

The present study is the first to compare the expression of *IL-4* on the mRNA level between different subtypes of NSCLC. Higher expression was observed in SCC than LCC; however, the difference was not statistically significant. Although our results should be interpreted with a degree of caution due to the small number of LCC cases, they confirm those of Huang et al. [28], who also report the expression of *IL-4* to be highest in an SCC line, but not significantly so. The same authors confirmed higher immunoexpression of IL-4 in SCC than AC at the protein level [28]. Our results indicate that *IL-4* mRNA expression varies depending on the NSCLC subtype; this is an important observation, considering that IL-4 mediates pleiotropic effects in the NSCLC microenvironment and demonstrates both stimulatory and inhibitory effects on antitumor immune responses and tumor proliferation [29–31]. Interestingly, it has been documented that IL-4 may decrease the proliferation of some solid tumor cell lines [32], including lung cancers [31], and may inhibit tumor vascularization [33]. On the other hand, IL-4 influences cancer-associated fibroblasts, which are recognized as key factors in cancer growth and progression [34]. Moreover, patients with advanced cancer often have reduced cell immunity associated with a 'switching' from Th1 to Th2 [35]. Tumor cells often demonstrate inhibition of the Thl cytokine response combined with an elevated type 2 (Th2) cytokine response (including IL-2) [36].

To better understand the biological role of IL-4 in cancer cells, the study compared *IL-4* mRNA expression between various stages of lung tumor development (TNM staging, AJCC classification). It should be emphasized that our work is the first study to address this issue. However, no statistically significant differences were found in IL-4 expression with regard to the TNM/AJCC classifications. On the other hand, a growing body of evidence suggests that overexpression of IL-4 receptor (IL-4R) may be observed in lung cancer cells as a tissue-specific molecular biomarker [37–39]. Based on this observation, IL-4 signaling through IL-4R has been recognized as a cytotoxic antitumor cytokine, regulating tumor cell survival via the activation of antiapoptotic proteins [31, 39–42]. Moreover, at the protein level, both IL-4R and IL-4 are considered prognostic biomarkers for various types of human epithelial cancer, including lung cancer [41–44].

The *IL-13* mRNA level was increased in all histopathological NSCLC subtypes and was significantly higher in SCC than LCC. Although SCC is thought to be associated with smoking behavior [45], this was not indicated by our findings. As only a small number of LCC samples could

be included in the present study, our findings should be confirmed in a larger group of patients diagnosed with this NSCLC subtype. LCC displays an aggressive phenotype, as it tends to grow rapidly and spreads more quickly than other NSCLC subtypes [46, 47]. Moreover, LCC frequently presents problems in terms of preoperative diagnosis and often requires additional histological examination. From this point of view, the results obtained seem to be encouraging for further research, in terms of the suitability of *IL-13* as a differentiating biomarker for NSCLC.

Similarly, increased *IL-13* protein and mRNA expression has been confirmed in NSCLC cell lines by Huang et al. [28]. Little is known about the importance of IL-13 in lung tumors, but it is claimed that *IL-4* and *IL-13* are important immunological antitumor response factors in lung cancer cells. In contrast to other human epithelial cancers, where the association with invasion, metastasis and poor prognosis has been confirmed [48, 49], the present study is the first to evaluate the significance of *IL-4* and *IL-13* expression as molecular markers in lung cancer (in relation to TNM/AJCC classification). Unfortunately, as no relationship was found between *IL-13* or *IL-4* expression and TNM/AJCC classification, it was not possible to confirm whether their mRNA expression could be used for NSCLC prognosis.

The study also found *STAT6* upregulation in all studied histopathological NSCLC subtypes (SCC, AC, LCC). These findings are similar to those of previous studies, which indicate the indirect significance of STAT6 in apoptosis resistance in NSCLC cells lines via cyclo-oxygenase (COX)-2 upregulation. Moreover, constitutive activation of STATs and their overexpression has been observed in vivo in many human tumors, including lung cancer [50–57]. It has been observed that cells harboring upregulated STAT6 displayed expression profiles supporting Th2 cytokine secretion, cell cycle promotion, anti-apoptosis, and pro-metastasis processes [51]. Our findings also confirm increased expression of the active form of STAT6 protein in lung cancer patients [58].

It should be pointed out that molecular studies on *STAT6* in lung cancer are rare and have received limited attention. This is surprising, given that among many STAT proteins, STAT6 acts as a positive regulator of cancer cell proliferation and tumor metastasis capacity [50, 51]. It is well-recognized that lung cancer, apart from genetic factors, which include JAK/STAT signaling, may be induced by smoking [59–61]. However, no published reports could be found on the relationship between *STAT6* expression and patient smoking behavior, and our findings do not reveal any such association between smoking history and *STAT6* expression. The reason may be the small number of patients studied. However, it is worth continuing this investigation of the relationship between smoking and changes in *STAT6* expression in view of raising awareness of the part played by STAT6 in

allergic airway chronic inflammation and the promotion of inflammatory-related cancer.

Similar to *IL-13*, the *STAT6* expression level was significantly higher in SCC than LCC samples, and these results should also be interpreted with caution because of the small number of LCC patients. Still, we hope that our observation might be beneficial for clinical practice because some authors have demonstrated a survival advantage for patients with the SCC subtype [45]. However, a more recent study has reported better prognosis for AC stage I than SCC stage I, and the opposite in stage II [62], while other studies indicate significantly more frequent metastasis occurrence in AC than SCC [46]. So, as the prognostic significance of different histological subtypes remains controversial [47, 63], there is a real need to search for the molecular differences between histological subtypes and the differentiating molecular markers.

The expression of *SOCS3* mRNA, a cytokine suppressor, was decreased in both the AC and SCC subtypes, with significant differences being observed between AC and LCC. Although, as mentioned earlier, our LCC group was restricted in size, the differences observed in *SOCS3*, *IL-13*, and *STAT6* expression between NSCLC subtypes can provide valuable information in the search for additional diagnostic markers in lung cancer. In future, an accurate subclassification based on molecular analysis could act as the basis for targeted therapies in NSCLC patients.

Reduced *SOCS3* expression in NSCLC has been reported in a number of previous studies [16, 17, 58, 64]. Significant downregulation of the SOCS3 protein or loss of gene expression in lung tumors or NSCLC cell lines due to promoter hypermethylation was revealed [16]. In the present study, more than 80% of the tested NSCLC samples, with the exception of the LCC subtype, had decreased *SOCS3* gene expression, confirming its role as a negative regulator of STAT. However, as *SOCS3* may be down- or upregulated by STATs, depending on tumor type [64–68], it is difficult to determine the diagnostic or prognostic value of *SOCS3* gene expression. As observed in the present study, the combined downregulation of *SOCS3* and upregulation of *IL-4*, *IL-13*, and *STAT6* indicate the presence of a functional relationship between *SOCS3* and the *IL-4/IL-13/STAT6* signaling pathway, which has been confirmed elsewhere [20, 52]. The recognized interaction of *STAT* and *SOCS3* genes by binding to SRE (STAT-response elements) in *SOCS3* promoter sequences seems to be responsible for IL-4/IL-13/STAT6 stimulation [20, 52].

The present study attempted to check whether the studied genes may be regarded as molecular diagnostic markers in NSCLC, based on an assessment of the relationship between gene expression and TNM/AJCC staging. The most interesting findings seem to be related to the *STAT6* and *SOCS3* genes, largely due to the paucity of reports focused

on similar correlations. The present study is the first to examine the correlation of *STAT6* expression with TNM/AJCC staging in lung cancer, and hence may be compared only with results from studies on gliomas and colon or prostate tumors, which indicate an association between *STAT6* expression and higher histological grades and larger tumor size, apoptosis resistance, metastasis, and shorter survival [20, 51, 57]. However, these ambiguous observations concerning NSCLC should not be surprising. It has been documented that depending on the cancer cell type, STAT6 can act as either a pro- or anti-apoptotic factor, and, by interacting with other transcription factors, plays a dual role as a STAT [69]. In addition, regarding the association between *SOCS3* mRNA expression and tumor progression, no statistically significant differences were found between TNM/AJCC stages. As in the case of *STAT6*, no comparable studies examining lung cancer can be found. However, Zhang et al. [17] report that restored expression of the SOCS3 protein in lung cancer cells increased apoptosis and decreased cell invasiveness, while significantly downregulated SOCS3 protein expression was correlated with more aggressive phenotypes and poor prognosis in other tumor types, such as breast and prostate cancers [64, 68, 69].

Interestingly, an inverse correlation was found between the *STAT6/SOCS3* relationship (as regulators of mutual expression) and tumor progression stage (TNM/AJCC) and NSCLC subtypes. It is therefore reasonable to assume that SOCS3 may be a negative regulator of STAT6, especially in the SCC histopathological subtype.

6 Conclusions

The present study presents some evidence that *STAT6/SOCS3* mRNA expression analysis could be useful as a support tool in NSCLC histopathological examination. However, our observations require further studies on larger numbers of patients diagnosed with non-small cell lung carcinoma.

Compliance with Ethical Standards

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