ORIGINAL ARTICLE

STAT3 rs3816769 polymorphism correlates with gene expression level and may predispose to nonsmall cell lung cancer: a preliminary study

Daria Domańska[1], Adam Antczak[2], Dorota Pastuszak-Lewandoska[1], Paweł Górski[3], Jacek Kordiak[4], Karolina Czarnecka[1], Monika Migdalska-Sęk[1], Ewa Nawrot[1], Justyna Kiszałkiewcz[1], Ewa Brzeziańska[1]

1 Department of Molecular Bases of Medicine, Medical University of Lodz, Łódź, Poland
2 Department of General and Oncological Pulmonology, Medical University of Lodz, Łódź, Poland
3 Department of Pneumonology and Allergy, 1st Chair of Internal Diseases, Medical University of Lodz, Łódź, Poland
4 Department of Chest Surgery, General and Oncological Surgery University Hospital No. 2, Medical University of Lodz, Łódź, Poland

KEY WORDS

nonsmall cell lung carcinoma, *STAT3,* gene expression, single nucleotide polymorphisms

ABSTRACT

INTRODUCTION The *STAT3* gene functions as both the oncogene and the regulator of immunity. Despite its important role in cancer development and regulation of the immune cells, studies of single nucleotide polymorphisms (SNPs) of the *STAT3* gene and the associated risk of lung cancer are sparse.

OBJECTIVES In the present study, we evaluated the association of SNPs (rs744 166 [AG] and rs3 816 769 [CT]) with predisposition to nonsmall cell lung cancer (NSCLC) development and their potential effect on *STAT3* expression.

PATIENTS AND METHODS DNA and RNA, isolated from lung tissue samples, were obtained from patients with diagnosed NSCLC (n = 71) and those without NSCLC, included in a control group (n = 104). *STAT3* SNP genotyping and relative expression were performed using TaqMan® probes.

RESULTS *STAT3* CC (rs3 816 769) and AA genotypes (rs744 166) were associated with lower lung cancer risk, whereas TT (rs3 816 769) and GG genotypes (rs744 166) were found to be associated with significantly elevated lung cancer risk. In the NSCLC group, odds ratio analysis showed that allele A was rare and might be linked with decreased while allele G with increased lung cancer risk. We demonstrated that overexpression of *STAT3* positively correlated with TT genotype (rs3 816 769) in NSCLC patients ($P = 0.0464$). Moreover, the differences in *STAT3* gene expression between squamous cell carcinoma and large cell carcinoma histopathological subtypes were observed.

CONCLUSIONS It has been shown that rs3816769 *STAT3* gene polymorphisms are associated with NSCLC susceptibility and might be regarded as having a significant functional and diagnostic value.

Correspondence to:
Ewa Brzeziańska, MSc, PhD, Zakład Molekularnych Podstaw Medycyny, Uniwersytet Medyczny w Łodzi, ul. Pomorska 251, 92-213 Łódź, Poland, phone: +48-42-675-77-15, fax: +48-42-675-77-12, e-mail: ewa.brzezianska@umed.lodz.pl
Received: August 20, 2013.
Revision accepted: December 3, 2013.
Published online: December 5, 2013.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2013; 123 (12): 672-679

INTRODUCTION Lung cancer, predominantly nonsmall cell lung cancer (NSCLC), is the leading cause of cancer-related death worldwide.[1,2] It has been documented that, especially in well-developed countries, the incidence and mortality due to lung cancer remain at a very high and constant level, particularly among men.[3,4] However, recently, the more frequent incidence among women has been observed.[5]

According to histopathological verification, lung cancer is classified into 2 major groups based on its biological phenotype, therapy, and prognosis: nonsmall cell lung cancer (NSCLC), including squamous cell carcinoma (SSC) and nonsquamous cell carcinoma (NSCC), which accounts for approximately 85% of all primary lung cancers and small cell lung cancer (SCLC), which constitutes about 15% of malignant cases. SCLC is widely recognized as a disease strongly associated with tobacco addiction.[6]

The prognosis in lung cancer is poor (5-year overall survival rate does not exceed 15%), and, moreover, the difficulties in diagnosis at an early stage of the disease are well-documented (up to

40% of the cases are diagnosed at the advanced stage IIB-IIIA).[7] Therefore, advances in identification of genetic factors that affect susceptibility to lung cancer, especially NSCLC, could be of great value.

Among many recognized genetic factors relevant for NSCLC, including mutations, polymorphisms, and overexpression of more than 30 genes, such as: *K-Ras, BRAF,TP53,MYC, cyclin D1, EGFR, C-erbB2 (Her-2/neu)* or *BCL2,*[6,8-11] the significance of *STAT3* molecular lesions have not been fully elucidated. As an important member of the STAT protein family, STAT3 participates in tumor cell survival, proliferation, migration, invasion, angiogenesis, and inhibition of apoptosis[12,13] in many types of human cancer, including lung cancer.[14-21] Moreover, the association between single nucleotide polymorphisms (SNPs) of *STAT3* and susceptibility to carcinogenesis has been confirmed.[22,23] Additionally, it has been recognized that increased expression of *STAT3* correlates with tumor progression and invasiveness; however, the causal mechanism of this molecular alteration and its prognostic value still remain unclear. Up to now, there have been few studies focused on SNPs in the *STAT3* gene and their effect on gene expression, as well as their correlation with NSCLC risk, especially in the European population. The recently published study has documented the correlation of *STAT3* expression with growth, survival, and radio resistance of NSCLC cells.[24] The significance of some *STAT3* SNPs in NSCLC and also in acute myeloid leukemia (AML) has been reported in the Asian population, but there was no correlation with gene expression.[25,26]

Therefore, in the present study we decided to examine whether the selected *STAT3* SNPs affect the NSCLC risk and/or expression of *STAT3*. We also evaluated their diagnostic value in NSCLC patients.

PATIENTS AND METHODS

Subjects The study was approved by the Ethical Committee of the Medical University of Lodz, Poland. We enrolled 71 patients with diagnosed NSCLC who had undergone lobectomy at the Department of Chest Surgery, General and Oncological Surgery University Hospital No. 2, Medical University of Lodz. Tumor tissue fragments (100–150 mg) from each patient were obtained from the center of lung lesion and the macroscopically unchanged lung tissue sample was obtained from the most distant site from the resected lesion. Lung tissue samples were collected into lysis buffer (Buffer RNA later) and frozen at –70°C until use, immediately after resection. Additionally, blood samples (5 ml) were obtained from each NSCLC patient and control subject for an SNP analysis. The control group consisted of 104 persons without NSCLC diagnosis, other tumors, or chronic disease. They were collected during the routine laboratory analysis. Clinical characteristics of the patients and control group are presented in TABLE 1.

Macroscopically unchanged lung tissue served as a reference standard (calibrator) in expression analysis. The smoking history of all studied patients (64 with diagnosed NSCLC and 82 as a control group) are available. Patients were divided into groups according to their smoking habits: the period of tobacco addiction and the amount of cigarettes smoked – the latter was presented as pack years and was calculated according to the NCI Dictionary of Cancer Terms (1 pack year is equal to smoking 20 cigarettes per day for 1 year).[29]

DNA extraction and SNP genotyping Two SNPs in the *STAT3* gene, rs744166, and rs3816769, have been selected based on the previously published reports regarding their associations with lung cancer risk[25] and a significant role in inflammation.[26] Genomic DNA from patients and the control group was isolated from blood samples using Qiagen QIAamp DNA Mini Kit, according to the manufacturer's procedure. The SNP analysis was performed using TaqMan SNP genotyping assays (Applied Biosystems, USA): C_3140282_10 (rs744166) and C_3140296_10 (rs3816769). SNPs were selected based on the previously published reports and related to *STAT3* genotyping.[26,30]

RNA extraction, relative expression of *STAT3* Total RNA was extracted from tissues, using RNeay Protect Kit (EURX, Poland) and according to the manufacturer's instructions. Complementary DNA (cDNA) was transcribed from 100 ng of total RNA, using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, United States) at a total volume of 20 µl per reaction. Reverse transcription (RT) master mix contained: 10 × RT buffer, 25 × dNTP Mix (100 mM), 10 × RT Random Primers, MultiScribe™ Reverse Transcriptase, RNase Inhibitor, and nuclease-free water. RT reaction was performed in a Personal Thermocycler (Eppendorf, Germany) under the following conditions: 10 minutes at 25°C, followed by 120 minutes at 37°C; then the samples were heated to 85°C for 5 s, and held at 4°C.

The relative expression of *STAT3* was assessed using TaqMan probes for *STAT3* (Hs01047580_m1) and *ACTB* (Hs99999903_m1), as the reference gene, in AppliedBiosystems 7900HT Fast Real-Time PCR System for 39 cycles. The PCR mixture contained: cDNA (1 to 100 ng), 20 × TaqMan® Gene Expression Assay, 2 × TaqMan® Gene ExpressionMaster Mix, RNase-free water in total volume of 20 µl. Macroscopically unchanged tissue served as a calibrator sample. The relative expression of each sample was assessed using the Comparative CT method (TaqMan Relative Quantification Assay) and presented as the relative quantification (RQ) value. The RQ values for *STAT3* were calculated using the delta CT method and adjusted to the β-actin expression and relative to the expression level of calibrator, for which RQ was equal 1.

TABLE 1 Clinical characteristics, characteristics of tobacco addiction and consumption (amount of cigarettes per day and pack years) for the studied patients and controls

Clinical and pathological features		Patients n = 71	Controls n = 104	Patients mean ±SD	Controls mean ±SD	P[a]
mean age	men	46	55	65 ±8.43	54 ±5.47	$P = 0.03$
	women	25	30	63 ±8.71	49 ±7.22	
age group	<60	20 (30)	47 (45)			
	60–70	35 (50)	55 (52)			$P = 0.01$
	>70	16 (20)	2 (2)			
histopathological types of NSCLC	SCC	41 (58)	0			
	NSCC	30 (42)	0			
	– AC	23 (32)	0			
	– LCC	7 (10)	0			
pTNM[b]	T1	19 (27)	0			
	T2	33 (47)	0			
	T3–4	19 (27)	0			
tobacco addiction and consumption		patients n = 64	controls n = 82			
smoking period, y	smokers	64 (90)	82 (79)			
	<25 years	11 (16)	17 (16)			
	25–39	29 (41)	39 (38)			
	≥40	24 (34)	26 (25)			
	nonsmokers	7 (10)	22 (27)			
pack years	<20	11 (17)	14 (17)			
	20–29	10 (16)	12 (15)			
	30–39	15 (23)	28 (34)			
	≥40	28 (44)	28 (34)			

Data are presented as mean ± standard deviation or number (percentage).

a Pearson χ^2 analysis
b postoperatively Tumor Node Metastasis Classification[27,28]

Abbreviations: AC – adenocarcinoma, LCC – large cell carcinoma, NSCLC – nonsquamous cell carcinoma, SCC – squamous cell carcinoma, SD – standard deviation

Statistical analysis The differences in the frequency of alleles and genotypes were assessed using the χ^2 and Fisher's tests. The compliance with Hardy–Weinberg equilibrium was assessed. Odds ratio (OR) and its 95% confidence intervals (95% CI) were calculated as an assessment of strength and direction of association between the individual genotypes and susceptibility to NSCLC. The Kruskal–Wallis test, Mann–Whitney test, Neuman–Keuls' multiple comparison test, and Spearman's rank correlation were used to assess the correlation between the polymorphic variants, as well as gene expression and NSCLC histopathological subtype, pTNM classification, patient age, sex, and smoking history. Linkage disequilibrium in the 2 studied polymorphic sites was evaluated using the Fisher's exact test. A P value of less than 0.05 was considered statistically significant.

RESULTS **Genotyping analysis** A statistical analysis of the frequency of the studied alleles and genotypes of *STAT3* confirmed the presence of significant differences between the studied groups (NSCLC vs. control). The frequencies of the studied genotypes (AA, AG, GG) and OR values for *STAT3* rs744166 polymorphism in NSCLC patients compared with controls are shown in **TABLE 2**.

The results revealed a significant association of GG genotype and G allele in rs744166 with increased lung cancer risk (OR, 2.60; $P = 0.04$; OR, 2.29; $P = 0.034$). We observed a statistically significant ($P < 0.05$) decrease in the frequency of AA genotype (OR, 0.41; $P = 0.012$) and A allele (OR, 0.44; $P = 0.034$) in NSCLC.

The frequencies of the studied genotypes (CC, CT, TT) and OR values for *STAT3* rs3 816 769 polymorphism in NSCLC patients as compared with controls are shown in **TABLE 3**.

Furthermore, the results revealed significant association of the CC genotype in rs3 816 769 with increased lung cancer risk (OR, 0.26; $P = 0.043$). We also observed a statistically significant

TABLE 2 Frequencies of *STAT3* rs744 166 genotypes (A/A, A/G, G/G) as well as A and G alleles and odds ratio in patients with nonsmall cell lung cancer compared with the control group

Genotype	Patients, n = 71		Controls, n = 104		OR (CI 95%)	*P*[a]
	number	frequency	number	frequency		
AA	18	0.25	47	0.45	0.41 (0.21–0.80)	0.012
AG	21	0.30	32	0.31	0.95 (0.50–1.83)	0.92
GG	32	0.45	25	0.24	2.60 (1.36–4.96)	0.04
	$\chi^2 = 3.49$		$\chi^2 = 3.41$			
allele A	57	0.40	126	0.60	0.44 (0.28–0.67)	0.034
allele G	85	0.60	82	0.40	2.29 (1.48–3.54)	0.034

a χ^2 test <3,991; $P > 0.05$

Abbreviations: OR – odds ratio, CI – confidence interval

TABLE 3 The frequencies of *STAT3* rs3 816 769 genotypes (C/C, C/T, T/T) as well as C and T alleles and odds ratio in patients with nonsmall cell lung cancer compared with the control group

Genotype	Patients, n = 71		Controls, n = 104		OR (CI 95%)	*P*[a]
	number	frequency	number	frequency		
CC	11	0.15	43	0.41	0.26 (0.05–0.72)	0.043
CT	24	0.34	35	0.33	1.01 (0.53–1.91)	0.86
TT	36	0.51	26	0.25	3.1 (1.6–5.9)	0.02
	$\chi^2 = 3.70$		$\chi^2 = 2.21$			
allele C	46	0.32	121	0.58	0.34 (0.22–0.54)	0.28
allele T	96	0.68	87	0.42	2.9 (1.86–4.54)	0.28

a χ^2 test <3,991; $P > 0.05$

Abbreviations: see TABLE 2

TABLE 4 Expression levels of the *STAT3* gene (RQ values) in individual histopathological subtypes of nonsmall cell lung cancer calculated by the delta-delta computed tomography method

Histopathological NSCLC subtype	Mean RQ value (range)	Number (percentage) of samples with	
		RQ value >1	RQ value <1
SCC (n = 41)	7.34 (0.01–137.98)	29 (71%)	12 (29%)
AC (n = 23)	2.75 (0.38–20.31)	17 (74%)	6 (36%)
LCC (n = 7)	1.73 (0.11–5.90)	5 (72%)	2 (28%)
total (n = 71)	3.94 (0.01–137.98)	51 (72%)	20 (28%)

Abbreviations: RQ – relative quantification, others – see TABLE 1

($P < 0.05$) decrease in the frequency of TT genotype (OR 3.1, $P = 0.02$) in NSCLC.

No association was found between the studied polymorphisms and NSCLC histopathological subtype, tumor clinical staging (pTNM classification) as well as patients' age, sex, and smoking history. A haplotype analysis showed no significant difference between the study and control groups.

The *STAT3* rs3816769 polymorphism is in a strong linkage disequilibrium (D' = 0. 923; $r^2 = 0.61$) with the neighboring rs744166 polymorphism in the studied Polish population.

Relative *STAT3* expression analysis The RQ values were compared between NSCLC patients with regard to histopathological NSCLC subtype, tumor staging, patients' age, sex, and smoking history, as well as to *STAT3* polymorphism. In each histopathological NSCLC subtype, the increased expression (RQ value >1) was observed for more than 70% of the cases. The highest expression level of *STAT3* in NSCLC was observed for the SCC subtype (mean RQ, 7.34) and the lowest in LCC (mean RQ, 1.73).The results of the relative quantification analysis in individual histopathological NSCLC subtypes, indicating mean RQ values, as well as the number of samples with increased (RQ >1) and decreased (RQ <1) *STAT3* expression are shown in TABLE 4.

A statistical analysis revealed the differences in RQ values between all studied histopathological

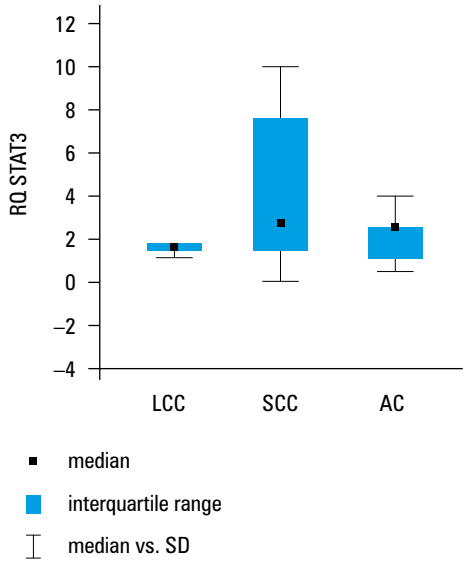

FIGURE 1 Box-and-whisker plots, representing the expression (median RQ levels) of the STAT3 gene in individual histopathological subtypes ($P = 0.0152$; Kruskal–Wallis test) Abbreviations: see TABLES 1 and 3

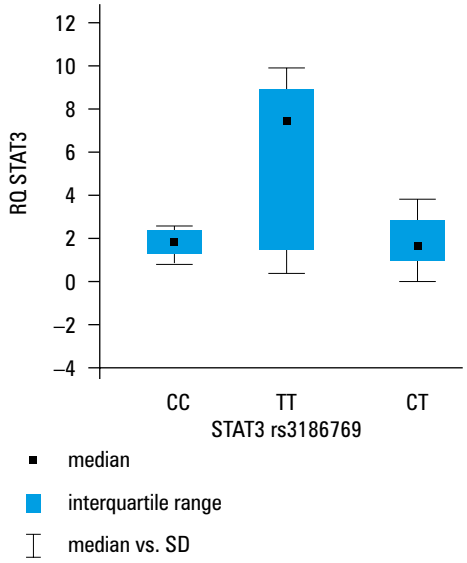

FIGURE 2 Box-and-whisker plots representing correlation between STAT3 expression and rs3816769 SNP ($P = 0.0464$; Kruskal–Wallis test)

subtypes ($P = 0.0152$; Kruskal–Wallis test), as presented in FIGURE 1. A significant difference was observed between squamous cell carcinoma (SCC) and large cell carcinoma (LCC) (Neuman–Keuls multiple comparison test).

There was no significant difference in RQ values and the clinical features of the studied NSCLC patients, i.e., patients' age, sex, and history of smoking (assessed as pack years) ($P > 0.05$; Kruskal–Wallis, Mann–Whitney's test, and Spearman's rank correlation coefficient).

The significant correlation between RQ values and *STAT3* rs3 816 769 SNP was observed for TT genotype ($P = 0.046$; Kruskal–Wallis test, Neuman–Keuls multiple comparison test), as presented in FIGURE 2. The analysis did not reveal any significant association between the expression level of *STAT3* and occurrence of rs744 166 SNP ($P = 0.132$; Kruskal–Wallis test).

DISCUSSION Numerous data have shown that oncogenic *STAT3* and its polymorphic variants are implicated in the development of various types of human cancer, including lung cancer.[22,26,31,32] It has been suggested that some inherited *STAT3* polymorphisms may correlate with *STAT3* expression and may be implicated via constitutive *SOCS3* expression in cancer progression as well as survival of cancer cells.[33,34] In the present study, we evaluated the association between 2 SNPs (rs744 166 and rs3 816 769) in the *STAT3* gene and the risk of NSCLC development in Polish patients and additionally assessed the effect of the studied SNPs on *STAT3* expression. According to our knowledge, it is the first study focused on European NSCLC patients.

Our study confirmed significant roles of both studied SNPs (rs744 166 and rs3 816 769) in the susceptibility to lung cancer development. The obtained results indicate that the presence of TT genotype (rs3 816 769) and GG genotype (rs744 166) might increase the risk of NSCLC development, while CC genotype (rs3816769) and AA genotype (rs744 166) might decrease the risk of lung cancer. In the Chinese population study, where several SNPs (e.g., rs4796793, rs7211777, rs12949 918, rs4796793, rs7211777, rs12949918, rs9912773, and rs3869550) were investigated, the association between SNP rs744166 and the risk of NSCLC development was also shown,[26] which is in line with our data. However, contrary to our results, the study performed by Jiang et al.[26] showed that carriers of the minor G allele (rs744 166) and GG genotype had a decreased risk of NSCLC, while the presence of A allele and AA genotype seemed to have a protective effect. Similarly, based on the study regarding Crohn's disease, a significantly decreased risk of this syndrome was observed in individuals carrying G allele in SNP rs744 166 of *STAT3*.[30]

The opposite results regarding the frequency of polymorphic variants of the *STAT3* gene (rs744166) and their association with the disease may suggest the population discrepancies. Additionally, these differences may suggest different types of inflammatory mechanisms, which are implicated in the induction of various types of tumors and diseases with chronic, nonspecific inflammation.[35] It is known that the enhanced production of pleiotropic interleukin 6 and tumor necrosis factor leads to inflammation and activation of the oncogenic transcription factor STAT3,[36,37] which plays a role in the immune system regulation. Moreover, the STAT3/IL-6 signaling pathway as a major mediator of the inflammatory response and host defense is involved in tumor cell survival owing to the progression of the neoplastic growth. Activated via phosphorylation, STAT3 upregulates tumor angiogenesis, metastasis, and invasion and inhibits tumor cell apoptosis.[37] In the light of this observation, *STAT3* polymorphic variants and their expression associated with high IL-6 levels seem to be important in chronic inflammatory disease as well as in inflammatory-related cancer, including lung cancer.

It should be stressed that the study by Ferguson et al.,[30] focused on Crohn's disease confirmed the significance of the *STAT3* gene SNPs in its ethiopatology. It also provides indirect evidence that *STAT3* gene variants may be important in the development of inflammatory-related cancer (e.g., colorectal malignances).[30] In particular, a significantly decreased risk of Crohn's disease was observed in individuals carrying the C allele and the genotype CC in rs3816769 SNP of the *STAT3* gene.[30] Considering this result, in our study, we decided to investigate if there is any correlation between rs3816769 SNP and lung cancer. Our results concerning this polymorphism are similar to those obtained by Ferguson et al.[30] in Crohn's disease. Unfortunately, we are unable to compare our results with respect to lung cancer or other types of human cancers, because there are no publications available focused on this issue.

We also evaluated the association between the studied SNPs and histopathological features of tumors (pTNM staging) and clinical characteristics of the patients and found no correlations. It is in contrast to the study performed by Jiang et al.[26] in the Chinese population, who found that AG or GG genotypes (rs744166) may have a protective value, especially in stage III/IV of lung cancer.

In our study, we focused also on the correlation of *STAT3* SNPs with gene expression. Results of our analysis suggest an association of rs3816769 with STAT3 expression. It should be stressed that our observation did not unambiguously confirm the functional significance of the studied SNP. We did not recognize the presence of the differences in STAT3 expression levels in correlation with the studied SNP separately for normal tissue. However, our findings are of value given the lack of similar studies concerning assessment of *STAT3* variants and their correlation with the gene expression level and NSCLC risk.

We also observed high linkage disequilibrium between the two studied SNPs in Polish NSCLC patients. The expression analysis assessing *STAT3* mRNA level in lung cancer patients revealed an increased gene expression in the majority of the studied NSCLC samples. To the best of our knowledge, the overexpression of *STAT3* at mRNA level has been observed in 2 types of human cancers, such as colorectal cancer and cervical squamous cell carcinoma.[15,20] The only study focused on *STAT3* expression in lung cancer regarded SCLC and concerning protein level has been published.[38] The results obtained in the above study showed positive correlations of STAT3 protein expression with lymph node metastasis, clinical stage, and tumor size.[38,39]

Additionally, the association between the overexpression of STAT3, pSTAT3, and some tumor parameters, such as the size and necrosis in different human soft tissue cancers was observed.[40] In our present study, a significantly increased *STAT3* expression was revealed in SCC, which is the most frequent and heterogeneous subtype of NSCLC. Regarding patient characteristics, we did not find any association with the *STAT3* expression level. Because there have been no similar studies, concerning NSCLC, we cannot compare our results with those of other investigators. However, *STAT3* overexpression and its positive association with SCC observed in our study might have an important diagnostic value, aiming at an accurate subclassification between SCC and adenocarcinoma, especially in the context of the latest research on new diagnostic algorithm based on molecular lesions and improving personalized treatment of NSCLC.[41,42]

Concluding, the presented results confirmed the association between the chosen *STAT3* polymorphisms and susceptibility to NSCLC development. It should be emphasized that the most important result of our analysis was the correlation found between the increased gene expression level and TT genotype (rs3816769), which additionally revealed the association with NSCLC risk in our study group. On the other hand, CC genotype (rs3816769) (association with decreased gene expression) might decrease lung cancer risk. The association between an increased *STAT3* expression and SCC histopathological subtype might draw attention to this gene as a potential diagnostic marker. It is worth noting that our study focus on the Polish population and may confirm universally accepted hypothesis concerning a significant role of *STAT3* gene as well as JAK3/STAT3 pathway in susceptibility to NSCLC development. It should be stressed that correlation between increased *STAT3* gene expression and TT genotype (rs3816769) of the *STAT3* gene in our patients may be useful in future diagnosis of NSCLC risk in the Polish population. However, further studies are needed on a larger group to draw firm conclusions.

Acknowledgments The study was funded by a grant from the Ministry of Science and Higher Education (grant no. 502/03-1-151/04/502-14-069).

REFERENCES

1 Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics. CA Cancer J Clin. 2002;: 74-108. http://globocan.iarc.fr/factsheet.asp. Accessed 2009

2 Greenlee RT, Murray T, Bolden S, et al. Cancer statistics. CA Cancer J Clin. 2000; 50: 7-33.

3 Wojciechowska U, Didkowska J, Zatoński W, et al. Prediction of cancer incidence and mortality in Poland up to the year 2025. Cancerregistration. 2009; 48: 35-39.

5 Alberg AJ, Samet JM. Epidemiology of lung cancer. Chest. 2003; 123: 21-49.

6 Ettinger DS, Akerley W, Bepler G, et al.; NCCN Non-Small Cell Lung Cancer Panel Members. Non-Small Cell Lung Cancer. J Natl Compr Canc Netw. 2010; 8: 740-801.

7 Deslauriers J, Tronc F, Grégoire J. History and current status of bronchoplastic surgery for lung cancer. Gen Thorac Cardiovasc Surg. 2009; 57: 3-9.

8 Devereux TR, Taylor JA, Barrett JC. Molecular mechanisms of lung cancer. Interaction of environmental and genetic factors. Giles F. Filley Lecture. Chest. 1996; 109: 14S-19S.

9 Sekido Y, Fong KM, Minna JD. Progress in understanding the molecular pathogenesis of human lung cancer. Biochim Biophys Acta. 1998; 19: 21-59.

10 Reissmann PT, Koga H, Figlin RA, et al. Amplification and overexpression of the cyclin D1 and epidermal growth factor receptor genes in non-small-cell lung cancer. Lung Cancer Study Group. J Cancer Res Clin Oncol. 1999; 125: 61-70.

11 Risch A, Plass C. Lung cancer epigenetics and genetics. Int J Cancer. 2008; 123,1-7.

12 Niu G, Wright KL, Huang M, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene. 2002; 21: 2000-2008.

13 Frank DA. STAT3 as a central mediator of neoplastic cellular transformation. Cancer Lett. 2007; 251: 199-210.

14 Chen SH, Murphy DA, Lassoued W, et al. Activated STAT3 is a mediator and biomarker of VEGF endothelial activation. Cancer BiolTher. 2008; 7: 1994-2003.

15 Takemoto S, Ushijima K, Kawano K, et al. Expression of activated signal transducer and activator of transcription-3 predicts poor prognosis in cervical squamous-cell carcinoma. Br J Cancer. 2009; 101: 967-972.

16 Lee CK, Raz R, Gimeno R, et al. STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. Immunity. 2002; 17: 63-72.

17 Coppo P, Gouilleux-Gruart V, Huang Y, et al. STAT3 transcription factor is constitutively activated and is oncogenic in nasal-type NK/T-cell lymphoma. Leukemia. 2009; 23: 1667-1678.

18 Liu X, He Z, Li CH, et al. Correlation Analysis of JAK-STAT Pathway Components on Prognosis of Patients with Prostate Cancer. Pathol Oncol. 2011. [Epub ahead of print].

19 Ryu K, Choy E, Yang C, et al. Activation of signal transducer and activator of transcription 3 (Stat3) pathway in osteosarcoma cells and overexpression of phosphorylated-Stat3 correlates with poor prognosis. J Orthop Res. 2010; 28: 971-978.

20 Morikawa T, Baba Y, Yamauchi M, et al. STAT3 expression, molecular features, inflammation patterns, and prognosis in a database of 724 colorectal cancers. Clin Cancer Res. 2011; 17: 1452-1462.

21 Slattery ML, Lundgreen A, Kadlubar SA, et al. JAK/STAT/SOCS-signaling pathway and colon and rectal cancer. Mol Carcinog. 2011; 10: 21 841.

22 Vaclavicek A, Bermejo JL, Schmutzler RK, et al. Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer. Endocr Relat Cancer. 2007; 14: 267-277.

23 Wang K, Zhou B, Zhang J, et al. Association of signal transducer and activator of transcription 3 gene polymorphisms with cervical cancer in Chinese women. DNA Cell Biol. 2011; 30: 931-936.

24 Yin ZJ, Jin FG, Liu TG, et al. Overexpression of STAT3 potentiates growth, survival, and radioresistance of non-small-cell lung cancer (NSCLC) cells. J Surg Res. 2011; 171: 675-683.

25 Zhong Y, Feng J, Chen B, et al. Signal transducer and activator of transcription 3 (STAT3) gene polymorphisms are associated with treatment outcomes in acute myeloid leukemia. Int J Lab Hematol. 2012; 34: 383-389.

26 Jiang B, Zhu ZZ, Liu F, et al. STAT3 gene polymorphisms and susceptibility to non small cell lung cancer. Genet Mol Res. 2011; 10: 1856-1865.

27 Sobin LH, Gospodarowicz MK, Wittekind C, et al. Lung and pleural tumours TNM classification of malignant tumours. 7th ed Oxford, England: Wiley-Blackwell. International Union Against Cancer. 2009; 20: 138-146.

28 Edge SB, Byrd DR, Compton CC, et al. AJCC cancer staging manual (7th ed). New York, NY: Springer. 2010; 23: 253-270.

29 http://www.cancer.gov/dictionary?CdrID=306 510

30 Ferguson LR, Han DY, Fraser AG, et al. Genetic factors in chronic inflammation: single nucleotide polymorphisms in the STAT-JAK pathway, susceptibility to DNA damage and Crohn's disease in a New Zealand population. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2010; 690: 108-115.

31 Ito N, Eto M, Nakamura E. STAT3 polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma. J Clin Oncol. 2007; 25: 2785-2791.

32 Kwon EM, Salinas CA, Kolb S. Genetic polymorphisms in inflammation pathway genes and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2011; 20: 923-933.

33 Brender C, Lovato P, Sommer VH, et al. Constitutive SOCS-3 expression protects Tcell lymphoma against growth inhibition by IFNalpha. Leukemia. 2005; 19: 209-213.

34 Brender C, Nielsen M, Kaltoft K, et al. STAT3-mediated constitutive expression of SOCS-3 in cutaneous T-cell lymphoma. Blood. 2001; 97: 1056-1062.

35 Phillips CM, Goumidi L, Bertrais S, et al. Dietary saturated fat modulates the association between STAT3 polymorphisms and abdominal obesity in adults. JNutr. 2009; 139: 2011-2017.

36 Ramos EJ, Xu Y, Romanova I, et al. Is obesity an inflammatory disease? Surgery. 2003; 134: 329-335.

37 Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. EurJCancer. 2005; 41: 2502-2512.

38 Zhao X, Sun X, Li XL. Expression and Clinical Significance of STAT3, P-STAT3, and VEGF-C in Small Cell Lung Cancer. Asian Pac J Cancer Prev. 2012; 13: 2873-2877.

39 Wang M, Chen GY, Song HT. Significance of CXCR4, phosphorylated STAT3 and VEGF-A expression in resected non-small cell lung cancer. Experimental Therapeutic Med. 2011; 2: 517-522.

40 David D, Rajappan LM, Balachandran K. Prognostic significance of STAT3 and phosphorylated STAT3 in human soft tissue tumors – a clinicopathological analysis. JExpClin Cancer Res. 2011; 16: 30-56.

41 Rossi G, Pelosi G, Graziano P. A reevaluation of the clinical significance of histological subtyping of non-small-cell lung carcinoma: diagnostic algorithms in the era of personalized treatments. Int J Surg Pathol. 2009; 17: 206-218.

42 Wichert P. Absorption of higher-molecular-weight substances via the lung. Pol Arch Med Wewn. 2012; 122 (Suppl 1): 78-83.

Polimorfizm genu *STAT3* rs3 816 769 wiąże się z poziomem jego ekspresji i może sprzyjać rozwojowi niedrobnokomórkowego raka płuca – badanie wstępne

Daria Domańska[1], Adam Antczak[2], Dorota Pastuszak-Lewandoska[1], Paweł Górski[3], Jacek Kordiak[4], Karolina Czarnecka[1], Monika Migdalska-Sęk[1], Ewa Nawrot[1], Justyna Kiszałkiewcz[1], Ewa Brzeziańska[1]

1 Zakład Molekularnych Podstaw Medycyny, Uniwersytet Medyczny w Łodzi, Łódź
2 Klinika Pulmonologii Ogólnej i Onkologicznej, Uniwersytet Medyczny w Łodzi, Łódź
3 Klinika Pneumonologii i Alergologii, I Katedra Chorób Wewnętrznych, Uniwersytet Medyczny w Łodzi, Łódź
4 Klinika Chirurgii Klatki Piersiowej, Chirurgii Ogólnej i Onkologicznej, Uniwersytecki Szpital Kliniczny im. Wojskowej Akademii Medycznej, Uniwersytet Medyczny w Łodzi, Łódź

SŁOWA KLUCZOWE

niedrobnokomórkowy rak płuca, *STAT3*, ekspresja genu, polimorfizmy pojedynczego nukleotydu

STRESZCZENIE

WPROWADZENIE Gen *STAT3* pełni funkcję zarówno onkogenu, jak i regulatora odporności. Pomimo jego istotnej roli w rozwoju raka i regulacji komórek układu immunologicznego, nieliczne są badania polimorfizmów pojedynczego nukleotydu (*single nucleotide polymorphysm* – SNPs) genu *STAT3* i związanego z tym ryzykiem wystąpienia raka płuca.

CELE W tym badaniu oceniano związek między SNP (rs744166 [AG] i rs3816769 [CT]) a predyspozycją do rozwoju NSCLC oraz ich potencjalny wpływ na ekspresję *STAT3*.

PACJENCI I METODY DNA i RNA zostało wyizolowane od pacjentów z rozpoznanym NSCLC (n = 71) oraz od osób bez rozpoznania nowotworu, zakwalifikowanych do grupy kontrolnej (n = 104). Do genotypowania SNP genu *STAT3* oraz do badania ekspresji użyto sond TaqMan®.

WYNIKI *STAT3* CC (rs3816769) oraz AA (rs744166) genu *STAT3* były związane z mniejszym ryzykiem wystąpienia nowotworu płuca, podczas gdy genotypy TT (rs3816769) i GG (rs744166) mogą je znacznie zwiększać. Analiza ilorazu szans (*odds ratio*) wykazała, że allel A może zmniejszać ryzyko NSCLC, natomiast allel G może to ryzyko zwiększać. Wykazano, że nadmierna ekspresja *STAT3* dodatnio koreluje z genotypem TT (rs3816769) u chorych na NSCLC (p = 0,0464). Ponadto zaobserwowano różnice w ekspresji genu *STAT3* między rakiem płaskonabłonkowym a rakiem wielkokomórkowym.

WNIOSKI Wykazano, że polimorfizm rs3816769 genu *STAT3* oraz różnice w poziomie jego ekspresji są związane z wrażliwością na NSCLC i mogą mieć duże znaczenie funkcjonalne oraz istotną wartość diagnostyczną.

Autor do korespondencji:
dr hab. med. Ewa Brzeziańska, Zakład Molekularnych Podstaw Medycyny, Uniwersytet Medyczny w Łodzi, ul. Pomorska 251, 92-213 Łódź, tel.: 42-675-77-15, fax: 42-675-77-12, e-mail: ewa.brzezianska@umed.lodz.pl

Praca wpłynęła: 20.08.2013.
Przyjęta do druku: 03.12.2013.
Publikacja *online*: 05.12.2013.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2013; 123 (12): 672-679