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SUPPRESSOR OF CYTOKINE SIGNALING MEMBERS IN LUNG ADENOCARCINOMA: UNVEILING EXPRESSION PATTERNS, POSTTRANSLATIONAL MODIFICATIONS, AND CLINICAL SIGNIFICANCE

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Abstract
The role of SOCS (Suppressor of Cytokine Signaling) members in cancer has garnered significant research interest. However, their specific role in lung adenocarcinoma awaits a recent report utilizing publicly available databases and software. Our analysis included an exploration of overall survival, expressions, correlation patterns, genetic alterations, prognostic values, network analysis, and gene ontology and posttranslational modification insights of SOCS members using bioinformatics tools. Our findings revealed that both SOCS2 and SOCS3 are downregulated in LUAD. Specifically, posttranslational modification analysis indicated that SOCS2 undergoes phosphorylation at serine 30 and ubiquitination at lysine 38. In the case of SOCS3, potential phosphorylation sites were identified at threonine 86 and tyrosine 165, 166, 221, and 204, with evidence of ubiquitination at lysine 6, 23, 195, and 206. We observed that SOCS genes exhibited a moderate net alteration frequency of 20% in LUAD patients. Additionally, a strong pairwise correlation between SOCS2 and SOCS3 was evident. Furthermore, our analysis revealed the integration of SOCS-related genes into kinase activity. Notably, decreased mRNA levels of SOCS1, SOCS3, SOCS4, and SOCS7, along with increased levels of SOCS5 and SOCS6, were significantly associated with longer overall survival. In conclusion, our study suggests that SOCS5 and SOCS6 function as tumor suppressor genes, while SOCS1, SOCS3, SOCS4, and SOCS7 act as tumor promoter genes in LUAD. Furthermore, our findings indicate that SOCS2 may represent a potential therapeutic target, and SOCS3 could serve as a valuable prognostic biomarker for LUAD patients.

Keywords: Lung adenocarcinoma, SOCSs, expression, prognosis, PTMs, therapeutic target

1. Introduction

Lung cancer is considered among highly frequent cancers, accounting for 11.4 % of cancer cases globally in 2020 [1]. The existing therapeutic strategies for lung cancer include chemotherapy, surgical cancerous tissue removal, and radiotherapy [2]. However, as lung cancer has such a high rate of recurrence and metastasis, the effects of its clinical progression are unsatisfactory, resulting in a usually poor patient prognosis [3]. Among lung cancers, adenocarcinoma arising from peripheral bronchi seems the most prevalent, with 40% cases. In addition, adenocarcinoma leads to pneumonitis, bronchiolar-alveolar cancers, and lobar atelectasis [4]. As a result, it is critical to find new targets for developing tailored and effective lung cancer treatment and innovative biomarkers to improve the patient's prognosis. As a result, the suppressor of cytokine signaling (SOCS) family members' aberrant expression regulates tumor formation and angiogenesis in malignancies. The SOCS family has eight members with SOCS 1–7 and cytokine-inducible SH2domain-containing proteins [5]. However, few studies have reported correlations of SOCS family gene expression with different cancers as cytokine signaling is involved in the progression of these malignancies [6].

It is well established that aberrant SOCSs expression signalling can initiate cancer development in multiple tissues, including immune cells in the tumor microenvironment [7]. One example is hypermethylation of SOCS3 in lung and head and neck cancers [8]. However, few documented reports have shown the role of SOCS family members in lung adenocarcinoma. SOCS1 inhibits the FAK-dependent signaling cascade by suppressing FAK tyrosine phosphorylation, according to Shimada and colleagues [9]. Zhou et al. studied SOCS2 expression levels in human lung cancer patients. They discovered that SOCS2 mRNA expression levels were substantially linked with histological subtype, lymph node metastasis, clinical stage, and survival time [10]. Although some information is available about the involvement of SOCS family proteins in lung cancer, the specific mechanism of action is not yet unraveled. Therefore, we performed this study using the data extracted from TCGA and other available datasets. Our investigation aimed to investigate more about the expression patterns, potential roles, and prognostic implications of SOCS family members in lung adenocarcinoma. In recent report, authors presented findings firmly rooted in bioinformatics. It is worth noting that some of the approaches we employed in this report have been previously utilized in our earlier work [11].

2. Materials and Methods

2.1.1 Comprehensive Analysis of SOCS Family Members in Lung Cancer: Insights from TCGA Datasets and Immunohistochemistry

For clinicopathological feature analysis we analyzed UALCAN [12]. In our study, expression data for SOCS members was obtained using the human protein atlas database

(https://www.proteinatlas.org/) for mRNA expression in healthy vs. cancerous tissues. Aside from that, we employed the ONCOMINE [13] and GEPIA [14] platforms to compare mRNA expression levels in healthy and malignant tissues (Rhodes et al., 2004) and the GEPIA tool, which is available online at (http://gepia.cancer.pku.cn/detail.php). We used the Human Protein Atlas tool to identify immunohistochemical photos of SOCS family members in lung cancer cells. More than five million tissue photos of immunohistochemically labeled cells are accessible. The principal staining patterns were negative, low, medium, and high. TCGA provided data for SOCS2/3 immunohistochemistry analysis (Normal 578 samples; Tumour 994 samples), which was then analyzed with Graph Pad PRISM6 (*P<0.05).

2.1.2. Exploring SOCS Family Gene Mutations and Correlation Networks in Lung Cancer: Insights from Transcriptome Data and Protein Interaction Analysis

To address SOCS family genes mutation and couples correlation analysis in lung cancer, we retrieved transcriptome datasets from 586 patients/samples (TCGA, Firehose Legacy) from cBioPortal [15]. We sorted all transcripts according to the Spearman correlation value with the coexpressed genes.

Furthermore, we obtained the SOCS relevant protein-protein interaction (PPI) network from STRING [16] as well as GENE MANIA [17] Cytoscape. Similarly, using GeneMANIA software, networks of SOCS members based on gene-to-gene interaction were also constructed. As a result, we identified the genes belonging to the PPI network and enriched the KEGG pathway presented in the bar graph.

2.1.3. Differential mRNA Expression of SOCS Genes in Cancer Patients: Impact on Overall Survival and Functional Insights through Gene Ontology and KEGG Pathways

We divided cancer subjects according to the mean expression of SOCSs mRNA into two groups, i.e., low and high expression groups. In addition, we assessed the relationship between the levels of mRNA expression in SOCS members and overall survival (OS). We used Kaplan–Meier curves and the log-rank test (Mantel-Cox test) [18] to assess survival. Using the ShinyGo [19], the identified SOCS genes in the network were tested for Gene Ontology. Based on a screening threshold of $p<0.05$, we selected the top 10 significant GO terms and KEGG pathways, and they are represented as a bubble chart.

2.1.4. SOCS Family Member Expression and Immune Infiltration in Cancer: Insights from TIMER Analysis and Transcriptional Determinants for Protein-Protein Interactions

We extracted data from TIMER [20] to know the association between the SOCS family members' expression level and immune infiltration. It infers the richness of tumor-infiltrating immune cells from gene expression profiles of multiple cancer types in TCGA. This tool includes numerous options for assessing immunological infiltration, such as TIMER, CIBERSORT, and EPIC. In the current investigation, we chose the TIMER results. We conducted an analysis using Enrichr [21] to identify transcriptional determinants associated with Protein-Protein Interactions (PPI) for SOCS genes.

2.1.5. Statistical analysis

To compare the means of two groups in the ONCOMINE database, we utilized a two-tailed student's t-test. For the analysis of clinicopathological features the student's t test was used to generate a p value (*0.05). For mRNA expression, we presented the data as fold change. Data of OS are presented as Kaplan-Meier plots with median selection, where we used the log-rank test to calculate p-values. In both cases, we considered the statistical significance at $p<0.05$.

3. Results

3.1.1. Exploring SOCS Family Member Expression in Various Cancer Types

We have presented the comprehensive summary and significance of our current work, as illustrated in Figure 1A. Additionally, Figure 1B provides a detailed list of features for the members of the SOCS family. Using Oncomine and GEPIA, we investigated the expressions of the seven SOCSs in different cancer types. Within the ONCOMINE, the mRNA expression of our proteins of interest in 19 distinct cancer types and comparisons to normal tissues are shown in Figure 2. In lung cancer, eight datasets showed decreased SOCS2 expression; however, no dataset showed increased expression. Two datasets showed significantly increased expression, and six datasets showed a significant decrease in SOCS3 expression comparing lung cancer and normal tissue. Two datasets showed that SOCS7 was overexpressed in lung cancer, and no dataset showed underexpression of SOCS7 in lung cancer. In GEPIA, the mRNA expression of seven SOCS proteins in Figure 2B. Compared to the normal, SOCS2 and SOCS3 were downregulated in tumor samples ($p<0.05$; Figure. 2A, B, C). We used the HPA database for immunohistochemical analysis to examine SOCS protein expressions (Figure. 3A, B). We also retrieved TCGA data for the term of SOCS2 and SOCS3 proteins at the tissue level and quantified it as described in (Figure. 3C, D). In LUAD patient tissues, SOCS2/3 expression was considerably lower than in normal counterpart.

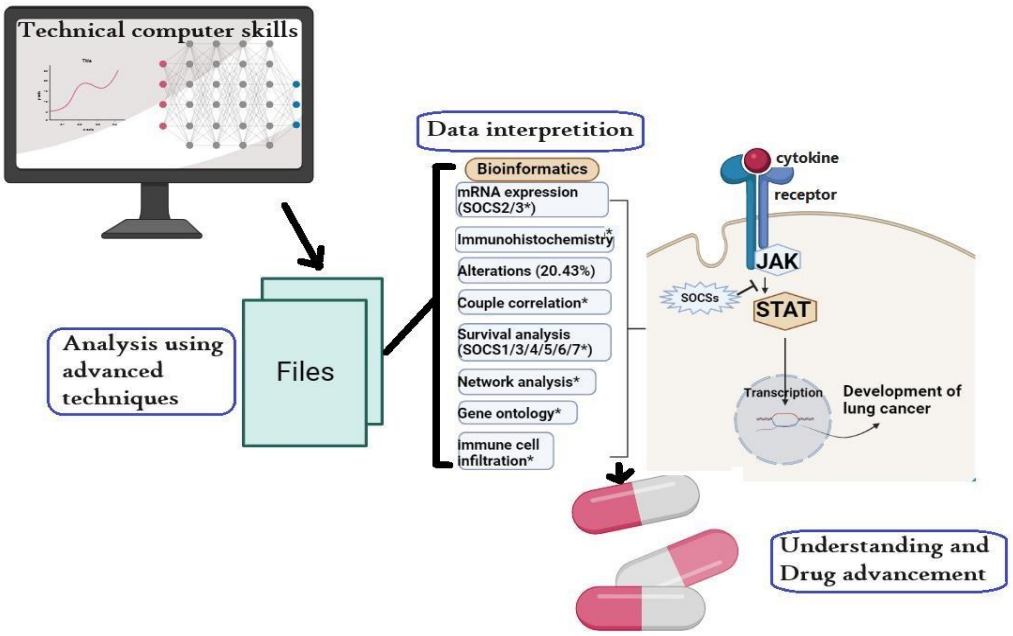

Figure 1A. Summary of the current work.

Gene name	Chromosomal Location	Subcellular Location	Subcellular Location Image	Domain	Major Function	Prognostic marker in cancer
SOCS1	16p13.13	Nucleus Cytoplasmic vesicle (Detected in perinuclear cytoplasmic vesicles upon interaction with FGFR3)		SH2 domain, SOCS box	Major regulator of signaling by interleukin 6 and leukemia inhibitory factor. Regulates interferon-gamma mediated sensory neuron survival. Negative regulator in IGF1R signaling pathway. Reversed regulation of cytokines that signal through the JAK/STAT3 pathway.	Renal (unfavorable) and head and neck cancer (favorable)
SOCS2	12q	Endoplasmic reticulum		SH2 domain, SOCS box	Negative regulator in the growth hormone/IGF1 signaling pathway. Probable substrate recognition component of a SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins.	Pancreatic and liver cancer (favorable)
SOCS3	17q25.3	Cytoplasm and plasma membrane		SH2 domain, SOCS box	Involved in negative regulation of cytokines that signal through the JAK/STAT pathway. Suppresses fetal liver erythropoiesis. Regulates onset and maintenance of allergic responses mediated by T-helper type 2 cells. Seems to recognize IL6ST.	Renal (unfavorable) and breast cancer (favorable)
SOCS4	14q22.1	Nucleoplasm and cytosol		SH2 domain SOCS box	Substrate-recognition component of a SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins. Inhibits EGF signaling by mediating the degradation of the Tyr-phosphorylated EGF receptor/EGFR.	Non.
SOCS5	2p21	Nucleoplasm, cytosol and plasma membrane		SH2 domain SOCS box	May inhibit IL6 and LIF signaling. Involved in the regulation of T-helper cell differentiation by inhibiting of the IL4 signaling pathway.	Liver and ovarian cancer (unfavorable)
SOCS6	18q22	Nuclear speckles and Cytosol			A high expression level of this gene has been found to play a key role in the development and survival of some types of leukaemia. The gene can be induced by GM-CSF and EPO in hematopoietic cells, as well as by other treatments such as immunotherapy.	Renal cancer (favorable)
SOCS7	17q12	Cytosol		SH2 domain SOCS box	Inhibits prolactin, growth hormone and leptin signaling by preventing STAT3 and STAT5 activation, sequestering them in the cytoplasm and reducing their binding to DNA. May be a substrate recognition component of E3 ubiquitin-protein ligase complex.	Breast and endometrial cancer (unfavorable)

Image green part representing the localization of SOCS protein

Figure 1. Features of SOCS Members.

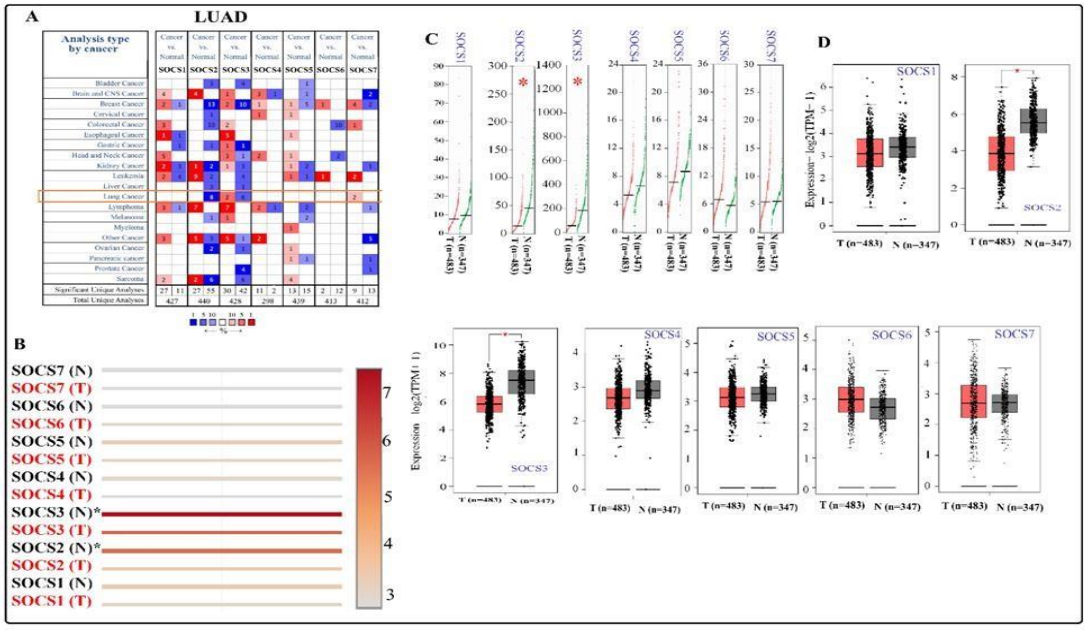

Figure 2. Expression of seven SOCS genes at mRNA level. (A) ONCOMINE analysis of mRNA expression of SOCS in different cancers. The best gene rank determines the cell color. Red color indicates copy gain or overexpression; blue represents copy loss or underexpression. Color intensity ranks the expression of the gene in analyses. The analysis that met our threshold is shown in each cell. (B) GEPIA database analysis for mRNA expression levels of SOCS genes in lung tumors and normal tissues. (C) Seven SOCS members mRNA expression profile (D) Box plot representation of mRNA expression of SOCS genes. The intensity of the hue shows the level of gene mRNA expression. Normal tissue is green, and tumour tissue is red. *P<0.05 and Log2 (foldchange) cutof=1.5. Log scale was used to show the RNA expression level.

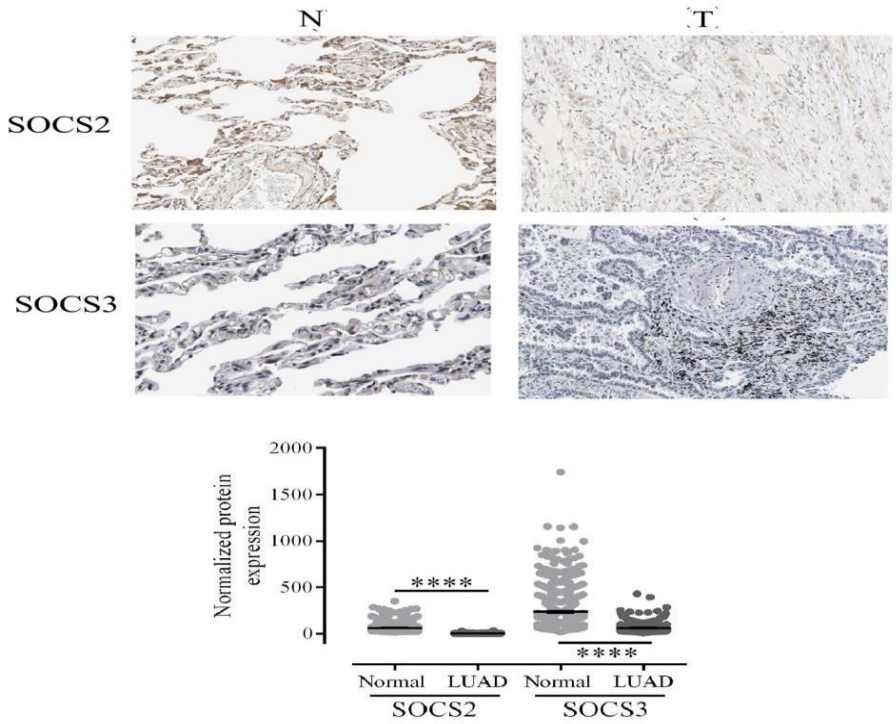

Figure 3. SOCS proteins expression in LUAD and tumor-adjacent normal tissues via HPA immunohistochemistry section. (A) SOCS2 and (B) SOCS3 protein expression in lung cancer and tumor-adjacent normal tissues. (C) Graph depicting quantification of SOCS2/3 protein in normal and LUAD patient's tissues from TCGA data.

3.1.2. Genetic Alterations and Co-expression Patterns of SOCS Family Proteins in Lung Adenocarcinoma

We retrieved genetic changes of the seven SOCS proteins using cBioPortal. We found a moderate alteration frequency (20%) in lung adenocarcinoma patients (Fig. 4A). Among seven studied SOCS family members, we found the highest frequency of patients (6.96%) with SOCS6 alteration having high mRNA levels. In addition, we studied co-expression correlations in the SOCS couples. We found a positive correlation between SOCS2 with SOCS3, SOCS5 with SOCS6, and a negative correlation between SOCS1 with SOCS7 (Fig. 4B). We found a similar correlation using "The Lung cancer explorer" (Fig. 4C), and the p values of the coefficients are depicted in (Fig. 4D).

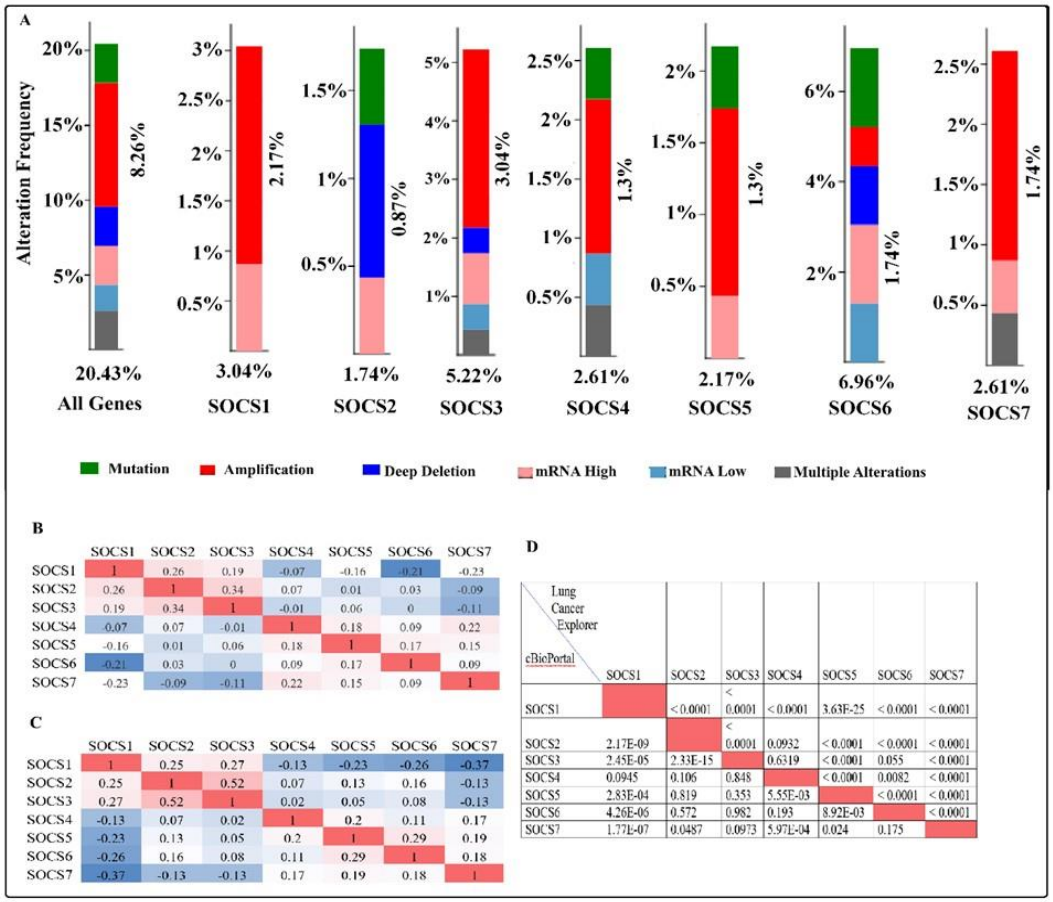

Figure 4. The alteration frequency, mechanisms and couple correlation for SOCS proteins in lung adenocarcinoma (cBioPortal and Lung Cancer Explorer). (A) Lung adenocarcinoma with 586 samples (RNA Seq V2) was investigated. The threshold was set at ±2.0 for mRNA expression zscore. (B) Pearson's correlations for mRNA expression of pairwise combinations of SOCS proteins in cBioPortal (C) and Lung Cancer Explorer. (D) Correlation p-values. The color scale represents the correlation coefficient value. (r) Pearson's correlation coefficient value; p-value; the number of patients.

3.1.3 Correlation between SOCS Family Genes and Clinicopathological Parameters in Lung Adenocarcinoma

Table 1 shows the clinicopathological parameters and associations derived from the analysis of LUAD patients in the TCGA datasets in UALCAN. Based on expression patients with high SOCS6 were more likely to have N and N0 lymph node metastasis and p53 mutations, whereas

SOCS6 was more likely to have N lymph node metastasis and p53 mutation based on methylation level. Low SOCS6 were more likely to have N and N3 lymph node metastasis and p53 mutations, whereas low SOCS3 were more likely to have N and N0 lymph node metastasis and p53 mutation

based on their expression. On the other hand, both SOCS2/3 were more likely to have N lymph node metastasis and p53 mutation based on their promoter methylation level. Stage I and II patients had higher SOCS1 expression compared with other stages. Table 1 shows the Association associations between all SOCS members and based on their expression and promoter methylation.

Table 1. SOCS family member's expression and promoter methylation profile based on patient's clinicopathological features.

Expression							
Feature	**Genes**						
	SOCS1	**SOCS2**	**SOCS3**	**SOCS4**	**SOCS5**	**SOCS6**	**SOCS7**
Tumor stage							
	0.05*	Non.	non.	non.	non.	non.	Non.
Nodal status							
Normal vs N0	N>N0* (1.809780E01)	N>N0 (5.1205994 9997479E-10)	N>N0 * (2.553299999 98555E-06)	N>N0 (4.371000E01)	N>N0 (9.822300 E-04)	N>N0 (9.65990 0E-02)	N>N0 (3.530100E02)
Normal vs N1	N>N1* (1.562840E01)	N>N1* (1.9988999 3446334E-10)	N>N1 (4.785899999 97059E-06)	N>N1 (9.929700E02)	N>N1 (7.415400 E-04)	N>N1 (4.12810 0E-02)	N>N1 (7.239400E01)
Normal vs N2	N>N2 (9.521400E01)	N>N2 (9.7895003 2148873E-10)	N>N2* (2.221499999 99921E-05)	N>N2 (3.902000E01)	N>N2* (1.399980 E-01)	N>N2 (9.09780 0E-01)	N>N2 (3.849000E01)
Normal vs N3	N3>N * (1.448140E02)	N3>N (6.399400E -01)	N3>N (5.841800E01)	N3>N (4.762800E01)	N>N3* (1.436990 E-01)	N>N3* (1.78957 0E-01)	N>N3* (2.914600E01)
N0 vs N1	N1>N0 (6.190400E01)	N1>N0 (3.804200E -01)	N0>N1 (8.315400E01)	N0>N1 (8.279500E03)	N0>N1 (3.458800 E-01)	N0>N1 (3.72960 0E-01)	N0>N1* (2.067600E01)
N0 vs N2	N0>N2 (4.735800E01)	N0>N2 (9.502000E -01)	N0>N2 (3.925000E01)	N2>N0 (7.785900E02)	N0>N2 (5.449200 E-01)	N0>N2 (3.51840 0E-01)	N0>N2 (5.509200E01)
N0 vs N3	N0>N3* (2.570000E01)	N3>N0 (4.707800E -01)	N0>N3* (1.751400E01)	N3>N0 (8.688200E02)	N0>N3 (5.537400 E-01)	N0>N3 (5.69900 0E-01)	N0>N3 (3.721800E01)
N1 vs N2	N1>N2 (3.474200E01)	N1>N2 (5.983200E -01)	N1>N2 (5.964600E01)	N2>N1 (4.454000E01)	N2>N1* (2.702200 E-01)	N1>N2* (1.79085 0E-01)	N2>N1 (6.564600E01)
N1 vs N3	N3>N1* (2.963400E01)	N3>N1 (4.620400E -01)	N1>N3 (3.263800E01)	N3>N1 (9.241500E03)	N1>N3 (6.712400 E-01)	N1>N3 (6.71060 0E-01)	N1>N3 (5.405200E01)
N2 vs N3	N3>N2 (3.611800E01)	N3>N2 (4.701400E -01)	N2>N3 (3.835400E01)	N3>N2 (4.538400E01)	N2>N3 (6.015200 E-01)	N2>N3 (6.03180 0E-01)	N2>N3 (4.402600E01)
P53 status							
	Mutated>WT (5.242400E01)	WT>mutat ed (9.648600E -04)	WT<mutated (5.466400E01)	WT<mutate d*(1.188460 E-04)	WT<muta ted (9.199800 E-01)	Mutated <WT (1.20119 0E-01)	WT<mutate d *(2.676300E -02)
Promoter methylation profile							
Nodal status							
Normal vs N0	N>N0 (4.198800E04)	N>N0 (8.3019999 9857228E-08)	N>N0 * (1.662430E02)	N>N0 * (1.28219999 995238E-06)	N>N0* (1.130090 E-02)	N>N0 (3.23850 0E-02)	N>N0 (4.050200E01)
Normal vs N1	N>N1 (5.542600E03)	N>N1 (4.216200E -03)	N>N1 (5.086000E02)	N>N1 (9.769400E04)	N>N1 (3.742300 E-03)	N>N1* (2.62340 0E-01)	N>N1 (8.296200E01)
Normal vs N2	N>N2* (1.035510E03)	N>N2* (1.573920E	N>N2 (3.684400E02)	N>N2 (3.412600E04)	N>N2 (4.601100	N>N2*(1 .6989499	N>N2 (5.704400E01)

		-02)			E-03)	9999531 E-05)	
Normal vs N3	Non.	Non.	Non.	Non.	Non.	Non.	Non.
N0 vs N1	N0>N1 (8.037000E01)	N0>N1 (3.579200E -01)	N0>N1 (8.217600E01)	N0>N1 (8.124800E01)	N0>N1 (4.988000 E-01)	N0>N1 (8.01180 0E-01)	N0>N1 (4.832400E01)
N0 vs N2	N0>N2 (7.382800E01)	N0>N2 (3.455000E -01)	N0>N2 (9.694000E01)	N2>N0 (8.641800E01)	N0>N2 (3.401600 E-01)	N0>N2 (6.15550 0E-03)	N0>N2 (8.243600E01)
N0 vs N3	Non.	Non.	Non.	Non.	Non.	Non.	Non.
N1 vs N2	N2>N1 (6.525800E01)	N1>N2 (9.104200E -01)	N1>N2 (8.892400E01)	N2>N1 (9.519800E01)	N1>N2 (7.614400 E-01)	N1>N2* (2.68600 0E-02)	N2>N1 (6.834800E01)
N1 vs N3	Non.	Non.	Non.	Non.	Non.	Non.	Non.
N2 vs N3	Non.	Non.	Non.	Non.	Non.	Non.	Non.
P53 status							
	WT>mutated (9.533600E01)	WT<mutat ed (8.986000E -01)	WT<mutated (8.986000E01)	WT>mutate d (4.148200E02)	Mutated< WT (7.859600 E-01)	WT<mut ated (5.95180 0E-02)	WT>mutate d (2.022800E01)

3.1.4. SOCS Members in Lung Cancer: Survival and Functional Insights

The Kaplan–Meier Plotter demonstrated that, except for SOCS2, all SOCS proteins exhibited predictive value for lung cancer patients' overall survival (OS). Reduced SOCS 1/3/4/7 mRNA levels and elevated SOCS 5/6 mRNA levels were associated with significantly longer overall survival (Fig. 5). We further depicted protein-protein interaction (PPI) networks from STRING and GENE MANIA. In the STRING, each constructed common protein network had 07 nodes, several edges 31 with PPI enrichment p-value < 1.0e-16, considered the moderate connected protein interactions (Fig. 6A). GeneMANIA revealed that the SOCS family's functions were primarily related to kinase activity and the receptor signaling pathway. There were 20 nodes surrounding the seven SOCS members, with 630 total links, showing co-localization, coexpression, shared protein domain interactions, prediction, and pathways (Figure 6B). We also identified the most significantly enriched Gene Ontology terms in the categories of biological processes, cellular components, and molecular functions, as well as the most significant KEGG pathway terms, as shown in Figure 6C-F. Additionally, we have demonstrated the post-translational modifications sites for SOCS2/3, as depicted in Figure 7.

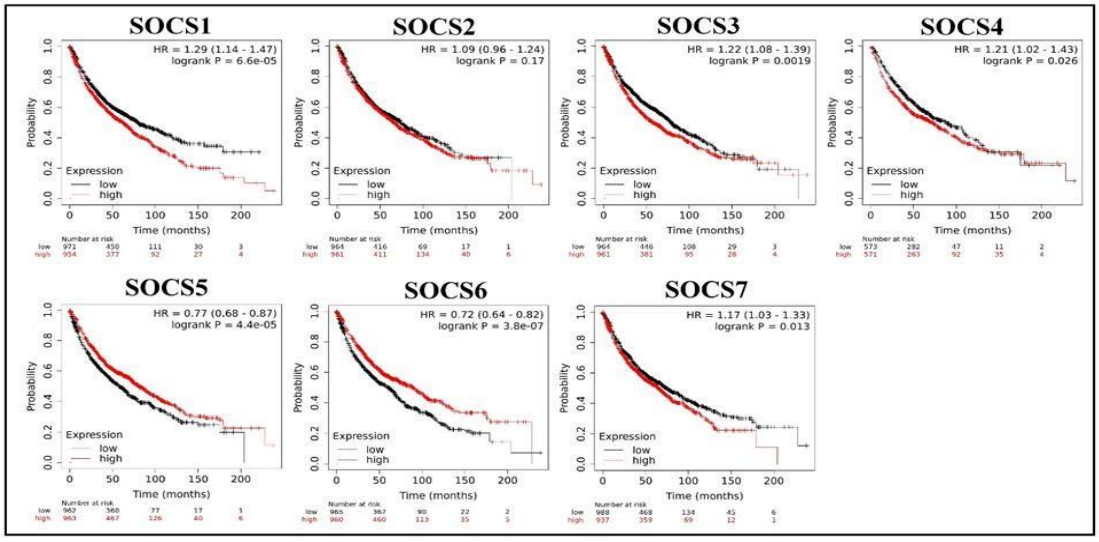

Figure 5. Prognostic values of SOCS members for overall survival (OS) in Kaplan-Meier plotter. LUAD patients are screened into high and low expression groups according to the median expression levels. *P<0.05.

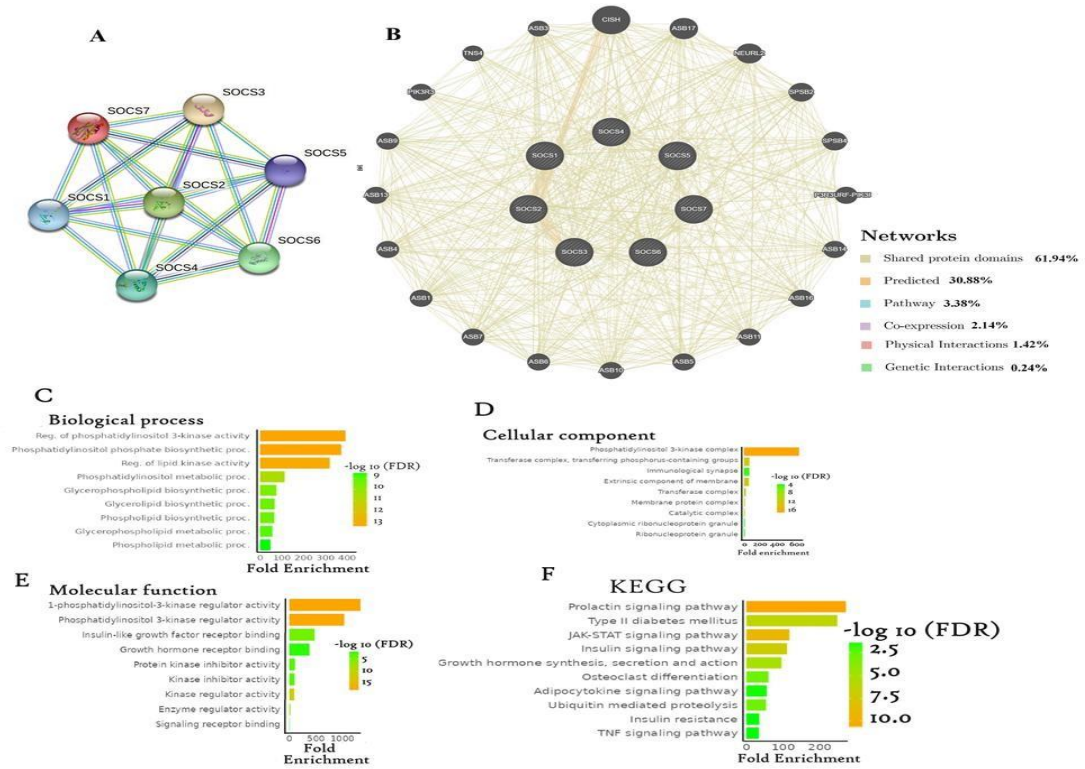

Figure 6. Protein-protein interaction network and The significant GO functions enriched for SOCS family members (A) Protein-Protein interaction via STRING (B) GeneMANIA (Gene to gene interaction network). (C) Biological process. (D) Cellular component. (E) Molecular function (D) KEGG pathway analysis. The dot indicates the gene cluster. Color intensity indicates more significant the GO term for each indication.

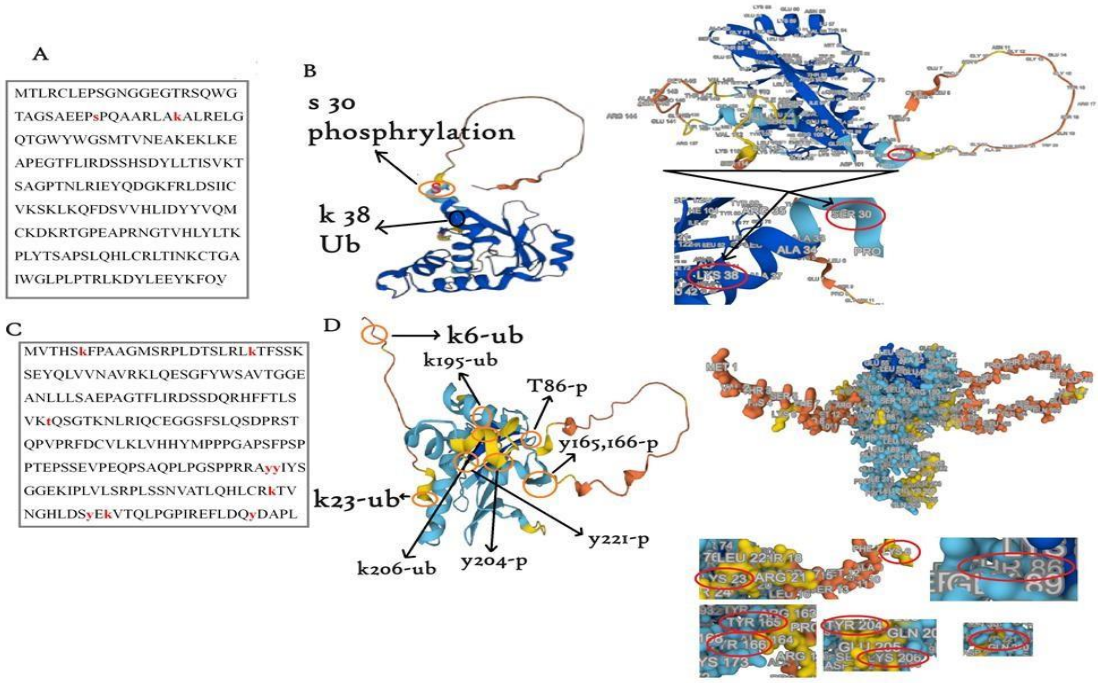

Figure 7. Structural and molecular description of SOCS 2 and SOCS3. A and C) Depict the SOCS2/3 sequences, emphasizing the locations of phosphorylation and ubiquitination sites. B and D) Provide a three-dimensional representation of SOCS2/3, with distinct sites clearly indicated for reference.

3.1.5. Exploring Transcription Factor Interactions and Regulation in the Context of Altered SOCS Family Expression in Lung Adenocarcinoma

Given that there is a significant difference in the expressions of SOCS family members in LUAD vs. healthy tissue, we investigated possible putative PP1 for transcription factors and transcription factor targets of the SOCS family members, as depicted in (Tables 2, 3). Seven transcription factors were identified to be linked to the transcription factor PPI, while 12 transcription factors were linked to SOCS regulation. The transcription factors STAT and IRF were the most abundant.

Table 2. PP1 transcriptional factors for SOCS members in human collected from several literature based databases.

TF	p-value	Adj.pvalue	Odds ratio	Combined score	Regulated genes
NOD2	0.01565	0.05711	75.56	314.16	SOCS3
BCLAF1	0.01875	0.05711	62.7	249.35	SOCS1
STAT3	0.003038	0.03342	32.38	187.67	SOCS3/7
BMI1	0.02425	0.05711	48.13	179	SOCS2
IRF3	0.02596	0.05711	44.86	163.8	SOCS1
STAT5A	0.03312	0.06073	34.91	118.95	SOCS7
NR4A1	0.03957	0.06218	29.06	93.87	SOCS4

Table 3. Key regulated factor in general for SOCS members in human (TRRUST).

TF	p-value	q-value	Regulated genes
IRF1	0.000133	0.001129	SOCS2/1
STAT3	0.001027	0.006983	SOCS3/1
STAT4	0.003844	0.014522	SOCS3
IRF3	0.005239	0.017345	SOCS2
GLI2	0.006284	0.017345	SOCS1
GLI1	0.006632	0.017345	SOCS1
STAT6	0.012534	0.025068	SOCS1
CEBPA	0.017717	0.030118	SOCS3
PPARG	0.022876	0.033816	SOCS7
HIF1A	0.028695	0.035258	SOCS1
STAT1	0.029036	0.035258	SOCS3
SP3	0.038891	0.045597	SOCS3

3.1.6. Examining the Relationship between SOCS Family Members and Immune Infiltration in Lung Adenocarcinoma

We performed the association between SOCS members and infiltrating immune cells via the TIMER tool. Table 4 depicts the relationships between SOCSs expression levels with tumor purity and tumor-infiltration levels across different immune cells. The puritycorrected partial Spearman's correlation and p-value were plotted. In lung adenocarcinoma, we found that SOCS1 is correlated with all types of immune cells, SOCS2 is correlated with macrophages and neutrophils, and SOCS3 is correlated with all immune cells except for B cells. In LUAD, SOCS4/5/6 are associated with all immune cells except B cells and macrophages, CD4+T cells and B cells, and CD4+T cells and dendritic cells; SOCS7 is not et al. (Table 4).

Table 4. SOCS members' expression correlates with tumor integrity and immune cell infiltration degrees in LUAD patients (TIMER).

	p.cor	***p-value***	**Sig**		**p.cor**	***p-value***	**Sig**
SOCS1				**SOCS2**			
Purity	-0.345280374	2.81E-15	*	Purity	-0.216449115	1.20E-06	*
Dendritic Cell	0.43172085	1.42E-23	*	Dendritic Cell	0.083773346	0.064440588	n.s.
Neutrophil	0.360093254	3.11E-16	*	Neutrophil	0.172334857	0.000141148	*
Macrophage	0.143753824	0.001502594	*	Macrophage	0.118933662	0.008746798	*
CD4+ T Cell	0.418160286	6.58E-22	*	CD4+ T Cell	0.069459223	0.127011074	n.s.
CD8+ T Cell	0.196302411	1.28E-05	*	CD8+ T Cell	0.07402085	0.102776668	n.s.
B Cell	0.374116231	1.59E-17	*	B Cell	0.081244425	0.074146452	*
SOCS3				**SOCS4**			
Purity	-0.207717669	3.23E-06	*	Purity	-0.060658673	0.178292629	n.s.
Dendritic Cell	0.215890815	1.48E-06	*	Dendritic Cell	0.155963034	0.000544844	*
Neutrophil	0.38323007	2.41E-18	*	Neutrophil	0.249513645	2.74E-08	*
Macrophage	0.19129008	2.22E-05	*	Macrophage	0.080797976	0.075452034	n.s.
CD4+ T Cell	0.129469614	0.00433109	*	CD4+ T Cell	0.148173277	0.001077575	*
CD8+ T Cell	0.111239748	0.014042823	*	CD8+ T Cell	0.143393773	0.001510266	*
B Cell	0.050721674	0.265404286	n.s.	B Cell	0.091617442	0.04394546	*
SOCS5				**SOCS6**			
Purity	0.017054011	0.70534769	n.s.	Purity	0.073604989	0.102253537	n.s.
Dendritic Cell	0.195311596	1.39E-05	*	Dendritic Cell	0.013773928	0.761501158	n.s.
Neutrophil	0.262812068	4.51E-09	*	Neutrophil	0.175750497	0.000103274	*
Macrophage	0.236802136	1.31E-07	*	Macrophage	0.217959027	1.26E-06	*
CD4+ T Cell	0.090930747	0.045559952	*	CD4+ T Cell	0.031008593	0.496131738	n.s.
CD8+ T Cell	0.247574456	3.10E-08	*	CD8+ T Cell	0.113370281	0.01229679	*
B Cell	0.02096485	0.645455446	n.s.	B Cell	-0.114840706	0.011460572	*
SOCS7							
Purity	0.078650012	0.080748163	n.s.				
Dendritic Cell	-0.001605733	0.971776041	n.s.				
Neutrophil	0.062720095	0.16875987	n.s.				
Macrophage	0.047473123	0.296773406	n.s.				
CD4+ T Cell	0.08555479	0.06000074	n.s.				
CD8+ T Cell	0.058818584	0.195044573	n.s.				
B Cell	0.095632585	0.035439602	*				

4. Discussion

Despite advances in understanding the critical functions of different SOCS family members, the complicated and unique actions of SOCSs still require exploration into carcinogenesis and prognosis of lung cancer. We analyzed the data using bioinformatics tools to understand the diverse characteristics of seven SOCS proteins in LUAD. Due to its high recurrence rate and metastasis, lung adenocarcinoma remained a problem [22]. In non-small lung cancer, the lung adenocarcinoma is considered primary lung cancer [23]. The JAK-STAT signaling pathway is at the top of the list, as our research found that members of the SOCS family and their related genes are heavily implicated in this signaling pathway. The distinctive involvement of SOCS genes in malignant processes has been documented in numerous studies [24]. The SOCS genes also regulate antitumor immune responses [25].

In this study, we found lower expression of both SOCS2 and SOCS3 in LUAD tissues compared to the adjacent normal tissues. In addition, we found that higher mRNA expressions of SOCS5 and SOCS6 genes and lower mRNA expression of SOCS 1/3/4/7 and all such expressions were associated with OS in LUAD patients. These findings could help improve lung cancer patients' therapy efficacy and prognosis accuracy. SOCS2 is a target recognition component of an E3 ligase, which belongs to

the ubiquitin ligases family [26]. In a mouse study, SOCS2 deletion enhanced the formation of spontaneous intestinal cancers [27]. Furthermore, reduced SOCS2 expression promotes lung adenocarcinoma invasion and metastasis in vivo and in vitro by modulating epithelial-mesenchymal transition (EMT), primarily dependent on the IGF1/IGF1R-stimulated STAT3/ STAT5 pathway [10]. In line with these findings, our research found reduced SOCS2 expression in LUAD patients. STAT3 regulated SOCS2 in the regulatory networks of SOCS genes. Overexpression of interleukin-6, a proinflammatory cytokine complexed with the epidermal growth factor receptor (EGFR), may activate the gp130/JAK/STAT3 pathway in primary human LUAD, suggesting it as a potential therapeutic target in lung cancer [27]. Another potent oncogenic molecule, STAT3, plays a vital role in causing LUAD in humans and mice [28]. These data suggest that lung cancer progression through underexpression of SOCS2, hence regulating STAT3 expression. Among other SOCSs, the promoter of SOCS3 was methylated in H2228 cells and three cases of 07 EML4-ALK-positive lung cancer tissues [29]. Increasing shreds of evidence link cancer-associated inflammation to loss or underexpression of SOCS3 that might lead to enhanced lung metastasis due to suppressive immunity [30].

Comparing LUAD tissue to normal, we found lower expression of SOCS3, and this low SOCS3 mRNA expression was anticipated to be linked to a longer OS in LUAD patients. In tumor initiation and development, various inflammatory signaling pathways, such as the JAK-STAT, are involved [31]. JAK2/STAT3 signaling abnormalities are seen in multiple cancers, including lung cancer [32]. The activities of SOCS4 in tumor growth and malignancy are far less well understood than those of SOCS2 and SOCS3. In breast cancer, SOCS4 was linked to a poor prognosis [31]. SOCS4 expression was lower in thyroid cancer cells in a recent study [33]. In contrast, an earlier study performed on breast cancer patients, early tumor stage, and better overall survival was linked to overexpression of SOCS4 [34]. However, in our study, low SOCS4 expression was linked to a prolonged OS in LUAD patients. Yoon et al. [35] studied SOCS5/6 expression in multiple human cancers and surrounding control tissues using the Cancer Profiling Array to its full potential. They found leveled expression of both SOCS5 and SOCS6 in cancer patients and healthy populations, indicating these two genes' transcriptional co-regulation. These findings support our results that expression of SOCS5 is significantly and positively linked to SOCS6 expression. Furthermore, elevated SOCS5 expression was linked to advanced tumors and substantially connected with LUAD patients' overall survival.

Similarly, when compared to adjacent normal tissues, SOCS5 was downregulated in tumor tissues in our study. Data on SOCS6 in lung cancer is lacking. However, reduced copy number and mRNA expression of SOCS6 was associated with disease recurrence in primary LUSC patients, suggesting that SOCS6 could be useful as a predictive biomarker [36]. Circular RNA circ 103820 suppresses lung cancer tumorigenesis by sponging miR-200b-3p to release LATS2 and SOCS6 [37]. However, in the current study, we noticed that SOCS6 was upregulated in LUAD tumor tissues, which was previously reported in breast cancer [36].

Further research is needed to better understand the role of SOCS6 in lung cancer. SOCS7 expression did not differ substantially between cancer and control samples; however, it was shown to be higher in LUAD tumor tissues when compared to adjacent normal tissues. LUAD had no information about SOCS7, despite studies on other cancer types being found. For instance, Sasi et al. [34] revealed that high SOCS7 expression was linked to early-stage malignancies and a better prognosis in breast cancer patients. Only a few researchers have attempted to prove the functional significance of SOCS family members in LUAD to yet. Initially, the current work focused solely on bioinformatics and imaging data (i.e., immunohistochemistry). We will need to conduct further prospective clinical studies and additional tests to confirm our findings. In addition, more research comparing COSC proteins to other cancer prognostic indicators is required. Future enhancements may incorporate novel ideas, such as incorporating wet lab methodologies and integrating hormonal, using extracts and nanomedicine approaches, building upon the insights gleaned from earlier reports [38, 39, 40, 41, 42, 43].

We investigated the expression, genetic changes, prognostic value, Gene Ontology enrichment, the association among SOCS family members, and immune infiltration of seven SOCS proteins using

multiple additional online tools. The functional importance of SOCS family members in LUAD was collected and identified in the current study.

Conclusions

In the context of LUAD, SOCS2 stands out as a potential candidate for therapeutic targeting, while SOCS3 appears to have promise as a prognostic factor for lung adenocarcinoma. Additionally, we identified that genes associated with SOCS3 play a role in the JAK-STAT signaling pathway. Furthermore, SOCS5 and SOCS6 exhibit characteristics of tumor suppressor genes, whereas SOCS1, SOCS3, SOCS4, and SOCS7 appear to be associated with promoting tumor growth. Consequently, our current findings validate and endorse the use of bioinformatics as a reliable initial approach for evaluating lung adenocarcinoma and discovering novel and advanced biomarkers and therapeutic targets in the treatment of lung cancer.

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Conflict of interest

The authors declare there is no conflict of interest.

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