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Original Article

Decoding signal transducer and activator of transcription 1 across various cancers through data mining and integrative analysis

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Abstract: Objectives: Using different online available databases and Bioinformatics tools, we extensively studied the role STAT1 across different cancers. Methods: STAT1 mRNA, protein expression, and promoter methylation were analyzed and validated using UALCAN, GENT2, Human Protein Atlas (HPA), and MEXPRESS. Furthermore, the potential prognostic values were evaluated through KM plotter. Then, cBioPortal was utilized to examine the STAT1-related genetic mutations, while pathway enrichment analysis was performed using DAVID. To identify STAT1 targeted microRNAs (miRNAs) and transcription factors (TFs) we used Enricher. Moreover, a correlational analysis between STAT1 expression tumor purity and CD8+ T immune cells and a gene-drug interaction network analysis was performed using TIMER, CTD, and Cytoscape. Results: In 23 major human cancers, STAT1 expression was notably up-regulated relative to corresponding controls. As well, the elevated expression of STAT1 was exclusively found to be associated with the reduced overall survival (OS) of Esophageal Carcinoma (ESCA), Kidney Renal Clear Cell Carcinoma (KIRC), and Lung adenocarcinoma (LUAD) patients. This implies that STAT1 plays a significant role in the development and progression of these three cancers. Further pathway analysis indicated that STAT1 enriched genes were involved in six critical pathways, while a few interesting correlations were also documented between STAT1 expression and promoter methylation level, tumor purity, CD8+ T immune cells infiltration, and genetic alteration. In addition, we have also predicted a few miRNAs, TFs, and chemotherapeutic drugs that could regulate the STAT1 expression. Conclusion: The current study revealed the shared oncogenic, diagnostic, and prognostic role of STAT1 in ESCA, KIRC, and LUAD.

Keywords: Cancer, diagnostic, CD8+ T immune cells, STAT1, OS analysis, prognostic

Introduction

Worldwide, cancer malignancy is the 2nd leading cause of death in humans behind ischemic heart disease [1]. According to an estimate in 2020, a total of >1.8 million new cancer cases and 606,520 cancer-related deaths were recorded around the globe [2]. It is also projected that by 2030, over >26 million new cancer cases and 17 million cancer-related deaths will be recorded per year [3]. Environmental factors, such as tobacco consumption, exposure to high energy rays including ultraviolet (UV) radiation, radon gas, and exposure to infectious agents have been considered as some of the responsible factors for this disease [4]. Cancer is also considered an outcome of somatic and germline mutations in various DNA repair and tumor suppressor genes including TP53, BRCA1/2, and APC [5]. Mutations are very prevalent in

those genes, for instance, and it is estimated that an average cancer patient harbors around 74 point mutations in these genes [6].

Furthermore, the worldwide distribution of different cancer subtypes, which predominantly continues to evolve, accounts for 50% of all the cancers worldwide; mainly in low and middle-income countries this proportion was at 55% in 2007 and is forecasted to hit 61% by 2050 [7]. Major cancer subtypes including lung cancer, breast cancer, lung, kidney, and colon/rectal cancers are no longer limited to Western countries but now are most prominent in other countries as well [7].

Prevention measures and treatment strategies particularly focusing on environmental factors have been used globally [8], but very little progress has been made in reducing cancer incidence. Hence, there is an urgent need to explore the underlying biological mechanisms of carcinogenesis and investigate the possible potential diagnostic and prognostic biomarkers that could be commonly employed to different cancers and help in managing the disease.

STAT1 (signal transducer and activator of transcription 1) and ISGF-3 (transcription factor) are key transcription factors (TF) involved in interferon (IFN)-related intracellular signaling [9]. Inside the nucleus, phosphorylation of STAT molecules is done by receptor-associated kinases, making them able to act as transcription factors. Among all the known STAT molecules, STAT1 is capable of being activated through several ligands including Interferon-alpha (IFN-α), Interferon-gamma (IFN-γ), and Epidermal Growth Factor (EGF), etc. [10]. It has been reported that STAT1 plays an anti-oncogenic role by up-regulating the caspases [11], Cyclin-dependent kinase inhibitor 1A [12], the IFN-regulatory factor 1 (IRF1), p53 pathway [13], and down-regulating the BCL2 family members [14].

Increasing evidence has shown the up-regulation of STAT1 in malignant tumors, such as breast and ovarian tumors [15]. Patients with high STAT1 expression levels were found to have worse clinical outcomes relative to patients having low expression levels of STAT1 expression [16]. However, on the other side of the coin, the loss of STAT1 expression was also found in colorectal and breast tumors [17]. Moreover, evidence from *in vivo* studies based on STAT1 knockout mice revealed that STAT1 deficiency may also increase the susceptibility of ovarian teratoma development [12, 13]. In summary, the mechanisms regarding the oncogenic role of STAT1 in human cancers is yet unclear and demands further derailed research.

Here in this study, we briefly analyzed the STAT1 expression, potential function, and its association with prognostic values of different cancer patients through publically available large databases and Bioinformatics tools. Furthermore, to reveal the potential role of STAT1 in cancer development and progression, we have also performed a series of additional analysis.

Materials and methods

UALCAN database

The UALCAN database (http://ualcan.path.uab.edu) has made TCGA cancer OMICS data easily accessible for cancer researchers [18]. We used this database for the pan-cancer gene expression analysis to document the differential mRNA expression level of STAT1 across 24 major subtypes of human cancer. In addition, we also utilized this platform to document the STAT1 targeted miRNAs expression and to perform the re-analysis of STAT1 expression in cancer patients with different clinicopathological features showing the significant dysregulation of STAT1. The transcription expression level of STAT1 was measured in terms of transcript per million (TPM) reads and a Student t-test was applied in UALCAN to compare the expression differences between normal and cancer groups. A *P*-value <0.05 was used to indicate the significant scores.

KM plotter

The KM plotter (http://kmplot.com/analysis/index.php?p=service) is based on mRNA expression and survival data of cancer patients obtained from the Gene Expression Omnibus (GEO) database [19]. In our study, we entered STAT1 into the search box of this database to obtain overall survival (OS) plots. Hazard ratios (HR) with 95% confidence intervals (CI) and log-rank *P*-value (<0.05) were determined and displayed.

GENT2 database

GENT2 database (http://gent2.appex.kr/) offers the reliable and accurate multi-omics analy-

sis of the cancer-related TCGA data [20]. In this study, for the validation of STAT1 expression across distinct cancer subtypes, we employed this tool to analyze the STAT1 differential expression patterns in new independent cancer. This database uses Student t-test to compare the expression differences between normal and cancer groups with a *P*-value <0.05 which was used to indicate the significant scores.

Data mining through human protein atlas (HPA)

Here in this study, the protein expression of STAT1 in cancerous tissues along with normal controls was examined using HPA (http://www.proteinatlas.org/) [21]. The data of protein expression is taken in this database from immunohistochemistry (IHC)-based experiments and its level is graded as not detected, low, medium, and high, depending on the staining intensity and proportion of the stained cells. A Student t-test was used to compare the expression differences between normal and cancer samples and *P*-value <0.05 was used to indicate the significant scores.

MEXPRESS database

MEXPRESS database (https://mexpress.be/) was developed to visualize the TCGA expression data and identify associations among the levels of promoter methylation and genes expression [22]. In this study, correlations among STAT1 mRNA expression levels and promoter methylation in distinct cancer subtypes were computed via this tool using Pearson correlation analysis. A *P*-value <0.05 was used to indicate the significant scores.

cBioportal database

The cBioPortal (http://cbioportal.org) database encompasses multi-omics data from more than 240 cancer studies [23]. We used this tool to evaluate the STAT1 genetic alterations in TCGA dataset of distinct cancers.

PPI network making and pathway analysis

STRING database (http://string-db.org/) is a valuable resource for making PPI of the genes of interest [24]. In the present study, we utilized this user-friendly resource to obtain the PPI network of the STAT1 enriched genes. Following that, the PPI network was visualized using Cytoscape [25] and the pathway analysis of the STAT1 enriched genes was performed through DAVID [26]. A *P*-value <0.05 was used to indicate the significant scores.

Enrichr database analysis

Enrichr (https://maayanlab.cloud/Enrichr/) [27] application in-house the many gene set libraries, which enables researchers to find different enrichment terms for examples pathway enrichment, gene-miRNA enrichment, and gene-specific transcription factors enrichment (TFs). In our study, we used this database to identify the STAT1 targeted miRNAs and the TFs. The top 10 significantly enriched items were displayed using Enrichr. A *P*-value was computed with the Fisher exact test and <0.05 score was considered as significant.

Correlations between STAT1 expression, tumor purity, and CD8+ T immune cells infiltration

TIMER (https://cistrome.shinyapps.io/timer/) is a valuable resource for computing the correlation between gene expression, tumor purity, and immune cells infiltration [28]. In the current study, we computed the correlations between the tumor purity, CD8+ T immune cells infiltration, and STAT1 expression in distinct cancer subtypes through Spearman analysis. A *P*-value <0.05 was used to indicate the significant scores.

Exploring STAT1-related chemotherapeutic drugs

The Comparative Toxicogenomics Database (CTD) [29] was used in this study for searching chemotherapeutic drugs that can reduce or increase the expression of STAT1 via gene-drug interaction network.

Results

STAT1 expression across different human cancers

Across 24 human cancers, the STAT1 expression level was analyzed via UALCAN platform. Among all the analyzed cancer samples, STAT1 was found to be significantly (P<0.05) down-regulated in kidney chromophobe (KICH)

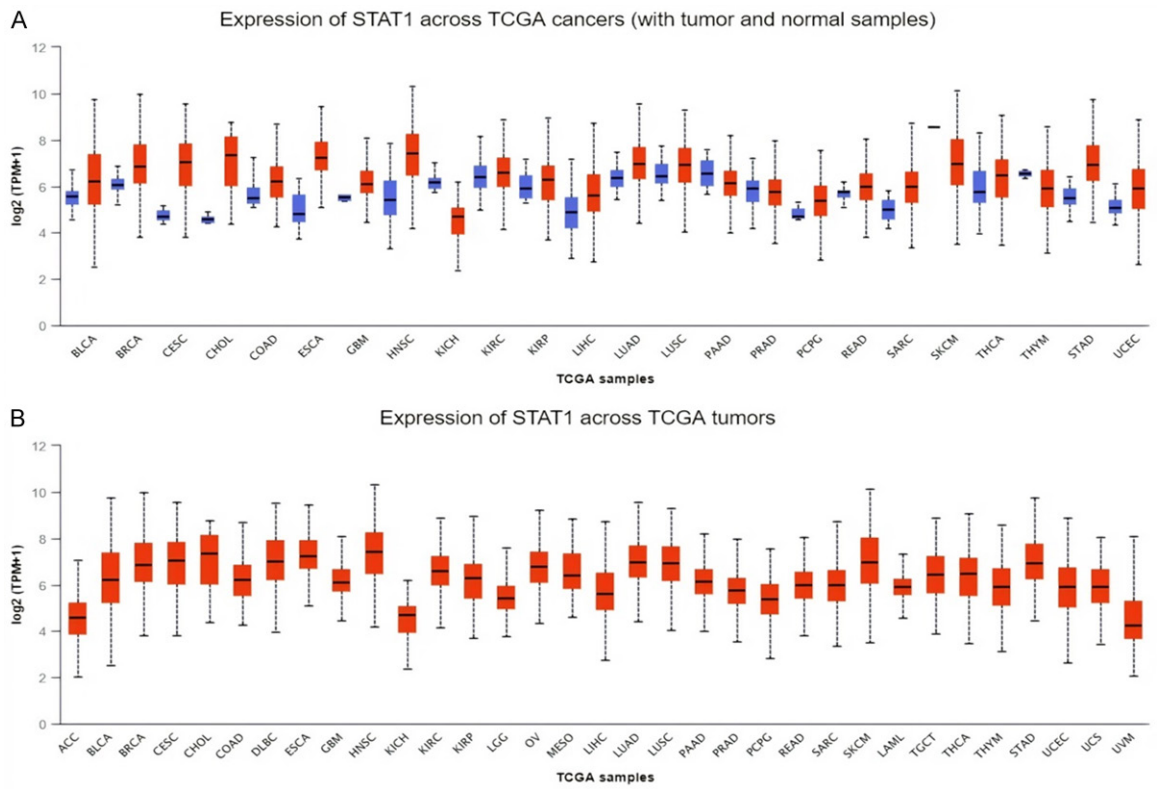


Figure 1. The pattern of STAT1 expression in different human cancers. (A) The relative pattern of STAT1 expression in cancer samples, and (B) the pattern of STAT1 expression in cancer samples relative to controls. A *P*-value <0.05 was used to indicate the significant scores.

samples relative to normal controls, while significantly (P<0.05) up-regulated in all the remaining 23 subtypes of the human cancers including esophageal carcinoma (ESCA), kidney renal clear cell carcinoma (KIRC), and lung adenocarcinoma (LUAD) (**Figure 1**).

Overexpressed STAT1 is correlated with reduced survival duration

We further explored the effect of overexpressed STAT1 on the OS duration of different cancer patients via KM plotter tool. Based on the median expression, the analyzed patients were categorized into high and low expression groups. In view of our results, from all the cancer patients having higher STAT1 expression, only ESCA, KIRC, and LUAD cancer patients had reduced survival duration than those having low STAT1 expression (**Figure 2**). Altogether, this data suggested that STAT1 might have a significant contribution to the development and progression of ESCA, KIRC, and LUAD, thus the next part of our study will mainly focus on the unique role of STAT1 in these 3 types of human cancers.

STAT1 correlated with cancer stages, patients genders, and nodal metastasis

Correlations among STAT1 expression and different clinicopathological characteristics including cancer stages, patient gender, and nodal metastasis were also analyzed via UALCAN. In view of our results, STAT1 was also found significantly (P<0.05) overexpressed in ESCA, KIRC, and LUAD patients of different clinicopathological characteristics relative to controls (**Figures 3**-**5**). A clinicopathological characteristics-wise ESCA, KIRC, and LUAD patients classification samples is summarized in **Tables 1** and **2**.

STAT1 expression validation on new independent cancer cohorts

For validating STAT1 expression in ESCA, KIRC, and LUAD, GENT2-based expression analysis was conducted in the present study on new independent cancer cohorts. Results of the analysis suggested that STAT1 expression was also significantly (P>0.05) elevated in patients with ESCA, KIRC, and LUAD samples relative to

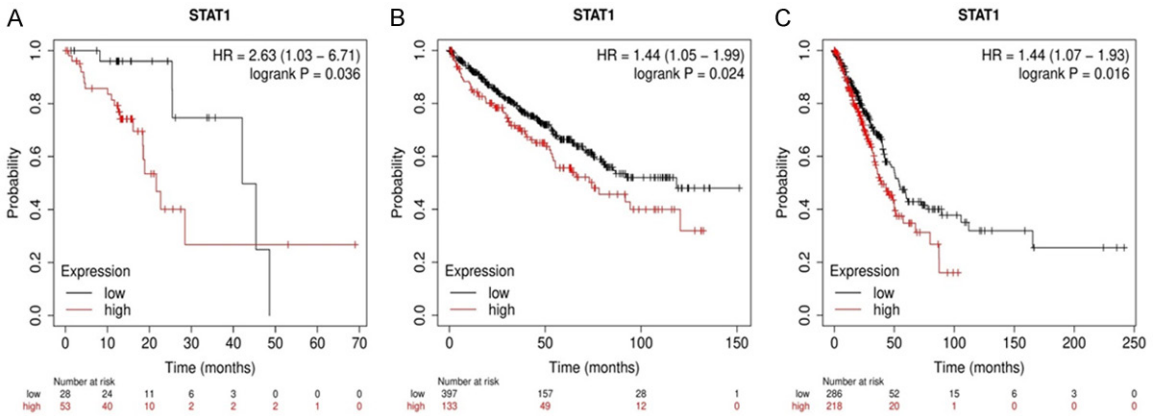


Figure 2. Overexpressed STAT1 is correlated with the reduced OS of different cancers. (A) Overexpressed STAT1 is correlated with the reduced OS of ESCA, (B) Overexpressed STAT1 is correlated with the reduced OS of KIRC, and (C) overexpressed STAT1 is correlated with the reduced OS of LUAD. A *P*-value <0.05 was used to indicate the significant scores.

corresponding controls (**Figure 6**). **Table 3** lists the ESCA, KIRC, and LUAD datasets that were utilized for STAT1 expression validation.

Expression analysis of STAT1 at the protein level in esophageal, kidney, and lung cancers

After evaluating the mRNA expression level of STAT1, its proteomics level was accessed using the HPA database. Results revealed that STAT1 was not detected in normal esophageal, kidney, and lung tissues whereas its medium expression was found in esophageal and higher expression was observed in lung, and kidney cancer tissues (**Figure 7**). Collectively, these results also suggested the STAT1 protein overexpression in ESCA, KIRC, and LUAD.

Promoter methylation analysis of STAT1

Earlier, it was reported that the dysregulation of functional genes due to hypermethylation of the promoter region leads to cancer [30]. To find the impact of promoter methylation on STAT1 expression, we herein analyzed the STAT1 promoter methylation status in ESCA, KIRC, and LUAD using MEXPRESS resource. Based on the results, promoter methylation values obtained from the different methylation probes in ESCA, KIRC, and LUAD were found to be significantly (P>0.05) negatively correlated with STAT1 expression levels (**Figure 8**).

Genetic mutations analysis of STAT1

ESCA-related genetic alterations information was obtained from a TCGA ESCA (TCGA, Nature 2017) dataset encompassing 559 cancerous samples, while in KIRC, the same information was explored via TCGA KIRC (TCGA, Nature 2013) dataset encompassing 446 cancerous samples. Finally in LUAD, a TCGA LUAD (TCGA, Nature 2014) dataset encompassing 230 cancerous samples were used to obtain the genetic alterations information of STAT1.

Results revealed that STAT1 harbors genetic alterations in only a small proportion of the analyzed samples, for instance, in 2.4% cases of the ESCA with maximum deep amplification genetic abnormality, in 1.9% cases of the KIRC, and 2.2% cases of LUAD with maximum missense mutations, and deep amplification genetic abnormalities, respectively (**Figure 9**).

PPI network making and pathway analysis

A PPI network of STAT1 was made via STRING database to recognize the STAT1 enriched genes. In total, one set of 10 STAT1 enriched genes was identified via this PPI network (**Figure 10A**). We next carried out the pathway analysis of these STAT1 enriched genes via DAVID tool. Results revealed that mostly STAT1 enriched genes were significantly involved in various diverse pathways including “Influenza A”, “Hepatitis B”, “Measles”, and “Coronavirus disease” (**Figure 10**).

Identification of miRNAs and TFs that potentially regulate STAT1

Enrichr database was utilized to predict the STAT1 targeted miRNAs and TFs. In total, there

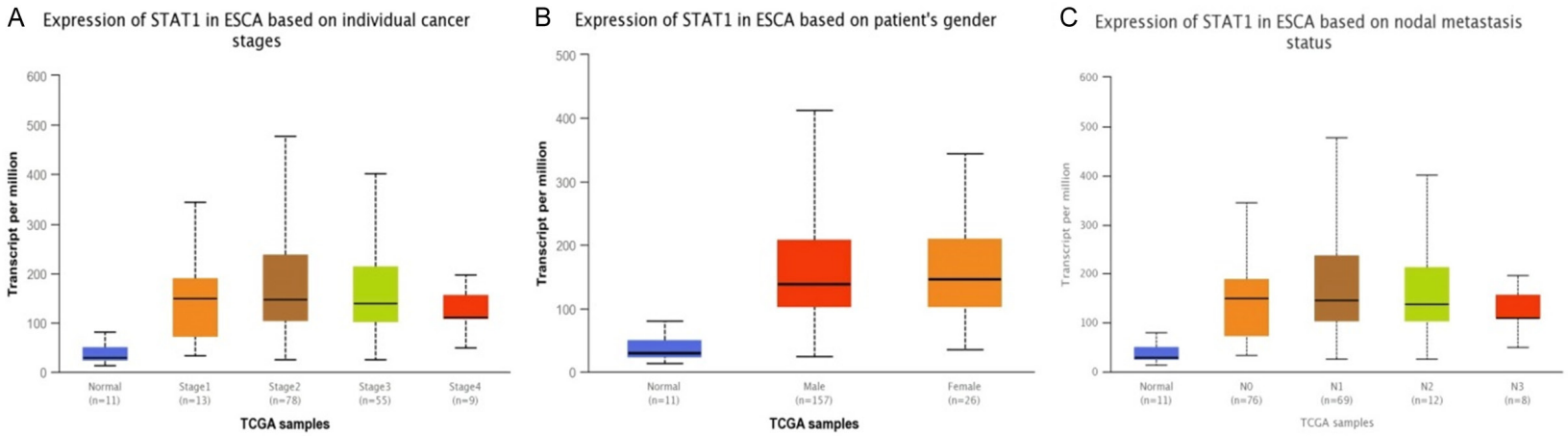


Figure 3. Correlations between STAT1 expression and ESCA patients with different clinicopathological parameters. (A) Correlation of STAT1 with ESCA patients of different cancer stages, (B) Correlation of STAT1 with ESCA patients of different genders, and (C) correlation of STAT1 with ESCA patients of different nodal metastasis statuses. A *P*-value <0.05 was used to indicate the significant scores.

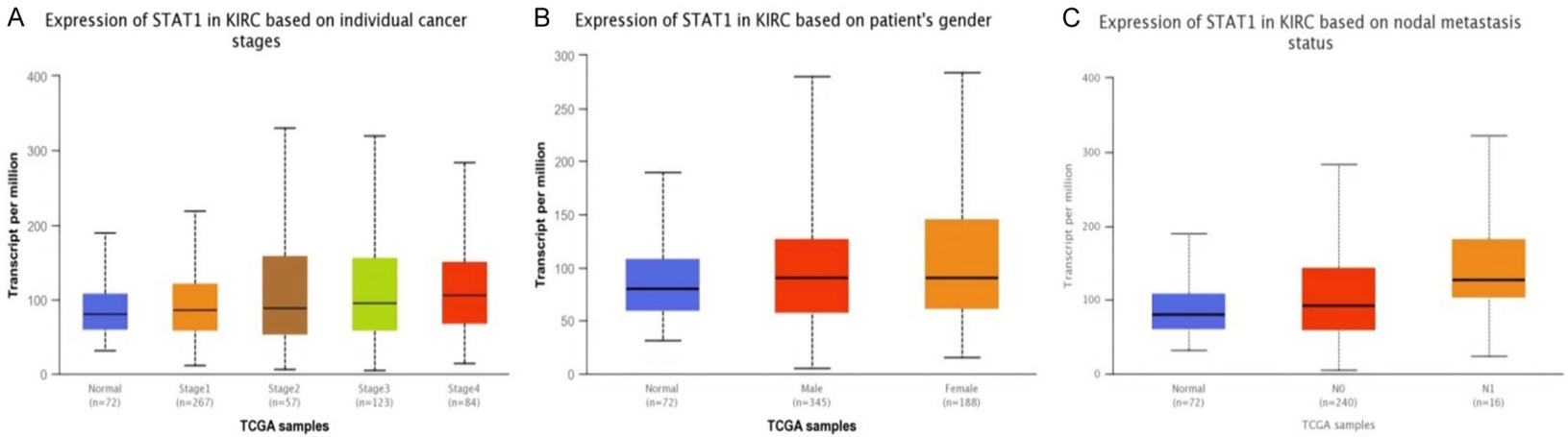


Figure 4. Correlations between STAT1 expression and KIRC patients with different clinicopathological parameters. (A) Correlation of STAT1 with KIRC patients of different cancer stages, (B) Correlation of STAT1 with KIRC patients of different genders, and (C) correlation of STAT1 with KIRC patients of different nodal metastasis statuses. A *P*-value <0.05 was used to indicate the significant scores.

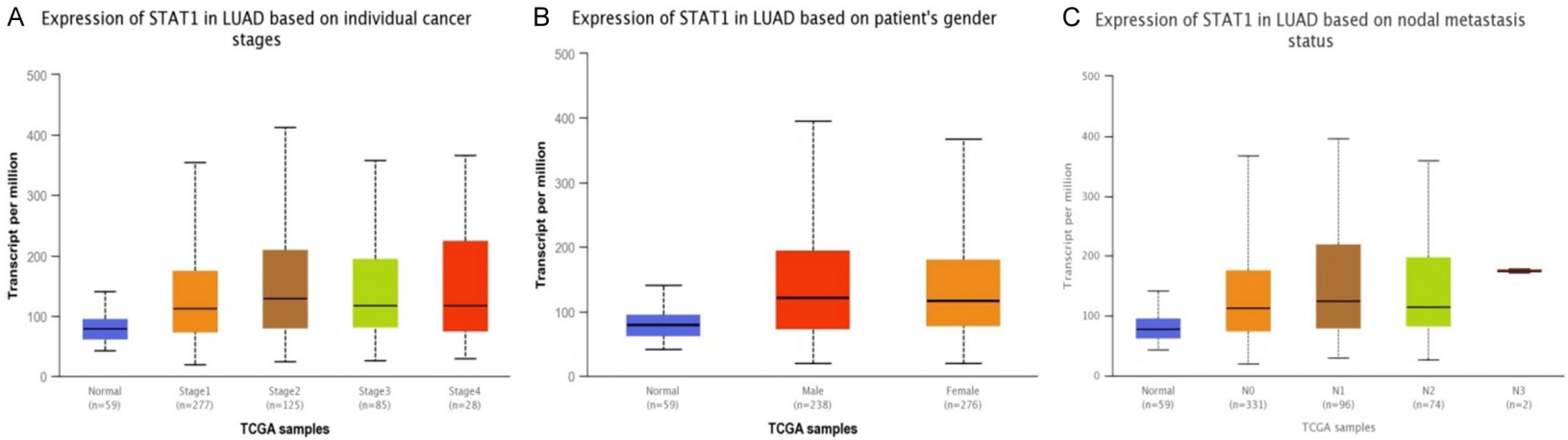


Figure 5. Correlations between STAT1 expression and LUAD patients with different clinicopathological parameters. (A) Correlation of STAT1 with LUAD patients of different cancer stages, (B) Correlation of STAT1 with LUAD patients of different genders, and (C) correlation of STAT1 with LUAD patients of different nodal metastasis statuses. A *P*-value <0.05 was used to indicate the significant scores.

Table 1. Clinicopathological parameters-based classification of ESCA and KIRC patients

Clinicopathological parameters-based classification of ESCA patients					
Sr. No	Clinicopathological parameter	Number of samples per parameter	Sum of ESCA samples	Sum of excluded samples due to missing details	Total number of samples undertaken analysis
1	Cancer stage distribution				
	Stage 1 (n)	13		29	155
	Stage 2 (n)	78			
	Stage 3 (n)	55			
	Stage 4 (n)	09			
2	Gender distribution				
	Male (n)	157	184	01	183
	Female (n)	26			
3	Nodal metastasis statuses based distribution				
	N0 (n)	76		24	160
	N1 (n)	69			
	N2 (n)	12			
	N3 (n)	03			
Clinicopathological parameters-based classification of KIRC patients					
Sr. No	Clinicopathological parameter	Sum of samples per parameter	Sum of KIRC samples	Sum of excluded samples due to missing details	Total number of samples undertaken analysis
1	Cancer stage distribution				
	Stage 1 (n)	267			
	Stage 2 (n)	57		02	531
	Stage 3 (n)	121			
	Stage 4 (n)	84			
2	Gender distribution				
	Male (n)	345	533	00	533
	Female (n)	188			
3	Nodal metastasis statuses based distribution				
	N0 (n)	240		177	356
	N1 (n)	16			

Table 2. Clinicopathological parameters-based classification of LUAD patients

Clinicopathological features of the LUAD cohort					
Sr. No	Clinicopathological parameter	Sum of samples per parameter	Sum of LUAD samples	Sum of excluded samples due to missing details	Total number of samples undertaken analysis
1	Cancer stage distribution				
	Stage 1 (n)	277		0	515
	Stage 2 (n)	125			
	Stage 3 (n)	85			
	Stage 4 (n)	28			
2	Gender distribution				
	Male (n)	238	515	01	514
	Female (n)	276			
3	Nodal metastasis statuses based distribution				
	N0 (n)	96		01	514
	N1 (n)	69			
	N2 (n)	74			
	N3 (n)	02			

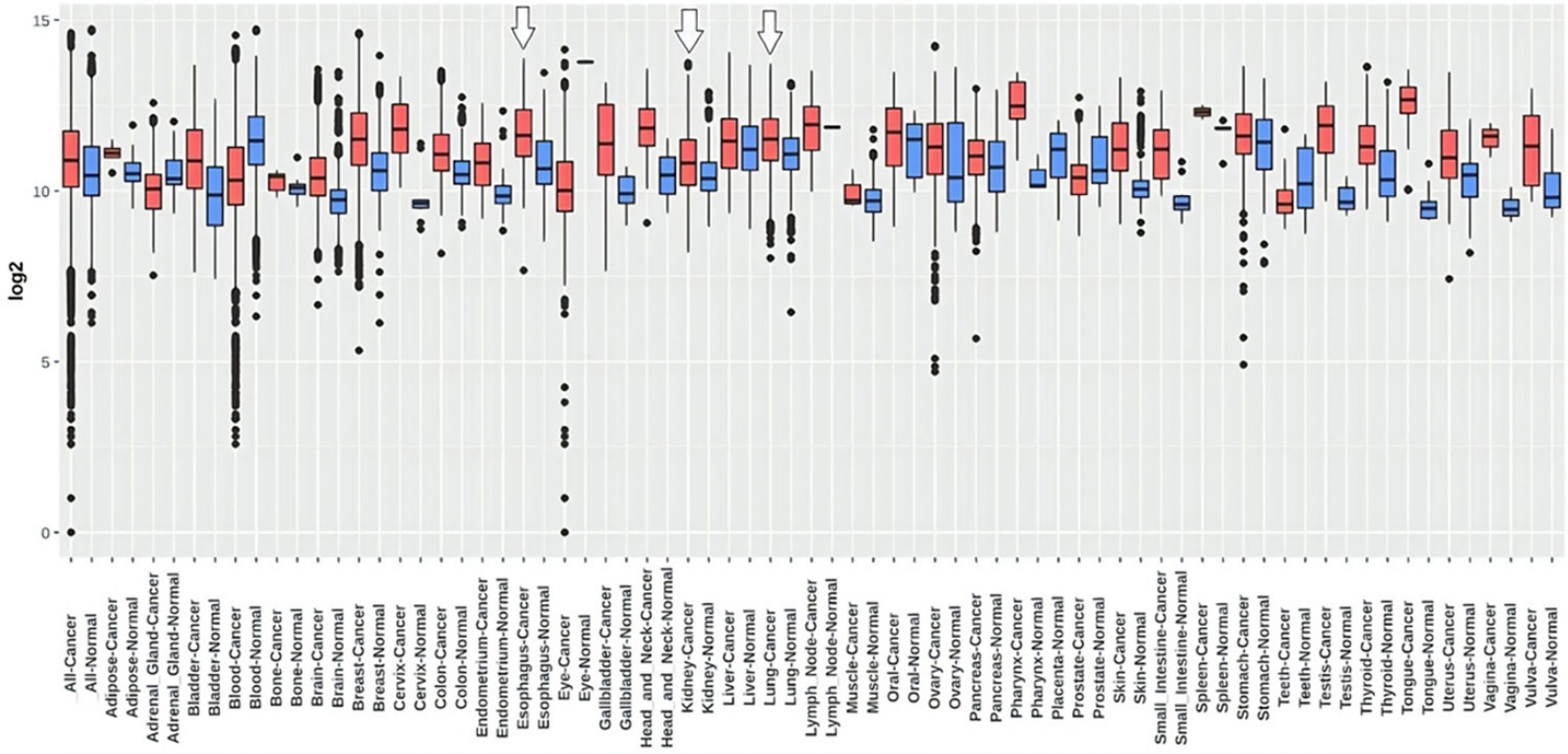


Figure 6. STAT1 expression in ESCA, KIRC, and LUAD patients belonging to new independent cohorts. Blue color; normal samples and red color; cancer samples. A *P*-value <0.05 was used to indicate the significant scores.

Table 3. Detail of ESCA, KIRC, and LUAD datasets utilized for the STAT1 expression validation

Sr. No	Cancer	Datasets	Source
1	ESCA	GSE63941, GSE2109, GSE34111, GSE45670, GSE17351, GSE51021, GSE63941, GSE21293, GSE22954, GSE26886, GSE33810, GSE17351, GSE43346, GSE45670, and GSE42363	Affymetrix U133A and U133 Plus2 microarray platforms
2	KIRC	GSE47352, GSE53224, GSE53757, GSE7023, GSE7392, GSE11151, GSE46699, GSE68629, GSE12606, GSE53757, GSE8271, GSE19982, GSE36895, GSE11045, GSE22541, GSE14762, GSE2109 GSE11151, and GSE12090	
2	LUAD	GSE37745, GSE40791, GSE5058, GSE43346, GSE43580, GSE10445, GSE40791, GSE2109, GSE77803, GSE50081, GSE30219, GSE33532, GSE63074, GSE64766, GSE19188, GSE27262, and GSE7307	

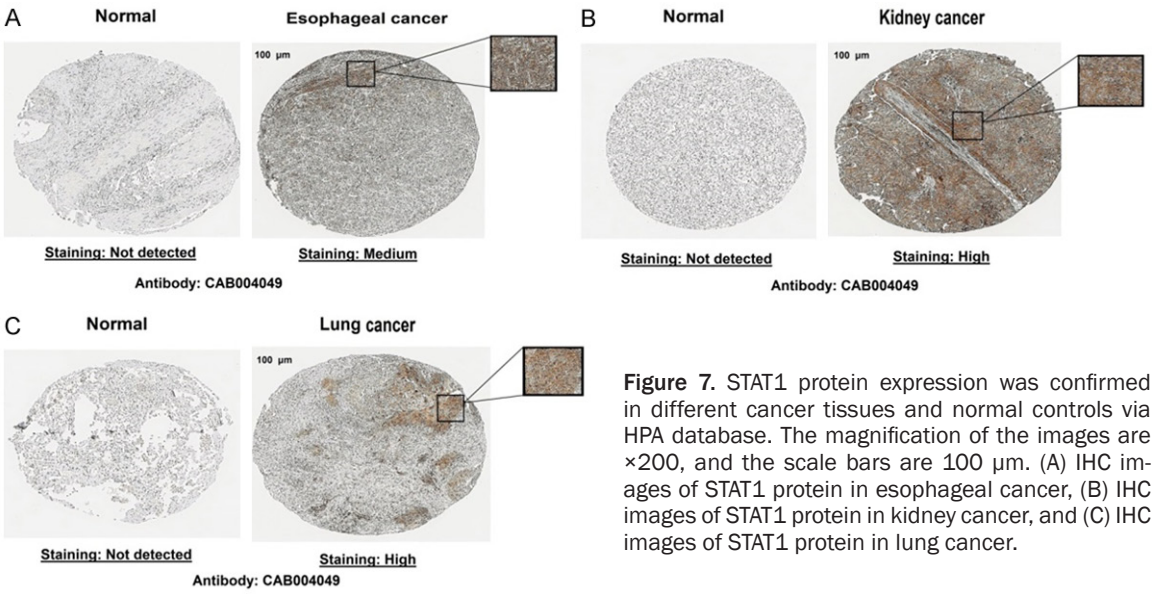


Figure 7. STAT1 protein expression was confirmed in different cancer tissues and normal controls via HPA database. The magnification of the images are ×200, and the scale bars are 100 µm. (A) IHC images of STAT1 protein in esophageal cancer, (B) IHC images of STAT1 protein in kidney cancer, and (C) IHC images of STAT1 protein in lung cancer.

were 10 most significant miRNAs (hsa-miR-450-5p, mmu-miR-3082-3p, mmu-miR-351-5p, mmu-miR-875-5p, mmu-miR-125a-5p, mmu-miR-23a-3p, mmu-miR-146a-5p, mmu-miR-3102-3p, mmu-miR-125b-5p, and hsa-miR-4693a-5p) and 10 TFs (CIITA, LMO2, IRF1, ATF3, BCL3, STAT2, STAT5B, STAT5A, VDR, and HSF1) that were identified which potentially regulate STAT1 expression (**Figure 11A**, **11B**). Taken together, these clues highlighted that STAT1 expression can be regulated by a variety of factors. Moreover, the latest research has shown that dysregulation of miRNAs is closely associated with cancer development [31]. In view of this, we further performed the expression profiling of STAT1 targeted miRNAs via UALCAN. Results of the analysis revealed that hsa-miR-450-5p is the only miRNA that is up-regulates significantly in ESCA, KIRC, and LUAD as a possible common modulator of oncogenesis (**Figure 11C**).

STAT1 has correlations with tumor purity and CD8+ T immune cells infiltration

The knowledge of tumor purity and CD8+ T immune cell infiltration in correlation with gene expression has laid the foundation of improved cancer immunotherapies presently [32]. Therefore, in this study, correlations among tumor purity, CD8+ T immune cells infiltration, and STAT1 in ESCA, KIRC, and LUAD were calculated using the TIMER database. Results revealed a significant ($P>0.05$) negative correlation between tumor purity and mRNA expression of STAT1 in ESCA, KIRC, and LUAD while a significant ($P>0.05$) positive correlation between CD8+ T immune cells infiltration and STAT1 expression (**Figure 12**).

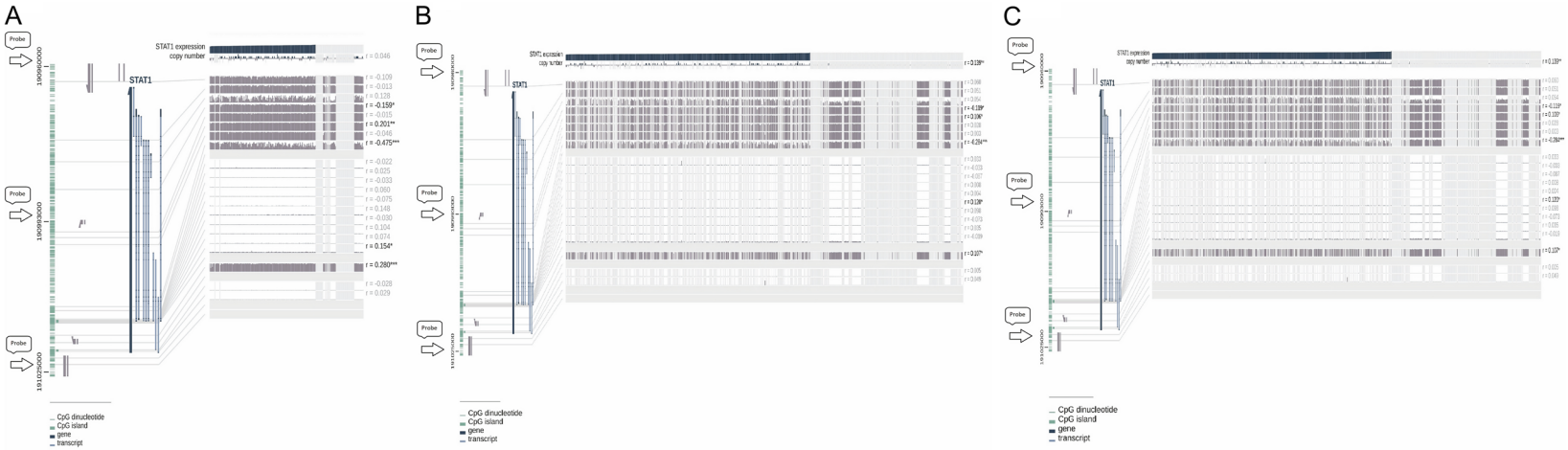


Figure 8. Correlations among STAT1 expression and its promoter methylation level in different cancers. (A) Correlations among STAT1 expression and its promoter methylation level in ESCA, (B) Correlations among STAT1 expression and its promoter methylation level in KIRC, and (C) correlations among STAT1 expression and its promoter methylation level in LUAD. A *P*-value <0.05 was used to indicate the significant scores. A minus sign represents the negative correlation.

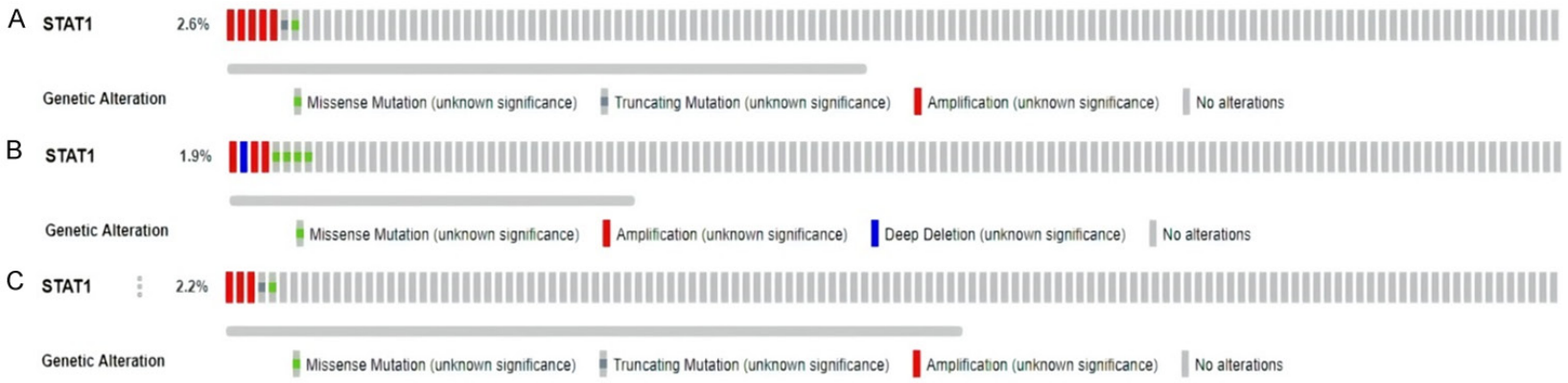
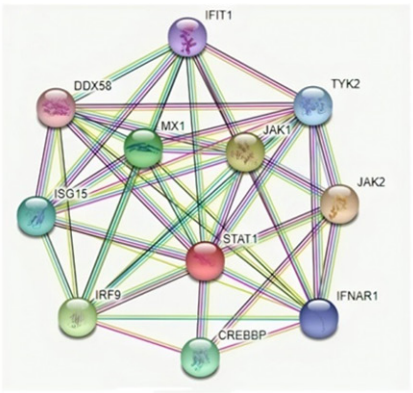


Figure 9. Genetic alterations analysis of the STAT1 in TCGA ESCA, KIRC, and LUAD datasets, (A) STAT1 genetic alterations analysis in TCGA ESCA dataset, (B) STAT1 genetic alterations analysis in TCGA KIRC dataset, (C) STAT1 genetic alterations analysis in TCGA LUAD dataset.

A



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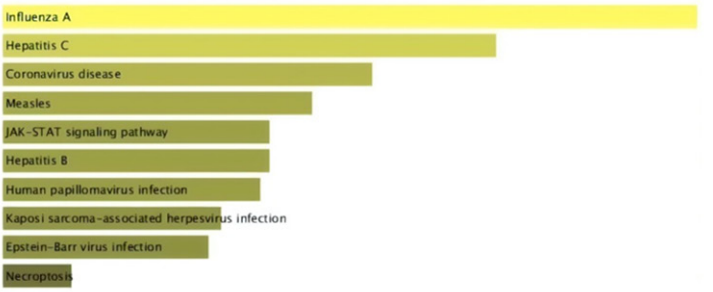


Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Influenza A	1.128e-17	6.545e-16	547.34	21359.04
2	Hepatitis C	1.947e-15	5.647e-14	355.08	12027.33
3	Measles	2.168e-13	3.143e-12	263.26	7676.54
4	Coronavirus disease	4.651e-14	8.992e-13	235.30	7223.44
5	JAK-STAT signaling pathway	6.446e-13	6.231e-12	223.93	6285.80
6	Hepatitis B	6.446e-13	6.231e-12	223.93	6285.80
7	Kaposi sarcoma-associated herpesvirus infection	2.231e-12	1.618e-11	186.32	4998.65
8	Epstein-Barr virus infection	3.080e-12	1.985e-11	177.64	4708.50
9	Human papillomavirus infection	8.174e-13	6.773e-12	162.36	4518.94
10	Necroptosis	1.026e-10	5.953e-10	155.58	3578.22

Figure 10. A PPI network and pathway analysis of STAT1-associated genes. (A) A demonstration of the PPI network, and (B) A heatmap of STAT1-associated genes enriched pathways.

Exploring STAT1-related chemotherapeutic drugs

Based on the gene-drug interaction network constructed via CTD database and Cytoscape, it was observed that STAT1 expression can be regulated by a variety of drugs. For example, trentinoin and lipopolysaccharides can elevate STAT1 expression while tinuvin and methotrexate can reduce STAT1 expression level (**Figure 13**).

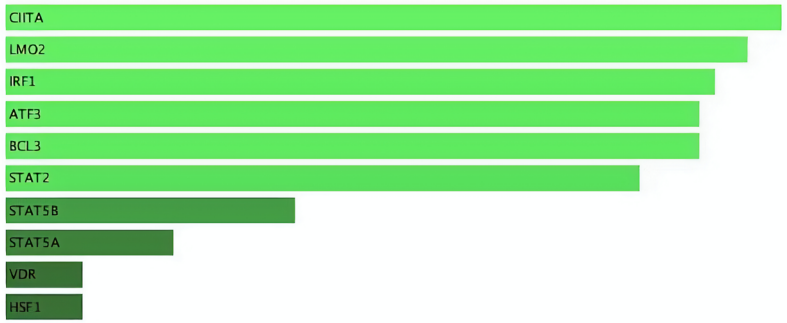
Discussion

Despite the great advances in early detection and accurate treatment, cancer is still a major threat to human survival worldwide and still, there is a lack of efficient biomarkers for its early diagnosis and predicting prognosis. Therefore, the discovery of shared biomarkers that could be used for detection, prediction of prognosis, and treatment of cancer patients without serious complications is needed [33].

STAT1 is a 91-kDa protein and an important regulator of interferon (IFN) signaling pathways [34]. It participates in cytokine-induced signaling pathways, and can further serve as a cell growth inhibitor and mediator of apoptosis [35]. Regarding the role of STAT1 in cancer development and progression, previous studies have reported conflicting results. For example, a few studies have reported that STAT1 restrains the proliferation of cancerous cells including lung, and colorectal cancer cells [36, 37]. Contrary to this, dysregulation of STAT1 has also been observed in different human cancers including pleural mesothelioma, renal cell carcinoma, and breast cancer [38, 39]. Prior to our knowledge, limited information is available in the medical literature regarding STAT1 dysregulation in other subtypes of human cancer, therefore, the current study was initiated to uncover the possible oncogenic, diagnostic, and prognostic roles of STAT1 in certain human cancers subtypes based on the data mining and integrative analyses.

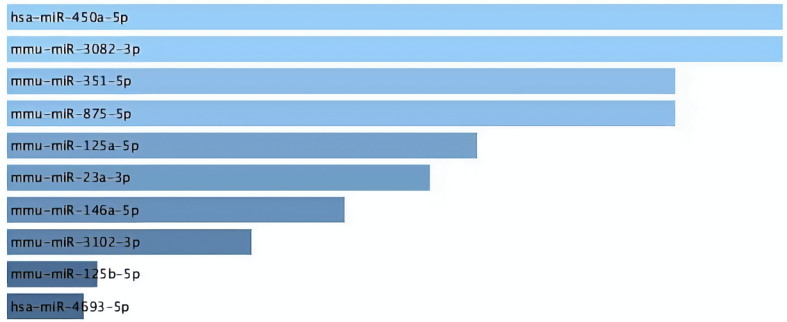
In this study, STAT1 expression in cancer patients and normal individuals were explored through computational methods, and in view of our results, STAT1 was found to be down-regulated in KICH samples while up-regulated in 23 different other cancer samples as shown in **Figure 1**. Next, via survival analysis of all the cancer patients, it was observed that STAT1 overexpression was significantly ($P<0.05$) correlated with the decreased OS duration of

A



Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	CIITA	0.002050	0.009583	19959.00	123544.89
2	LMO2	0.002150	0.009583	19957.00	122581.99
3	IRF1	0.002250	0.009583	19955.00	121662.50
4	ATF3	0.002300	0.009583	19954.00	121217.83
5	BCL3	0.002300	0.009583	19954.00	121217.83
6	STAT2	0.002500	0.009583	19950.00	119530.06
7	STAT5B	0.004050	0.01202	19919.00	109734.82
8	STAT5A	0.004800	0.01202	19904.00	106270.50
9	VDR	0.005450	0.01202	19891.00	103674.93
10	HSF1	0.005450	0.01202	19891.00	103674.93

B



Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	hsa-miR-450a-5p	0.0008500	0.009428	19983.00	141285.77
2	mmu-miR-3082-3p	0.0008500	0.009428	19983.00	141285.77
3	mmu-miR-351-5p	0.001000	0.009428	19980.00	138017.41
4	mmu-miR-875-5p	0.001000	0.009428	19980.00	138017.41
5	mmu-miR-125a-5p	0.001350	0.009428	19973.00	131975.02
6	mmu-miR-23a-3p	0.001450	0.009428	19971.00	130534.69
7	mmu-miR-146a-5p	0.001650	0.009428	19967.00	127928.56
8	mmu-miR-3102-3p	0.001900	0.009500	19962.00	125080.30
9	mmu-miR-125b-5p	0.002400	0.009800	19952.00	120356.53
10	hsa-miR-4693-5p	0.002450	0.009800	19951.00	119939.12

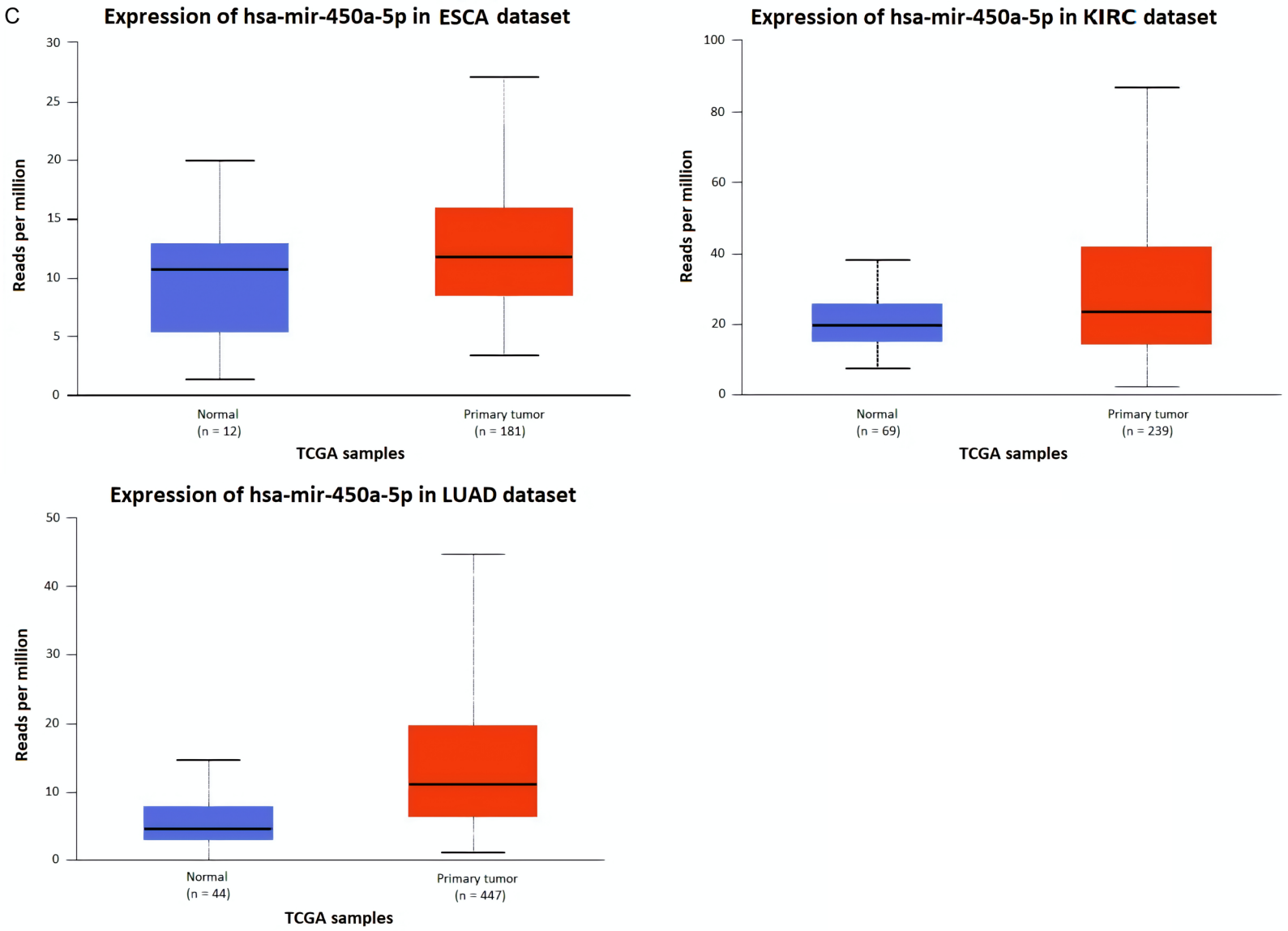


Figure 11. STAT1 targeted TFs, miRNAs, and expression analysis of miRNA (hsa-miR-450-5p) in human cancers. (A) STAT1 targeted TFs, (B) STAT1 targeted miRNAs, and (C) UALCAN-based expression analysis of STAT1 targeted miRNA (hsa-miR-450-5p) across ESCA, KIRC, and LUAD. A *P*-value <0.05 was used to indicate the significant scores.

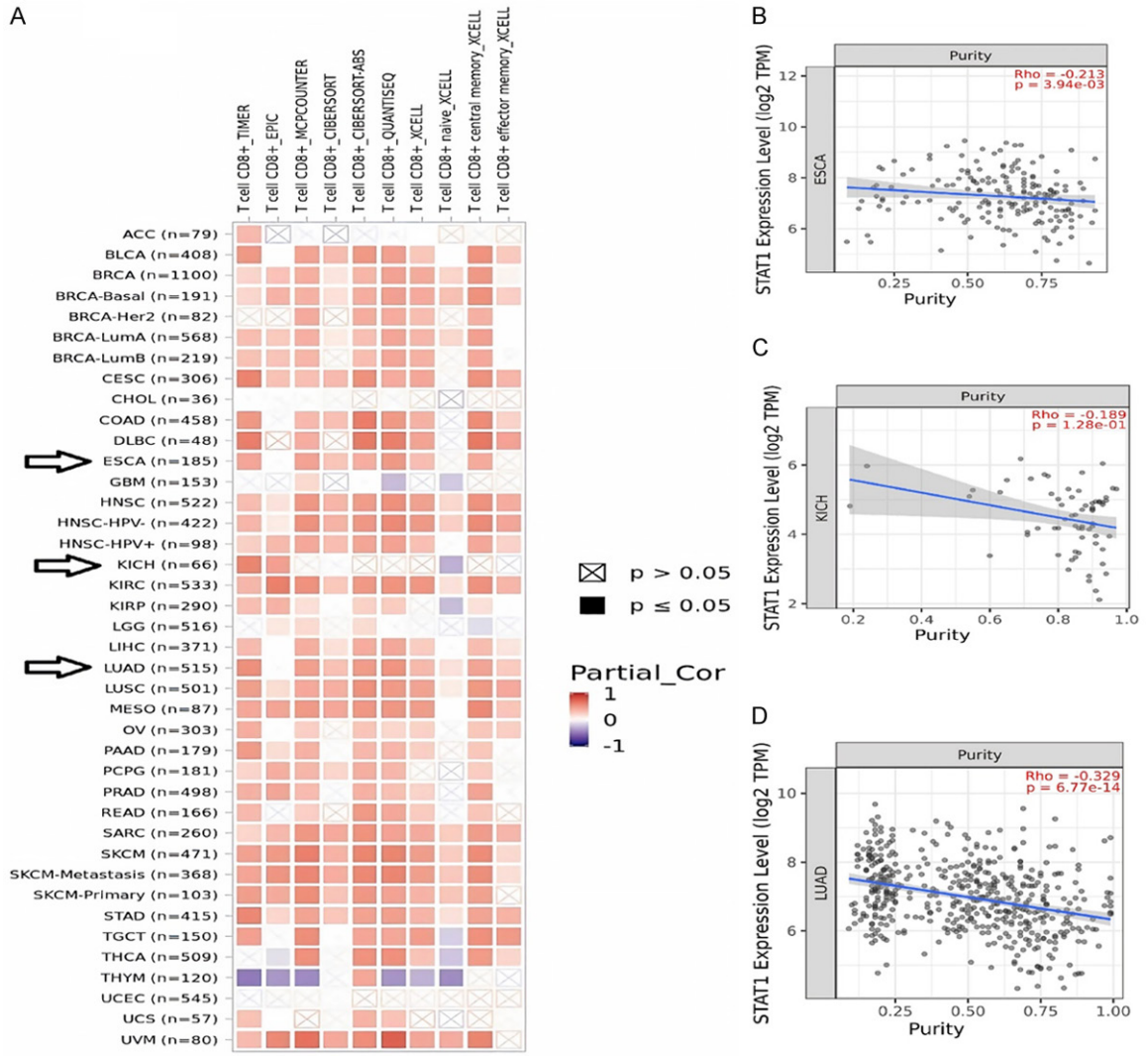


Figure 12. Correlational between the tumor purity, CD8+ T immune cells infiltration, and STAT1 expression in ESCA, KIRC and LUAD. (A) Spearman correlational between CD8+ T immune cells infiltration and STAT1 expression in ESCA, KIRC and LUAD, (B) Spearman correlational between tumor purity and STAT1 expression in ESCA, (C) Spearman correlational between tumor purity and STAT1 expression in KIRC, (D) Spearman correlational between the tumor purity and STAT1 expression in LUAD. A *P*-value <0.05 was used to indicate the significant scores.

ESCA, KIRC, and LUAD patients. Taken together, these data suggested that STAT1 might serve a crucial role in the pathogenesis of ESCA, KIRC, and LUAD; therefore, in this study, we mainly focused these three cancer subtypes. Furthermore, STAT1 expression was also found to be positively correlated with different clinicopathological features including cancer stage, patient gender, and nodal metastasis status at the same time in ESCA, KIRC, and LUAD patients. To the best of our knowledge, we are the first to report that STAT1 expression elevates regardless of clinicopathological features in ESCA, KIRC, and LUAD through this study.

To further identify the possible causes of STAT1 overexpression, we performed the correlation analysis of STAT1 overexpression with its promoter methylation level and mutational spectrum in ESCA, KIRC, and LUAD patients. STAT1 was found to be enriched in the deep amplification and missense genetic abnormalities in very small proportions (2.4% and 1.9%) of the ESCA and KIRC patients, respectively. Similarly, STAT1 has also shown enrichment in deep amplification genetic abnormalities in a small proportion (2.2%) of the LUAD patients as well. Therefore, it is unlikely that genetic mutations participate in the expression regulation of the STAT1. Furthermore, the re-

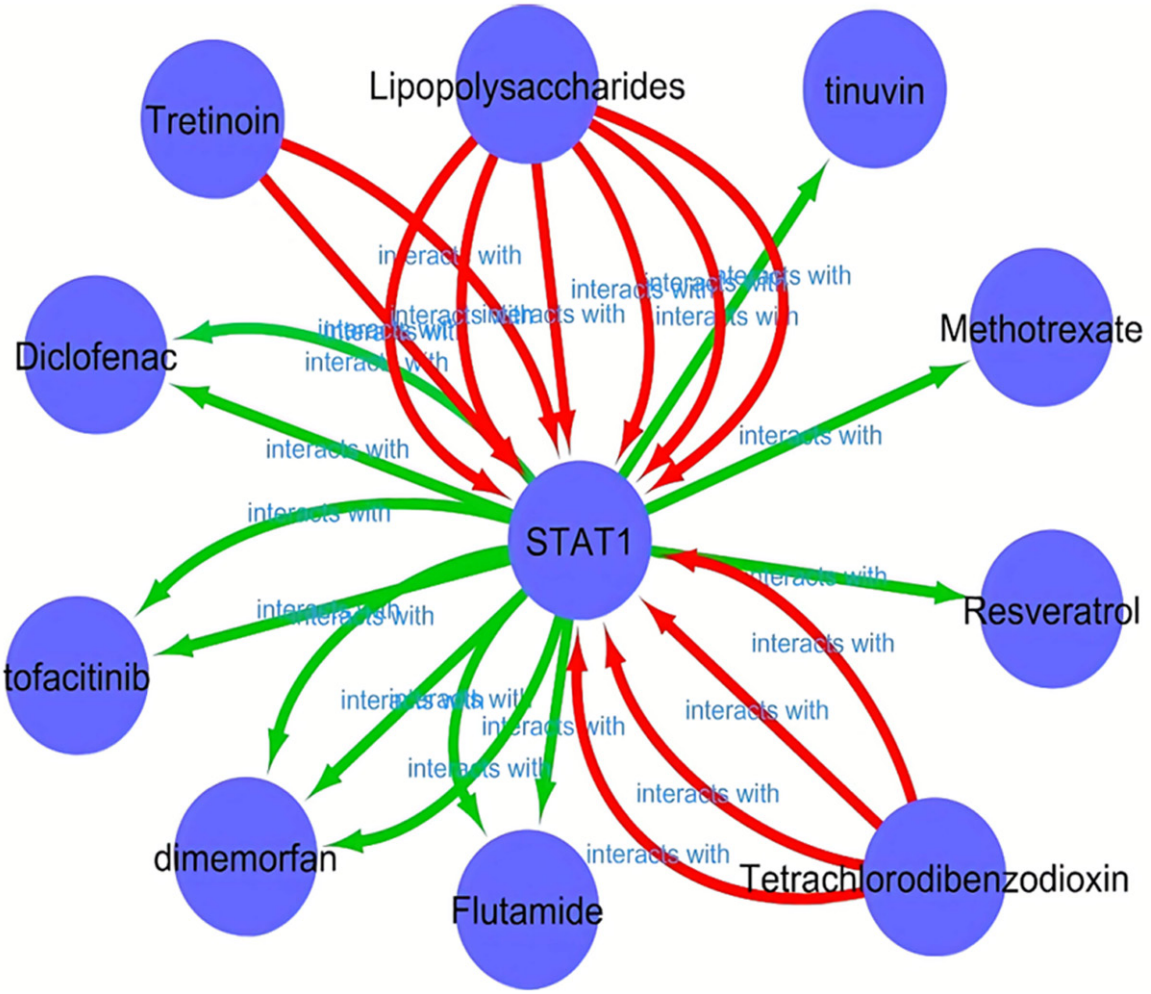


Figure 13. Gene-drug interaction network of STAT1. Red arrows: chemotherapeutic agents that can increase the expression of STAT1; green arrows: chemotherapeutic agents that can decrease the expression of STAT1. The count of arrows in this network between chemotherapeutic drug and gene represents the number of studies that have supported the interaction.

sults of STAT1 promoter methylation revealed an expected negative correlation between STAT1 overexpression and its promoter methylation level in ESCA, KIRC, and LUAD samples relative to normal controls. Taken together, our results revealed that promoter hypomethylation has a solid impact on the up-regulation of STAT1 in ESCA, KIRC, and LUAD.

Recently, various studies have been proposed to explore ESCA-related molecular biomarkers. For example, *Yin et al.* revealed that rs18050-34 T>C polymorphism in the RANK gene was strongly associated with ESCA development [40]. *Pan et al.* proposed the up-regulation of the CASC9 gene as a potential biomarker of ESCA [41]. Similarly, *Long et al.* provided evidence of SLC52A3 involvement in ESCA [42]. However, none of these or any other biomarkers have been generalized so far in ESCA patients of different clinicopathological features. Via this study, we revealed the significant ($P<0.05$) up-regulation of STAT1 expression in ESCA patients with different clinicopathological including different cancer stage, patient gender, and nodal metastasis. We have also shown that STAT1 overexpression is significantly ($P<0.05$) associated with decreased OS in ESCA patients. Overall these outcomes suggested STAT1 up-regulation as a novel reliable diagnostic and prognostic biomarker of ESCA.

Accurate diagnosis is essential for the survival of KIRC patients, and until now, the expression of various genes including ALDH6A1, HADH,

PCCA, AUH, ACADSB, CTLA4, and ACAA1 have been significantly correlated with the accurate diagnosis and survival of the KIRC patients [43, 44]. However, none of these or any other biomarkers have been generalized so far in KIRC patients of different clinicopathological features. In our study, we have shown the significant ($P<0.05$) up-regulation of STAT1 expression in KIRC patients of different clinicopathological features including cancer stage, patient gender, and nodal metastasis. Furthermore, the degree of methylation in the STAT1 promoter, as well as its OS information, has also shown the potential of this gene to be a novel potential diagnostic and prognostic biomarker of KIRC patients.

So far, several LUAD specific diagnostic and prognostic biomarkers have been identified by previous studies. For example, *Sudbanshu Shukla et al*. have carried out the first prognostic biomarkers analysis in LUAD patients through RNA sequencing technique and generated prognostic features through a Cox model [45]. Subsequently, *Li B et al.* utilized the RNA sequencing dataset to identify a few immune signatures that can predict the prognosis and OS of nonsquamous non-small cell lung cancer patients [46]. Furthermore, *Zheng S et al.* have used the Cox model and developed an 8-lncRNA-based diagnostic and prognostic signature for LUAD patients [47]. However, similar to ESCA and KIRC, none of these or any other LUAD biomarker have been generalized so far in LUAD patients of different clinicopathological features. In the current study, we have revealed the significant ($P<0.05$) up-regulation of STAT1 expression in LUAD patients of different clinicopathological features including cancer stage, patient gender, and nodal metastasis. Furthermore, the degree of methylation in the STAT1 promoter, as well as its OS information, has also shown the potential of this gene to be a novel potential diagnostic and prognostic biomarker of LUAD patients.

The miRNAs and TFs play an important role to control gene expression at the post-transcriptional level and are also involved in carcinogenesis [31, 48]. To know the role of miRNAs and TFs in the dysregulation of STAT1, we predicted the potential miRNAs and TFs of STAT1 using Enrichr. Our results revealed 10 most significant miRNAs and TFs that can potentially regulate the STAT1 expression, including hsa-miR-450-5p, mmu-miR-3082-3p, mmu-miR-351-5p, mmu-miR-875-5p, mmu-miR-125a-5p, mmu-miR-23a-3p, mmu-miR-146a-5p, mmu-miR-3102-3p, mmu-miR-125b-5p, and hsa-miR-4693a-5p miRNAs, and CIITA, LMO2, IRF1, ATF3, BCL3, STAT2, STAT5B, STAT5A, VDR, and HSF1 TFs. Finally, via expression analysis through UALCAN, we also explored hsa-miR-450-5p as the only miRNA from the predicted miRNAs that up-regulate significantly in ESCA, KIRC, and LUAD as a possible shared modulator of oncogenesis. This valuable information can also help to understand the STAT1 oncogenic role in more detail.

Tumor purity and CD8+ T immune cells infiltration are two of the most essential components of immunotherapy. The intriguing correlations observed in our study between tumor purity, CD8+ T immune cells infiltration, and STAT1 expression lead to novel therapeutic options for ESCA, KIRC, and LUAD patients who do not respond to currently available immune checkpoint inhibitors/regulators.

In the present study, the interaction network of STAT1-associated genes was constructed and visualized. In total, one set of 10 STAT1 associate genes were identified. Pathway enrichment analysis STAT1-associated genes revealed their involvement in various signaling pathways including “Influenza A”, “Hepatitis B”, “Measles”, and “Coronavirus disease”. These findings are in line with previous research, in which the carcinogenic role of identified pathways is well established [49, 50]. Furthermore, we have also looked for a few possible chemotherapeutic drugs that might be effective in treating ESCA, KIRC, and LUAD by modulating STAT1 expression; however, further experimental validation is required to be done.

Conclusion

In the current study, we utilized different online expression databases and Bioinformatics tools to comprehensively analyze STAT1 in different types of human cancers and verified its oncogenic, diagnostic, and prognostic roles in ESCA, KIRC, and LUAD patients. However, further voluminous testing is required for the full clinical implications of these findings.

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Disclosure of conflict of interest

None.

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