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Original Article

Expression profile and prognostic values of STAT family members in non-small cell lung cancer

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Abstract: Non-small cell lung cancer (NSCLC) is a highly malignant type of cancer with a poor 5-year survival rate. The development of prognostic biomarkers and novel drug targets are required in order to improve the survival for NSCLC patients. Signal transducer and activator of transcription (STAT) proteins are cytoplasmic transcription factors known to play key roles in many cellular biological processes. However, the roles of STAT family members in the development and progression of NSCLC have not yet been apparently determined. Our study investigated the roles of STATs in the prognosis of NSCLC using cBioPortal, Human Protein Atlas, ONCOMINE, and Kaplan-Meier Plotter databases. High mutation rate of STATs existed in both lung adenocarcinoma (ADE) patients and squamous cell carcinoma (SCC) patients. High mRNA expression of STAT2 was significantly associated with shorter overall survival (OS) in NSCLC patients, while increased STAT5 and STAT6 were associated with better OS in NSCLC patients. We further found that increased mRNA expressions of STAT2 and STAT3 predicted unfavorable overall survival (OS) while high mRNA expression of STAT5B and STAT6 related to favorable OS for lung ADE patients. However, no significant correlation was identified for lung SCC patients. In stratified survival analysis, high expression of STAT2 predicted poor prognosis in stage II NSLCC patients, surgical margins negative patients and female patients. Taken together, our results illustrated that STAT5B and STAT6 could be effective prognostic biomarkers for survivals of NSCLC patients. And STAT2 might be a promising therapeutic target for the treatment of NSCLC as well as ADE.

Keywords: STAT, non-small cell lung cancer, online database, prognosis, therapeutic target

Introduction

Lung cancer ranks first among the causes of cancer deaths in men and women worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for 80-85% of all cases of lung cancer and classifies into two distinct histological subtypes: squamous cell carcinoma (SCC) and the more common adenocarcinoma (ADE) [2]. Despite advances in the early detection and therapeutic strategies, median overall survival of advanced NSCLC was marginally improved and the overall 5-year survival rate of NSCLC remains low [3]. Approximately 25% of NSCLC patients are first diagnosed with stage III NSCLC and most of these patients have unresectable disease [4]. Even in patients with early-stage and resectable disease receiving definitive chemoradiation, up to 90% of patients eventually relapse [3]. NSCLC remains a challenge to cure, thus investigations and attempts at identifying novel molecular target therapy to improve patients' outcomes still continue.

The Signal transducer and activator of transcription (STAT) family members mediate the effects of cytokines, growth factors, several hormones and various pathogens. STAT family comprises seven sub-members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6), and each family member possesses unique functions in signal transduction [5]. Inactive STATs are located in the cytosol under basal conditions [6]. Once activated, STAT proteins form dimers and/or tetramers, and STAT pathway is connected upstream with Janus kinases (JAK)

family protein. They transmit signals from the cell surface to the nucleus where they bind to specific promoter regions of DNA to regulate the transcription of target genes [7, 8]. Their variety of functions include proliferation, angiogenesis, inflammation, immunity and survival [9-11].

Numerous studies have reported that overexpression of STAT proteins enhances carcinogenesis and affect the prognosis of cancer patients [12]. STAT family members notably STAT3 and STAT5 play important roles in tumor progression whereas STAT1 is conversely involved in suppressing tumor growth in certain instances [13]. The functions of STAT signaling on tumor initiation and progression make the STAT proteins favorite targets for drug development and cancer therapy. STAT proteins are critically involved in tumorigenesis in various cancers, including lung cancer [14]. Previous studies have found that disruption of STAT3 signaling promotes KRAS-induced lung tumorigenesis [15] and downregulation of JAK/STAT3/SOCS3 signaling pathway by MiR-410 induced the apoptosis of lung cancer A549 cells [16]. A Recent study revealed that JAK/STAT inhibitor ruxolitinib, in combination with vesicular stomatitis virus, would enhance oncolytic virotherapy for NSCLC cancer through preventing PDL-1 expression of tumor cells in response to virotherapy [17]. These evidences suggested that targeting STATs might enhance the effect of other therapy approaches and improve outcomes for NSCLC patients. Nevertheless, the prognostic value of each STAT family member in NSCLC is not well established.

In the present study, we addressed this problem for the first time by analyzing the expression and mutations of different STAT members, and the correlation between STAT members' mRNA expressions and OS of NSCLC patients. We further evaluate the clinical data including clinical stages, pathological grade, smoking history, gender, surgical margins status, and therapy approaches to systemically explore the roles of STATs. The comprehensive study of distinct STAT members in NSCLC will help to provide perspectives on new biomarkers for predicting the prognosis of NSCLC, and highlight the noteworthy therapeutic targets for NSCLC treatment.

Materials and methods

Oncomine

Oncomine database (www.oncomine.org) is an integrated online cancer microarray database and web-based data mining platform by which the transcriptome data of most cancers and respective normal tissues could be compared [18]. Transcriptional expression of STAT members between NSCLC tissues and normal tissues were observed by Oncomine. Cut-off of *p* value and fold change were as following: *p* value: 0.05, fold change: 1.5, gene rank: 10%, data type: mRNA. The 10th, 25th, 50th, 75th and 90th percentile data of each STAT member in both cancer and normal tissues were plotted.

cBioPortal

cBioPortal (www.cbioportal.org) is an open-access website resource for exploring and visualizing multidimensional cancer genomics dataset [19]. We selected Lung Adenocarcinoma (TCGA, Provisional) dataset and Lung Squamous Cell Carcinoma (TCGA, provisional) dataset to analyze genetic alterations of STAT family genes in NSCLC. The genomic profiles included mutations, putative copy-number alterations, and mRNA expressions (RNA Seq V2 RSEM with z-scores = ±2).

Human Protein Atlas

The Human Protein Atlas (HPA, http://www.proteinatlas.org) is a database that contains transcriptomes, proteomes and immunohistochemistry-based expression data. This database consists of Tissue Atlas, Cell Atlas and Pathology Atlas [20]. We got the Fragments Per Kilobase of exon per Million fragments mapped (FPKM) values and protein expression patterns of each STAT gene in NSCLC patients from HPA database.

Kaplan-Meier plotter

The Kaplan-Meier Plotter (www.kmplot.com) is an online database comprises gene expression information and clinical outcome parameters of liver cancer, breast cancer, ovarian cancer, lung cancer and gastric cancer [21]. The prognostic value of mRNA expression of distinct STAT

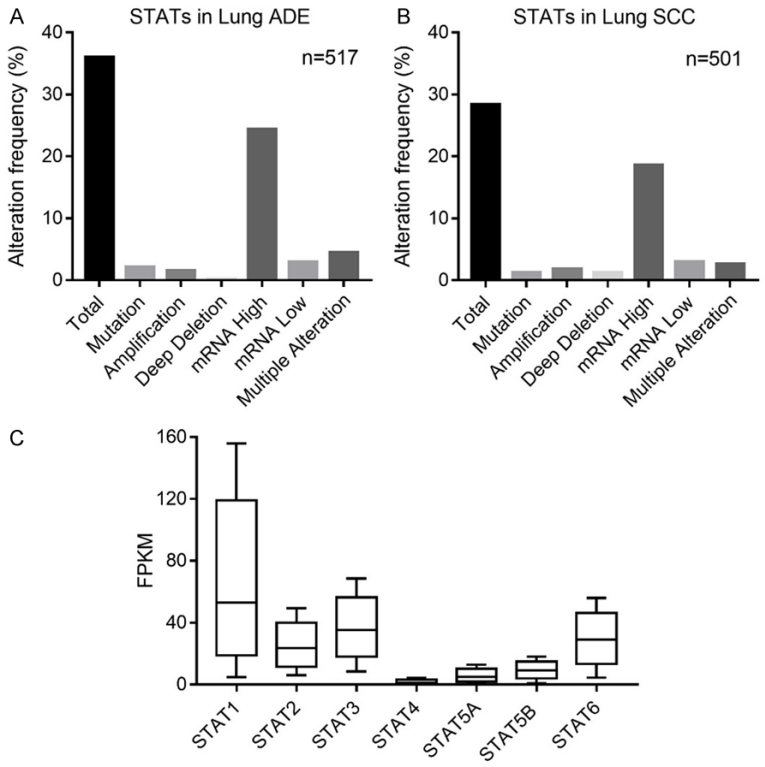

Figure 1. Genetic alterations of STAT family members in NSCLC. (A, B) Alteration frequency of STATs in lung ADE (A) and SCC (B) via cBioPortal. (C) The FPKM value of each STAT member in NSCLC via HPA.

members in Lung cancer was analyzed using Kaplan-Meier plotter. In Kaplan-Meier plotter, patients with lung cancer were divided into two groups by median expression (high vs. low expression) and validated by Kaplan-Meier survival curves. The Affymetrix ID of each STAT gene was valid (Supplementary Table 1). In this study, "array quality control" was selected "exclude biased arrays".

Results

Genetic mutations and mRNA expression of STAT family members in NSCLC patients

Firstly, we analyzed the genetic alteration of individual STAT members in Lung cancers via cBioPortal database. As shown in **Figure 1A** and **1B**, high mutation rates of STATs were observed in both lung ADE patients (36%) and lung SCC patients (28%). Among all types of mutations, mRNA deregulation was the most common alteration. We also compared the mRNA level of different STAT family members in NSCLC through HPA database. The STAT1 mRNA level was the relative highest in all the genes, whereas STAT4 expressions was very low (**Figure 1C**). Given that STAT4 was mainly involved in autoimmune and inflammatory disorders through modulating type 1 T helper cells differentiation [22], we assessed the STAT genes except STAT4 in the following analysis.

Next, mRNA expressions of seven STAT family members in lung cancer were measured and compared to normal tissues by ONCOMINE database. Among all the STAT members, only higher mRNA expressions of STAT1 were found in lung cancer tissues in multiple datasets (Supplementary Table 2). In Okayama Lung dataset [23], overexpression of STAT1/2/3 was found in lung ADE tissues compared with normal tissues, while STAT5A expression in lung ADE tissues was lower and STAT5B/6 exhibited similar expression level (**Figure 2**). In Hou Lung database [24], mRNA expressions of STAT1/2/5B were found to be significantly up-regulated in lung SCC tissues compared to normal samples, whereas STAT3/5A/6 expression levels in lung SCC tissues were lower (**Figure 3**). We also assessed mRNA expression of individual STAT members in other datasets including Selamat [25], Landi [26] and Talbot [27]. STAT genes expression in normal lung tissues and different subtypes of NSCLC tissues were summarized in **Table 1**. The mRNA levels of STAT1/2 in lung ADE tissues were higher than in normal tissues in Selamat and Landi Lung databases. STAT1/3/6 in lung SCC tissues showed increased expression levels in Talbot database. The inconsistent conclusions from these different datasets most probably due to sample size, study design and detection methods. It is notable that STAT1/2 expressions were markedly higher in these two types of NSCLC compared with that in normal controls.

We further explored the protein expression patterns of STATs in NSCLC by the HPA database

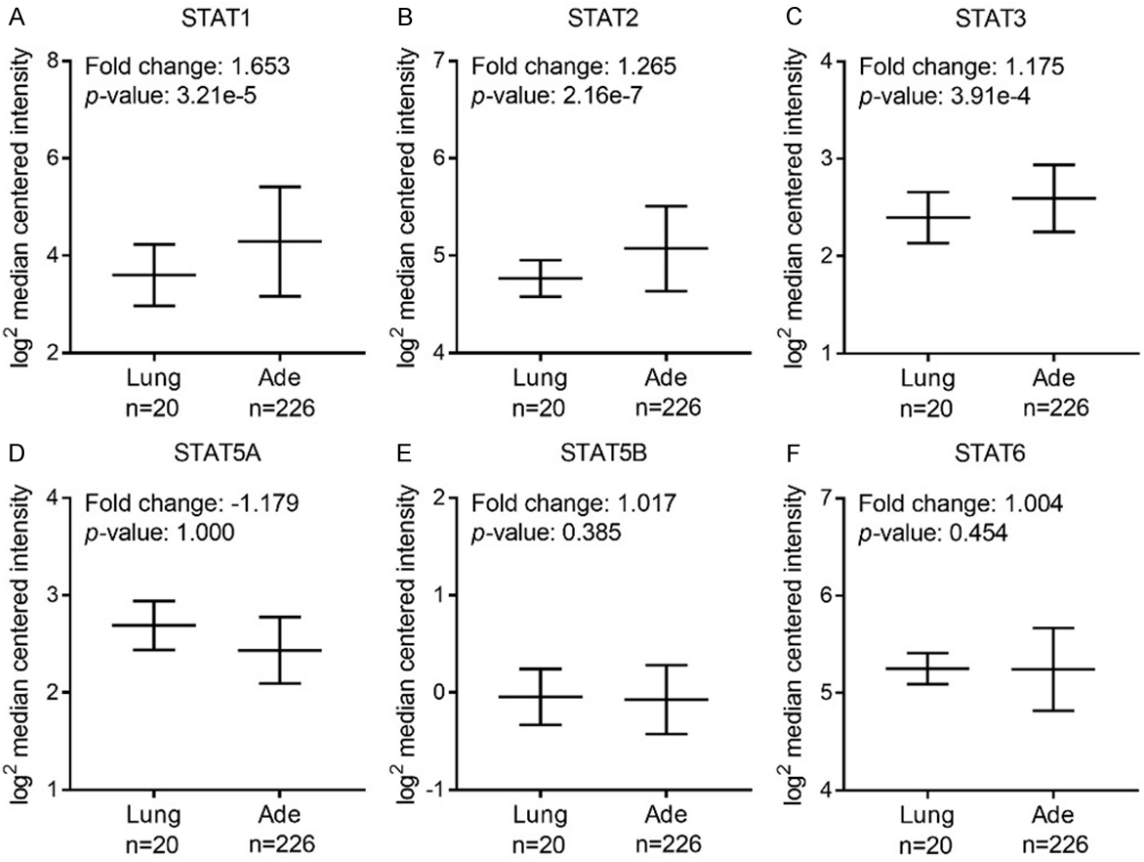

Figure 2. mRNA expression of distinct STAT members in lung ADE and normal tissues using Oncomine (threshold: *p*-value = 0.05, 1.5 fold change, top 10% gene rank).

and summaried in Supplementary Table 3. However, these data could not well explain the protein expression of STATs in NSCLC due to the small sample size.

Prognostic values of STAT family members in NSCLC patients

The prognostic values of STAT mRNA expression were evaluated in the Kaplan-Meier Plotter database and the OS curves were plotted for NSCLC patients. As shown in **Figure 4**, mRNA expressions of most STAT family members were significantly associated with NSCLC patients' prognosis. Increase in STAT2 mRNA expression level (HR = 1.19, 95% CI: 1.05-1.36, *P* = 0.0057) was significantly associated with shorter OS for all NSCLC patients, while higher mRNA expression of STAT5A/5B/6 significantly related to favorable OS (STAT5A, HR = 0.87, 95% CI: 0.77-0.99, *P* = 0.033; STAT5B, HR = 0.75, 95% CI: 0.66-0.86, *P* = 1.4e-05; STAT6, HR = 0.69, 95% CI: 0.6-0.78, *P* = 7.7e-09). However, mRNA expression of STAT1/3 showed no correlation with prognosis of NSCLC patients (STAT1, HR = 0.95, 95% CI: 0.84-1.07, *P* = 0.4; STAT3, HR = 0.97, 95% CI: 0.86-1.1, *P* = 0.64). These results revealed that mRNA expressions of STAT2/5A/5B/6 were significantly associated with NSCLC patients' prognosis and they might be exploited as useful biomarkers for predicting NSCLC patients' survival. Most notably, STAT2 might be a potential therapeutic target for NSCLC treatment.

We also analyzed the prognostic values of STAT genes in lung ADE patients and SCC patients. High expression of STAT2/3 mRNA were significantly associated with poor rates of OS in lung ADE patients (STAT2, HR = 1.6, 95% CI: 1.26-2.02, *P* = 8e-05; STAT3, HR = 1.33, 95% CI: 1.05-1.69, *P* = 0.017; **Figure 5B** and **5C**), while high STAT5B/6 mRNA expression showed correlation with favorable OS in ADE patients (**Figure 5E** and **5F**). In addition, STAT1/4/5A expression exhibited no correlation with OS for ADE

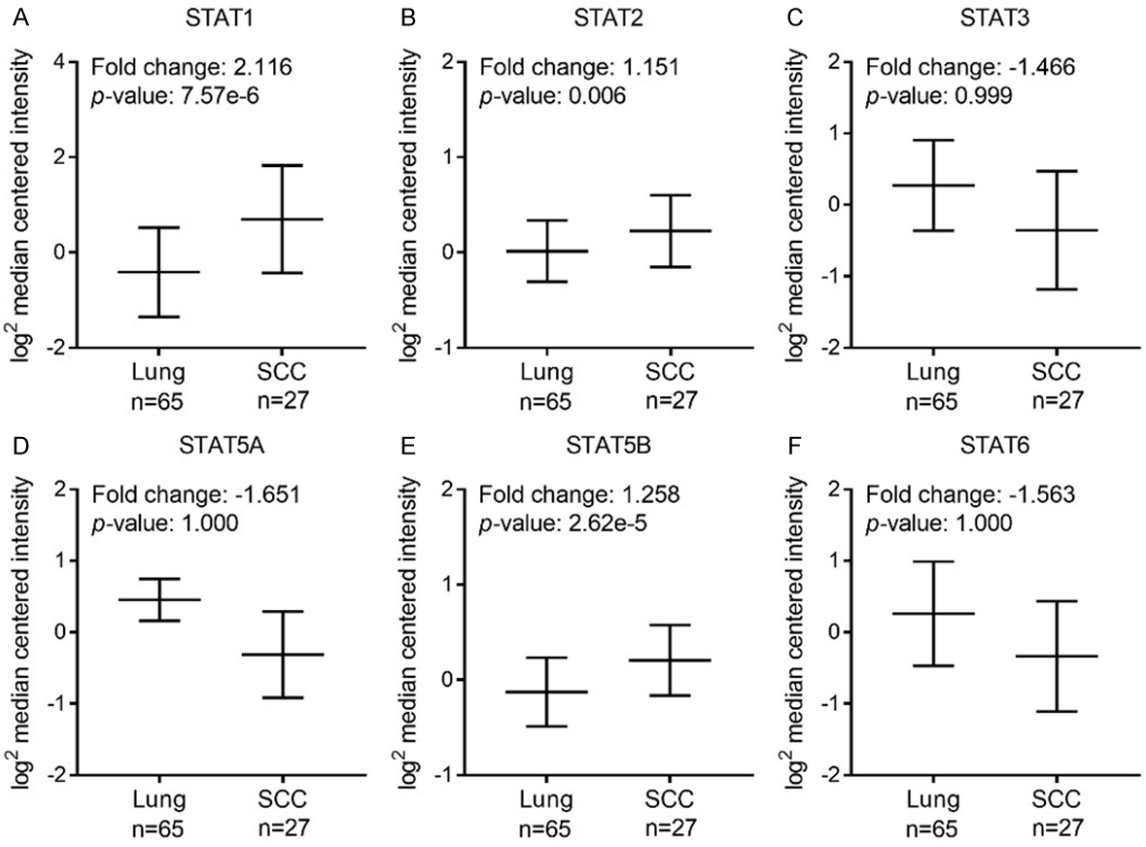

Figure 3. mRNA expression of distinct STAT members in lung SCC and normal tissues using Oncomine (threshold: *p*-value = 0.05, 1.5 fold change, top 10% gene rank).

patients (**Figure 5**). As for lung SCC patients, there was no significant association between all the STAT members and OS rates (**Figure 6**). These results implied that mRNA expression of STATs could significantly affect lung ADE patients' prognosis.

Prognostic values of STATs in NSCLC patients with different clinicopathological features

To assess whether the prognostic value of the mRNA expression status of individual STAT family members depend on other clinicopathological characteristics, we analyzed the influence of high vs. low STAT expression on OS for NSCLC patients with differenet clinicopathological characteristics including clinic stage, pathological grade, smoking history, gender, surgical margins status, different treatment approaches. As illustrated in **Table 2**, we found that overexpression of STAT2 was correlated with unfavorable OS in stage II NSCLC patients, and high STAT3 was associated with worse OS in stage I NSCLC patients. Increased expressions of STAT4/5B/6 were correlated with better OS in stage I NSCLC patients, and high STAT2 predicted better OS in stage III patients.

In **Table 3** we investigated the association between STATs expression and grades in NSCLC patients. No significant association with OS was identified for any of the STAT members in patients with different pathological grades. **Table 4** showed prognostic significance between STAT mRNA expression and smoking history in NSCLC patients. High mRNA expression of STAT2 was correlated with worse OS in patients with and without smoking habit. STAT3 overexpression also predicted unfavorable OS in no smoking patients. However, high STAT5B predicted worse OS in both smoking and no smoking patients. Overexpression of STAT6 was associated with favorable OS in smoking patients.

The results in **Table 5** revealed prognostic value between STAT mRNA expression and gender in NSCLC patients. STAT2 expression related to

Table 1. Elevated STAT family genes expression in NSCLC cancer

Gene	*p*-value	Fold Change	Dataset	Normal	Cancer	Total
Normal vs. ADE						
STAT1	1.09e-15	2.516	Selamat	58	58	116
	7.23e-8	1.756	Landi	49	58	107
	7.09e-9	1.453	Selamat	58	58	116
STAT2	0.013	1.069	Landi	49	58	107
	0.049	1.080	Selamat	58	58	116
STAT3	0.587	-1.010	Landi	49	58	107
	1.000	-1.415	Selamat	58	58	116
STAT5A	1.000	-1.258	Landi	49	58	107
	1000	-1.277	Selamat	58	58	116
STAT5B	0.572	-1.005	Landi	49	58	107
	0.998	-1.176	Selamat	58	58	116
STAT6	0.726	-1.127	Landi	49	58	107
Normal vs. SCC						
STAT1	2.63e-10	2.625	Talbot	2	34	36
STAT2	0.095	1.803	Talbot	2	34	36
STAT3	8.23e-5	1.382	Talbot	2	34	36
STAT5A	0.994	-1.112	Talbot	2	34	36
STAT5B	0.992	-1.140	Talbot	2	34	36
STAT6	1.20e-7	1.483	Talbot	2	34	36

Different subtypes of lung cancer were analyzed and *p*-values, fold changes, datasets and the number of clinical specimen were included.

nancy in human [13]. Despite some members of STAT have already been confirmed to play key roles in NSCLC, the prognostic value of each STAT member mRNA expression in NSCLC patients, to our knowledge, had not been clarified by any previous study thus far. Personalized treatment decisions based on the genetics of the individual tumor will be paramount to combat malignancies in the future. Therefore, understanding the prognostic values of STAT family members in NSCLC might show light in discovering new biomarkers and noteworthy therapeutic targets for NSCLC. The aim of our study was to indicate the influence of STATs expression status on the OS rate of NSCLC patients, and the association between STATs and the clinical features of NSCLC.

unfavorable OS for female NSCLC patients. Overexpression of STAT4/5A were significantly correlated with favorable OS in male patients. STAT5B/6 showed correlations with better OS in both female and male patients. In **Table 6** we analyzed the correlation between STATs expression and surgery margins status in NSCLC patients. Overexpression of STAT2/3 were correlated with worse OS in patients with negative surgery margins, while STAT5B was associated with better OS in patients with negative surgery margins. Lastly, **Tables 7** and **8** demonstrated the correlation of STATs mRNA expression with OS in NSCLC patients who received chemotherapy or radiotherapy. We found that high expression of STAT2 predicted favorable OS in patients receiving chemotherapy and increased STAT5A was associated with better OS in patients without radiotherapy.

Discussion

Being important components of signaling transduction, STAT family proteins are implicated in the development of multiple cancers, including NSCLC. However, not all STAT proteins participate in the initiation and progression of malig-

Previous studies demonstrated that activated STAT1 seemed to exhibit pro-apoptotic and anti-proliferative effect since STAT1-null mice had higher risk of tumor development than controls [28, 29]. Modified STAT1 that was hyperresponsive to interferons (IFN) improved the antitumor response of IFNs in lung cancer cells [30]. STAT1 is unlikely to promote tumor cell growth in human and has the tumor-suppressing properties like TP53. The role of STAT1 was puzzling for the reason that STAT1 acted as a tumor promotor in other researches [31, 32]. This probably attributes to cancer specific. Here, although STAT1 mRNA expression was significantly higher in NSCLC tissues than that in normal tissues, no correlation of STAT1 on OS rate of NSCLC patients was observed.

STAT2 is a well-known pivotal and specific effector of type I interferon (IFN-I) signaling which is important in tumor immunosurveillance [33, 34]. A deficiency in STAT2 impaired IFN-I mediated anti-tumorigenic effects in dendritic cells thus allowing tumors to thrive. It has already reported that the growth of melanoma and colon adenocarcinoma were accelerated in $STAT2^{-/-}$ mice [35]. Similarly, loss of STAT2 mark-

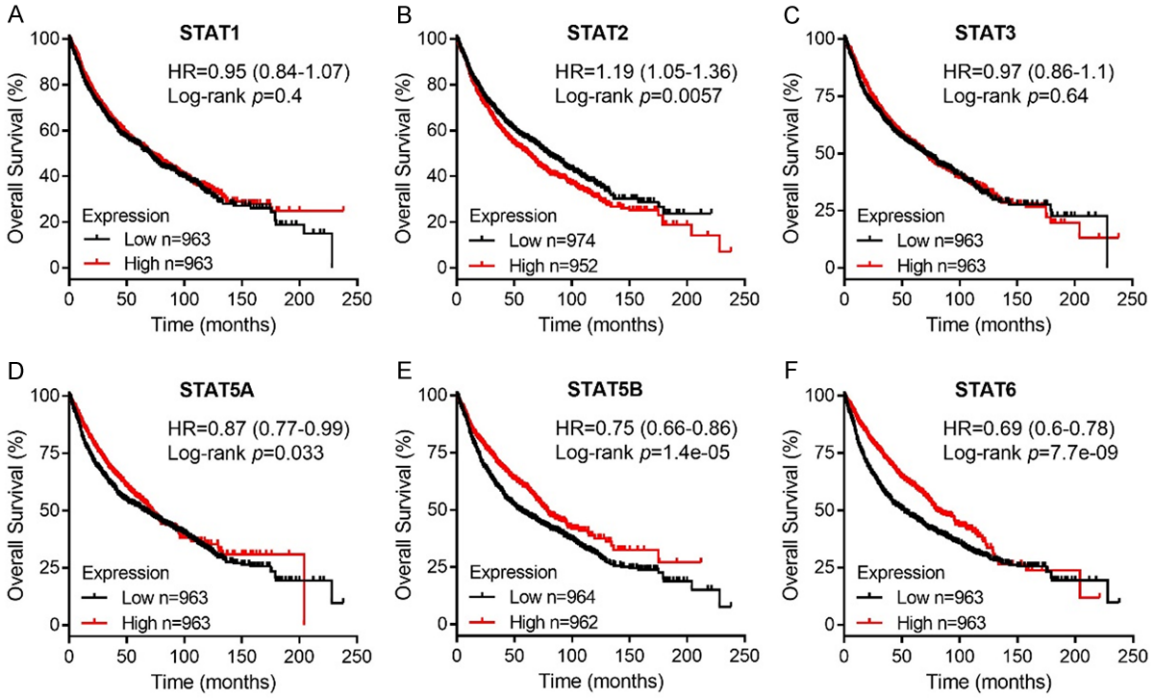

Figure 4. Prognostic value of STAT family members in NSCLC patients using Kaplan-Meier plotter.

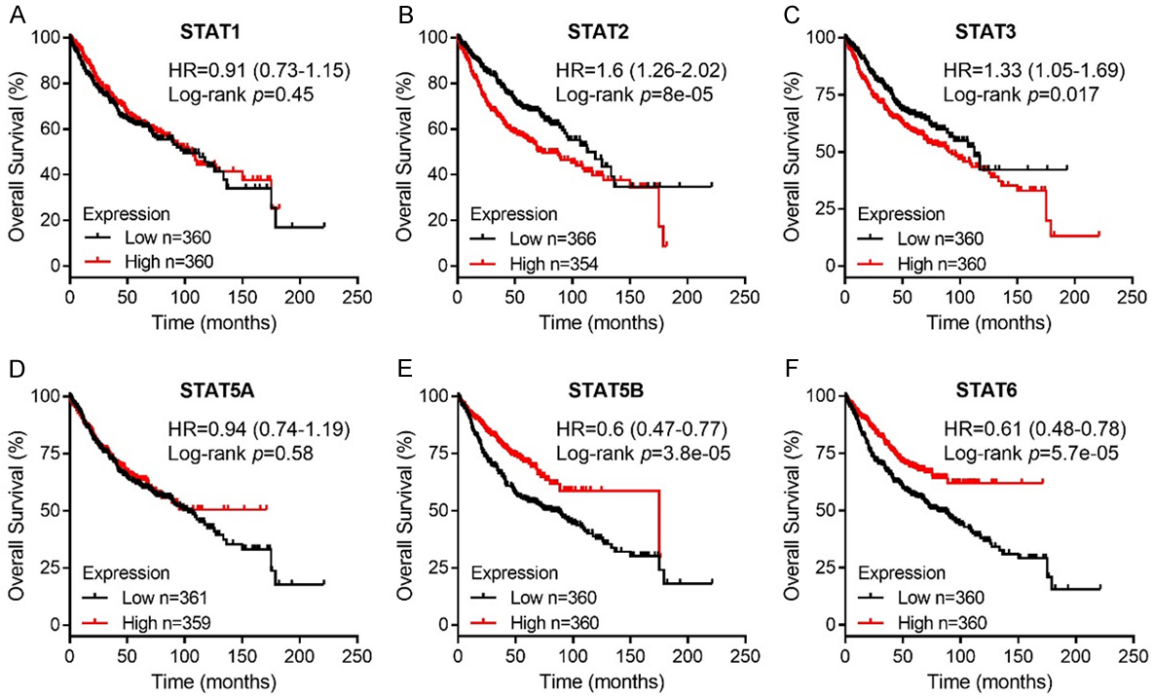

Figure 5. Prognostic value of mRNA expression of STAT family members in lung ADE patients using Kaplan-Meier plotter.

edly inhibited the ability of inflammatory breast cancer cells to proliferate, migrate, invade, and form 2-D colonies [36]. And STAT2 as an important molecular target has been confirmed in skin squamous cell carcinoma cells [37]. These were similar to discoveries made in our study, in which low mRNA expression of STAT2 was associated with better prognosis in NSCLC

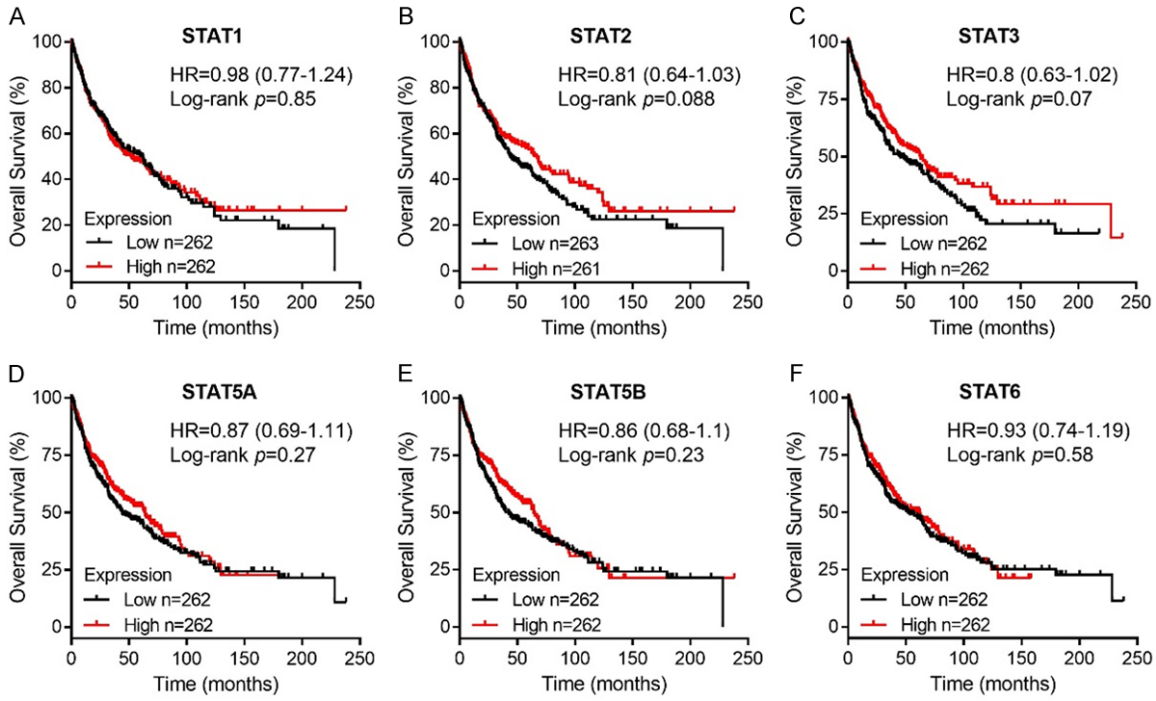

Figure 6. Prognostic value of mRNA expression of STAT family members in lung SCC patients using Kaplan-Meier plotter.

Table 2. Correlation of STAT family members with tumor stages of NSCLC patients

Gene	Stages	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	I	288	289	0.83 (0.63-1.09)	0.18
	II	122	122	0.75 (0.52-1.08)	0.12
	III	35	35	0.79 (0.46-1.36)	0.39
STAT2	I	293	284	1.25 (0.96-1.64)	0.1
	II	122	122	1.64 (1.14-2.37)	0.0078*
	III	35	35	0.55 (0.32-0.96)	0.033*
STAT3	I	288	289	1.38 (1.05-1.81)	0.02*
	II	122	122	1.45 (1-2.09)	0.049*
	III	35	35	1.57 (0.91-2.7)	0.1
STAT5A	I	288	289	0.76 (0.58-1)	0.052
	II	122	122	0.9 (0.63-1.3)	0.59
	III	35	35	0.93 (0.53-1.61)	0.79
STAT5B	I	288	289	0.45 (0.33-0.6)	6.5e-08**
	II	122	122	0.83 (0.57-1.2)	0.32
	III	35	35	0.67 (0.39-1.16)	0.15
STAT6	I	288	289	0.45 (0.33-0.6)	3.7e-08**
	II	122	122	0.89 (0.62-1.29)	0.55
	III	35	35	0.51 (0.29-0.89)	0.016*

Low/High (cases): low/high expression of the corresponding gene (patient number).
*$P < 0.05$, **$P < 0.01$.

patients, apart from SCC patients. In addition, STAT2 could regulate the expression of genes involved in the differentiation and recruitment of immune effector cells to the tumor site [35]. To date, little is known about the function of STAT2 in NSCLC. Our data suggested that STAT2 might be a crucial drug target for the treatment of lung ADE. However, the converse prognostic values of STAT2 in stage II and stage III NSCLC patients possibly due to specimen's specific and sample size. Importantly, existing therapeutic approaches plus STAT2 inhibition might effectively improve outcomes of lung ADE patients. Future mechanistic researches and clinical trials are needed to explore the clinical application of STAT2 in the treatment of NSCLC.

Among all the STAT family members, STAT3 has been the most researched and investigated member in human cancers. The aberrant activation

Table 3. Correlation of STAT family members with tumor grades of NSCLC patients

Gene	Grades	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	I	100	101	0.94 (0.66-1.35)	0.75
	II	155	155	0.93 (0.68-1.28)	0.67
	III	38	39	1.14 (0.59-2.2)	0.7
STAT2	I	101	100	1.18 (0.82-1.69)	0.37
	II	156	154	1 (0.73-1.37)	1
	III	38	39	0.78 (0.4-1.51)	0.46
STAT3	I	100	101	0.96 (0.67-1.37)	0.81
	II	155	155	0.95 (0.69-1.3)	0.76
	III	38	39	0.72 (0.37-1.39)	0.32
STAT5A	I	100	101	0.98 (0.69-1.4)	0.92
	II	155	155	0.86 (0.63-1.17)	0.33
	III	38	39	0.56 (0.29-1.08)	0.079
STAT5B	I	100	101	1.22 (0.85-1.75)	0.28
	II	155	155	0.91 (0.66-1.24)	0.55
	III	38	39	0.67 (0.35-1.29)	0.23
STAT6	I	100	101	0.94 (0.66-1.35)	0.76
	II	155	155	0.94 (0.69-1.29)	0.71
	III	38	39	0.67 (0.34-1.29)	0.23

Low/High (cases): low/high expression of the corresponding gene (patient number). $*P < 0.05$, $**P < 0.01$.

of STAT3 occurs in many solid tumor [38-42] and hematopoietic malignancies [43, 44]. STAT3 Activation was observed in nearly 50% of Lung cancers [45]. Previous studies found that STAT3 inhibitor regressed human breast and lung cancer xenografts [46], and direct inhibition of STAT3 even induced apoptosis in NSCLC cells with acquired Erlotinib resistance [47]. Moreover, STAT3 has been thought to play a tumor-promoting role in NSCLC and during acquired drug resistance [48, 49]. Targeting STAT3 is currently proposed as therapeutic intervention. A number of potent small-molecule inhibitors of STAT3 have already been published [46, 50-52]. In our study, STAT3 mRNA was overexpressed in lung ADE tissues compared with that in normal controls. Increased STAT3 was significantly associated with a poorer OS rate only in ADE patients but not in SCC patients. Taken together, STAT3 might be a therapeutic target for lung ADE not SCC. It should be noted that most STAT3 inhibitors reported to date have not undergone an *in vivo* efficacy, pharmacology or toxicity testing. There is the need to further assess the role of STAT3 in lung ADE cancer and identify suitable anti-STAT3 agents for development into clinically useful anti-ADE therapeutics.

STAT5A and STAT5B, two isoforms of STAT5, are transcribed from separate genes, but share 94% structural homology [53]. STAT5 proteins are of particular interest for their critical roles in cellular functions such as proliferation, differentiation, and survival [54]. In addition, STAT5 proteins critically regulate the maintenance of normal immune function and homeostasis [53], and has prominent roles in mammary development and function [55]. Similar to STAT3, constitutive activation of STAT5 is the leading causes of tumorigenesis [56]. STAT5 activation was essential for cancer progression in chronic myelogenous leukemia (CML) and myeloproliferative disease [57, 58]. There are strong evidences showing that targeting STAT5 with small molecules could interfere with a substantial proportion of human tumors [59, 60]. A variety of STAT5 inhibitors have been identified that induce antitumor cell effects [61-63]. Several STAT5 inhibitors for hematological malignancy have already advanced to clinical studies [13]. However, our data indicated that STAT5B was associated with an improved prognosis in all NSCLC patients combined and ADE patients alone. These results suggested that STAT5A and STAT5B might be prognostic biomarkers not therapeutic targets for NSCLC.

The function of STAT6 mainly involved in immune function, tumor immunosurveillance, lymphomagenesis and inflammatory-related tumorigenesis [56, 64]. STAT6 is constitutively stimulated in a number of human cancers, such as colon and prostate cancer, mediastinal large B-cell lymphoma and Hodgkin's lymphoma [65-67]. Thus, STAT6 is considered as a therapeutic target for various cancer types. For instance, Binnemars-Postma K et al. observed that targeting the STAT6 pathway in tumor-associated macrophages reduced tumor growth and metastatic niche formation in breast cancer [68]. Leon-Cabrera SA et al. demonstrated that lack of STAT6 attenuated inflam-

Table 4. Correlation of STAT family members with smoking history of NSCLC patients

Gene	Smoking history	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	Never smoked	103	102	0.73 (0.41-1.28)	0.27
	Smoked	410	410	0.92 (0.75-1.13)	0.41
STAT2	Never smoked	103	102	1.88 (1.06-3.34)	0.027*
	Smoked	410	410	1.27 (1.03-1.56)	0.024*
STAT3	Never smoked	102	103	2.17 (1.21-3.91)	0.0079**
	Smoked	410	410	1.08 (0.88-1.33)	0.48
STAT5A	Never smoked	102	103	1.12 (0.64-1.96)	0.68
	Smoked	410	410	0.89 (0.73-1.1)	0.28
STAT5B	Never smoked	102	103	0.33 (0.18-0.6)	0.00013**
	Smoked	410	410	0.81 (0.66-1)	0.044*
STAT6	Never smoked	102	103	0.87 (0.5-1.51)	0.61
	Smoked	410	410	0.77 (0.63-0.95)	0.013*

Low/High (cases): low/high expression of the corresponding gene (patient number). *$P < 0.05$, **$P < 0.01$.

Table 5. Correlation of STAT family members with gender of NSCLC patients

Gene	Gender	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	female	358	357	1.21 (0.96-1.52)	0.11
	male	550	550	0.9 (0.77-1.06)	0.2
STAT2	female	361	354	1.33 (1.05-1.68)	0.016*
	male	557	543	1.13 (0.97-1.33)	0.12
STAT3	female	358	357	1.06 (0.84-1.33)	0.64
	male	550	550	0.94 (0.8-1.1)	0.43
STAT5A	female	359	356	0.96 (0.76-1.21)	0.71
	male	551	549	0.76 (0.65-0.89)	0.00086**
STAT5B	female	358	357	0.69 (0.54-0.87)	0.0018**
	male	554	546	0.83 (0.71-0.97)	0.022*
STAT6	female	359	356	0.73 (0.58-0.93)	0.0092**
	male	550	550	0.71 (0.6-0.83)	1.9e-05**

Low/High (cases): low/high expression of the corresponding gene (patient number). *$P < 0.05$, **$P < 0.01$.

Table 6. Correlation of STAT family members with negative surgical margins of NSCLC patients

Gene	Surgical margins	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	negative	363	363	0.81 (0.65-1.02)	0.071
STAT2	negative	364	362	1.54 (1.22-1.94)	0.00022**
STAT3	negative	364	362	1.62 (1.28-2.05)	5.9e-05**
STAT5A	negative	363	363	1.06 (0.84-1.33)	0.63
STAT5B	negative	364	362	0.65 (0.52-0.82)	0.00025**
STAT6	negative	363	363	0.82 (0.66-1.04)	0.096

Low/High (cases): low/high expression of the corresponding gene (patient number). **$P < 0.01$.

matory responses and cell recruitment, which in turn prevented the early development of colitis-associated colon cancer [69]. A recent study also revealed that direct inhibition of STAT6 induced prostate cancer cell apoptosis [70]. However, the function of STAT6 in NSCLC is less clear. Here, STAT6 mRNA expression was correlated with an improved prognosis in all NSCLC patients combined, as well as ADE patients alone. In detail, increased STAT6 predicted better OS in stage I patients.

All the individual STAT members were not significantly associated with pathological grades of NSCLC patients. Overexpression of STAT2 was correlated with favorable OS in stage III patients, suggesting that STAT2 inhibitors might not be suitable for advanced NSCLC patients. No STAT member was identified to be a valid prognostic factor in NSCLC patients with a smoking history. We also investigated the association between STATs expression and therapy strategies in NSCLC patients. Increased STAT2 expression exhibited correlations with better OS when patients received chemotherapy. Given that most lung

Table 7. Correlation of STAT family members with chemotherapy of NSCLC patients

Gene	Chemotherapy	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	yes	88	88	1.07 (0.71-1.61)	0.74
	no	155	155	0.9 (0.65-1.26)	0.56
STAT2	yes	88	88	0.61 (0.4-0.91)	0.015*
	no	158	152	1.05 (0.75-1.47)	0.76
STAT3	yes	88	88	1 (0.66-1.52)	1
	no	155	155	0.78 (0.56-1.09)	0.14
STAT5A	yes	88	88	0.96 (0.64-1.43)	0.82
	no	155	155	0.75 (0.54-1.05)	0.094
STAT5B	yes	89	87	1.13 (0.75-1.69)	0.56
	no	155	155	1.06 (0.76-1.49)	0.72
STAT6	yes	88	88	0.89 (0.59-1.33)	0.56
	no	155	155	0.82 (0.59-1.15)	0.25

Low/High (cases): low/high expression of the corresponding gene (patient number). *$P < 0.05$.

Table 8. Correlation of STAT family members with radiotherapy of NSCLC patients

Gene	Radiotherapy	Low (cases)	High (cases)	HR (95% CI)	*p*-value
STAT1	yes	35	35	1.07 (0.63-1.82)	0.81
	no	136	135	0.94 (0.66-1.34)	0.72
STAT2	yes	35	35	1.18 (0.69-2.01)	0.54
	no	136	135	0.94 (0.66-1.34)	0.74
STAT3	yes	35	35	0.74 (0.43-1.28)	0.28
	no	136	135	0.8 (0.56-1.14)	0.21
STAT5A	yes	35	35	1.47 (0.86-2.52)	0.16
	no	136	135	0.69 (0.48-0.99)	0.043*
STAT5B	yes	35	35	1.43 (0.84-2.45)	0.19
	no	138	133	1 (0.7-1.42)	0.99
STAT6	yes	35	35	0.95 (0.56-1.63)	0.86
	no	136	135	1.02 (0.71-1.45)	0.93

Low/High (cases): low/high expression of the corresponding gene (patient number). *$P < 0.05$.

cancer patients would be treated with chemotherapy, it should be paid more attention to the usage of inhibitors targeting STAT2. Conversely, patients with negative surgery margins should to consider combined with STAT2 or/and STAT3 inhibitors to improve therapeutic efficacy of NSCLC including ADE.

In conclusion, our data showed that mRNA expression of STAT1/2/3 were increased in lung ADE and STAT1/2/5B were up-regulated in lung SCC. Increased mRNA expression of STAT2 was significantly associated with shorter OS in NSCLC patients, while increased expressions of STAT4/5A/5B/6 were significantly related to favorable OS. In lung ADE patients, high STAT2/3 predicted unfavorable OS and high STAT5B/6 indicated favorable OS. A stratified analysis for all NSCLC patients combined was performed, but included patients with SCC, no correction between STAT members and OS was observed. These data suggested that STAT5B/6 could be potential biomarkers for the prognosis of lung ADE and STAT2 might be promising therapeutic targets for NSCLC treatment. Considered that all the data in our study was obtained from online databases, further studies consist of larger sample sizes are required to validate our findings and to investigate the possible underlying mechanism between distinct STATs and NSCLC. Importantly, the potential function of STAT2 in lung ADE should be verified with more evidence.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The desired Affymetrix ID of STAT family genes in www.kmplot.com

Gene	Affymetrix ID
STAT1	200887_s_at
STAT2	217199_s_at
STAT3	208991_at
STAT4	206118_at
STAT5A	203010_s_at
STAT5B	212549_at
STAT6	201331_s_at

Supplementary Table 2. The number of datasets significantly correlated with STAT genes up-regulation and down-regulation in lung cancer tissues versus normal tissues, was displayed at *p*-value 0.05, fold change 1.5, gene rank: top 10%

Gene	Up-regulation (case number)	Down-regulation (case number)
STAT1	8	1
STAT2	0	0
STAT3	0	1
STAT4	0	2
STAT5A	0	6
STAT5B	0	5
STAT6	0	3

Supplementary Table 3. Protein expression patterns of STATs in NSCLC by the Human Protein Atlas

Gene	Antibody	High	Medium	Low	Not-detected	Total
STAT1	CAB004049	1	3	1	7	11
STAT2	HPA018888	0	2	1	8	11
STAT3	HPA058603	1	6	0	4	12
STAT4	CAB013108	2	2	3	5	12
STAT5	HPA049883	2	1	0	7	10
STAT6	HPA049883	0	3	5	4	12