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Research Paper

Systematic analysis of gene expression alterations and clinical outcomes of STAT3 in cancer

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ABSTRACT

Accumulated studies have provided controversial evidences of prognostic value for signal transducer and activator of transcription proteins 3 (STAT3) in cancers. To address this inconsistency, we performed a systematic analysis to determine whether STAT3 can serve as a prognostic marker in human cancers. STAT3 expression was assessed using Oncomine analysis. cBioPortal, Kaplan-Meier Plotter, and Prognoscan were performed to identify the prognostic roles of STAT3 in human cancers. The copy number alteration, mutation, interactive analysis, and visualize the altered networks were performed by cBioPortal. We found that STAT3 was more frequently overexpressed in lung, ovarian, gastric, blood and brain cancers than their normal tissues and its expression might be negatively related with the prognosis. In addition, STAT3 mutation mainly occurred in uterine cancer and existed in a hotspot in SH2 domain. Those findings suggest that STAT3 might serve as a diagnostic and therapeutic target for certain types of cancer, such as lung, ovarian, gastric, blood and brain cancers. However, future research is required to validate our findings and thus promote the clinical utility of STAT3 in those cancers prognosis evaluation.

INTRODUCTION

Cancer is one of the major causes threatening human health and life [1]. Despite significant advances in diagnostic and treatment modalities, the average five-year survival rate for cancer patients is still extremely poor [2]. Stepwise accumulation of somatic genetic alterations is the basis for cancer which involved in base insertions, deletions, substitutions, translocation events, and copy number alteration [3–5]. The fact that targeted therapy has been successful in part of cancers calls for a better comprehension of the pathological mechanisms responsible for these oncogenic alterations leading to cancer.

Signal transducer and activator of transcription proteins 3 (STAT3), a member of STAT family, is well demonstrated to exerts an important effect on tumorigenesis and tumor-related inflammation [6]. Aberrant expression and persistent activation of STAT3 is implicated in cell proliferation, differentiation, apoptosis and immune escape, inducing and maintaining a pro-carcinogenic inflammatory

microenvironment [7]. Continuously activated STAT3 has been found in many human tumors, such as lung cancer [8], gastric carcinoma [9], cervical carcinoma [10], and meningiomas [11]. Moreover, mounting evidence have demonstrated STAT3-targeted therapy could effectively inhibit tumor development in various human cancers [12]. However, the prognostic value of STAT3 overexpression in human tumors is still controversial. Therefore, in the current study, we carried out a systematic analysis combining thousands of gene expression or copy number variation analysis published online, to evaluate the expression pattern, potential functions and distinct prognostic value in cancer of STAT3.

RESULTS

To explore the role of STAT3 in cancers, we compared the transcription levels of STAT3 in cancers with that in normal tissues, using Oncomine database and found that the mRNA expressions of STAT3 were significantly over-expressed in certain types of tumors and lower in others as compared to that of the normal sample. As show in Figure 1, STAT3 may work either oncogenic or anti-oncogenic function based on the cancer types. Therefore, detailed analyses of STAT3 were described below.

The transcript expression of STAT3 in different cancer types

We conducted cDNA microarray analysis by using the Oncomine database to explore gene expression of STAT3 in cancer types. The Oncomine database was queried for STAT3 expression in cancer and normal tissues. Our analysis revealed that STAT3 was over-expressed in brain and cns, gastric, head and neck, melanoma, myeloma cancers, but was under-expressed in breast, leukemia, liver, lymphoma, and sarcoma cancers as compared to that in normal tissue (Table 1, Figure 2A–2C. Supplementary Figures 1–3) [13–28]. These observations are in agreement with the previously published reports on STAT3 expression [29]. For instance, our study indicated that STAT3 is highly expressed in glioblastoma (Figure 2B) [29], elevated in hepatocellular cancer (Supplementary Figure 2B) [30].

Genetic alterations of STAT3 and overall survival (OS)

Using the comprehensive Kaplan-Meier survival analysis platform, we discovered that decreased mRNA expression of STAT3 is an unfavorable prognostic factor of overall survival for patients with breast adenocarcinoma (Figure 3). Contrastingly, lung, ovarian, and gastric cancers showed the relationship between overexpression of STAT3 and overall low survival rates (Figure 3).

The prognostic value of STAT3 expression was reported by PrognoScan database (Figure 4, Table 2). The poor prognosis in ovarian cancer patients with higher STAT3 expression (Figure 5) was in line with the data from Kaplan–Meier plotter analysis (Figure 3). Using the comprehensive survival analysis platforms Kaplan-Meier plotter, Oncomine, and PrognoScan, we have demonstrated the oncogenic role of STAT3 in ovarian, lung, blood, and brain cancer, however, which is not clear in breast cancer.

Protein components of nodes across the STAT3

STAT homologs in mammals are comprised of six conserved structural domains, as follows: N-domain (ND), coiled-coil, DNA binding, linker, Src homology 2 (SH2), and transcriptional activation domain [31, 32]. Under normal physiological conditions, the activation of STATs is strictly regulated, which could regulate cell proliferation, survival and other critical cellular functions by modulating the expression of specific target genes. In cancer, by contrast, STAT protein, especially STAT3, become activated constitutively, thereby driving the malignant phenotype of cancer cells. We selected the functional protein partners of STAT3 based on previous publications and curated databases [6, 33–37]. Hence, the following nine predicted proteins, including cyclin D1 (CCND1), epidermal growth factor receptor (EGFR), Interleukin-6 (IL6), Janus kinase 1 (JAK1), Janus kinase 3 (JAK3), mitogen-activated protein kinase 1 (MAPK1), myelocytomatosis oncogene (MYC), suppressor of cytokine signaling3 (SOCS3), SRC were chose for further analysis of STAT3 (Figure 6).

Unbiased cross cancer subtypes relationships by cBioPortal data

When compared with the high frequency of STAT3 genetic deletions, there were few, frequent STAT3 gene mutations in 87 studies examined using cBioPortal Web. As show in Figure 7, a total of 300 mutation sites were detected and located between amino acids 0 and 770. STAT3 mutation mainly occurred in uterine cancer and existed in a hotspot in SH2 domain. In addition, we used cBioPortal tool to analyze the 10 gene of mutations and CNAs with 87 different cancer studies. The results analyzed 20 different cancer studies representing 8513 samples that contained >40% alteration frequency and at least 100 samples in the dataset (Figure 8, Table 3). From the lowest to highest dominance hierarchy, the ratio of alteration ranged over 40.1–67.9%. The particular interest constituted the predominant pattern of amplification occurring in neuroendocrine prostate cancer (NEPC).

Furthermore, we applied the OncoPrint from a query for alterations in CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC, and STAT3 genes. The percentages of alterations in these genes among NEPC

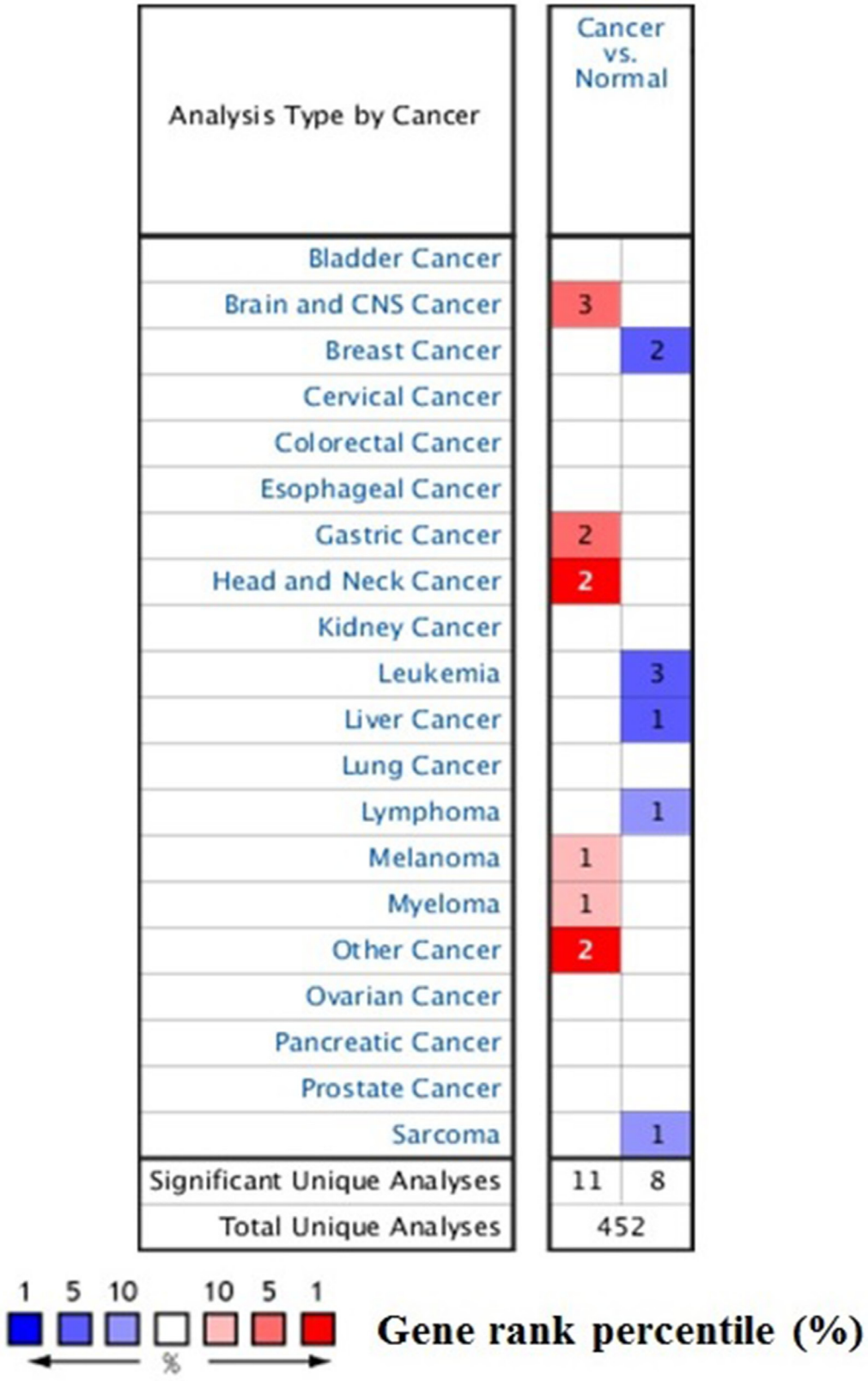

Figure 1: The transcription levels of STAT3 in different types of cancers. This graphic was generated from Oncomine, indicating the numbers of datasets with statistically significant ($p < 0.01$) mRNA over-expression (Red) or down-expression (Blue) of STAT3 (different types of cancer vs. corresponding normal tissue). The threshold was designed with following parameters: *p*-value of 1E-4, fold change of 2, and gene ranking of 10%.

Table 1: STAT3 expression in cancers

Cancer	Cancer subtype	*p*-value	Fold change	Rank (%)	Sample	Reference
Brain	Glioblastoma	2.81E-7	2.076	4	31	[13]
	Glioblastoma	2.30E-10	2.270	7	104	[14]
Breast	Ductal Breast Carcinoma	1.00E-8	–2.176	2	47	[15]
	Invasive Breast Carcinoma Stroma	1.21E-15	–11.013	10	59	[16]
Gastric	Gastric Mixed Adenocarcinoma	6.45E-6	2.190	3	35	[17]
	Gastric Intestinal Type Adenocarcinoma	2.26E-10	2.252	3	57	[17]
Head and Neck	Salivary Gland Adenoid Cystic Carcinoma	7.94E-8	2.560	2	22	[18]
	Tongue Carcinoma	1.81E-7	2.284	1	37	[19]
Leukemia	B-Cell Acute Lymphoblastic Leukemia	3.69E-41	–2.179	3	221	[20]
	B-Cell Acute Lymphoblastic Leukemia	1.76E-10	–2.669	5	93	[21]
	T-Cell Acute Lymphoblastic Leukemia	1.86E-6	–3.149	7	17	[21]
Liver	Hepatocellular Carcinoma	4.66E-9	–2.290	3	57	[22]
Lymphoma	Burkitt's Lymphoma	9.06E-5	–2.746	8	42	[23]
Melanoma	Cutaneous Melanoma	8.09E-5	8.442	10	52	[59]
Myeloma	Monoclonal Gammopathy of Undetermined Significance	1.14E-5	2.213	7	66	[25]
Other	Teratoma, NOS	1.34E-10	2.490	1	20	[26]
	Pleural Malignant Mesothelioma	6.05E-5	3.884	4	49	[27]
Sarcoma	Myxoid/Round Cell Liposarcoma	2.12E-5	–2.229	9	29	[28]

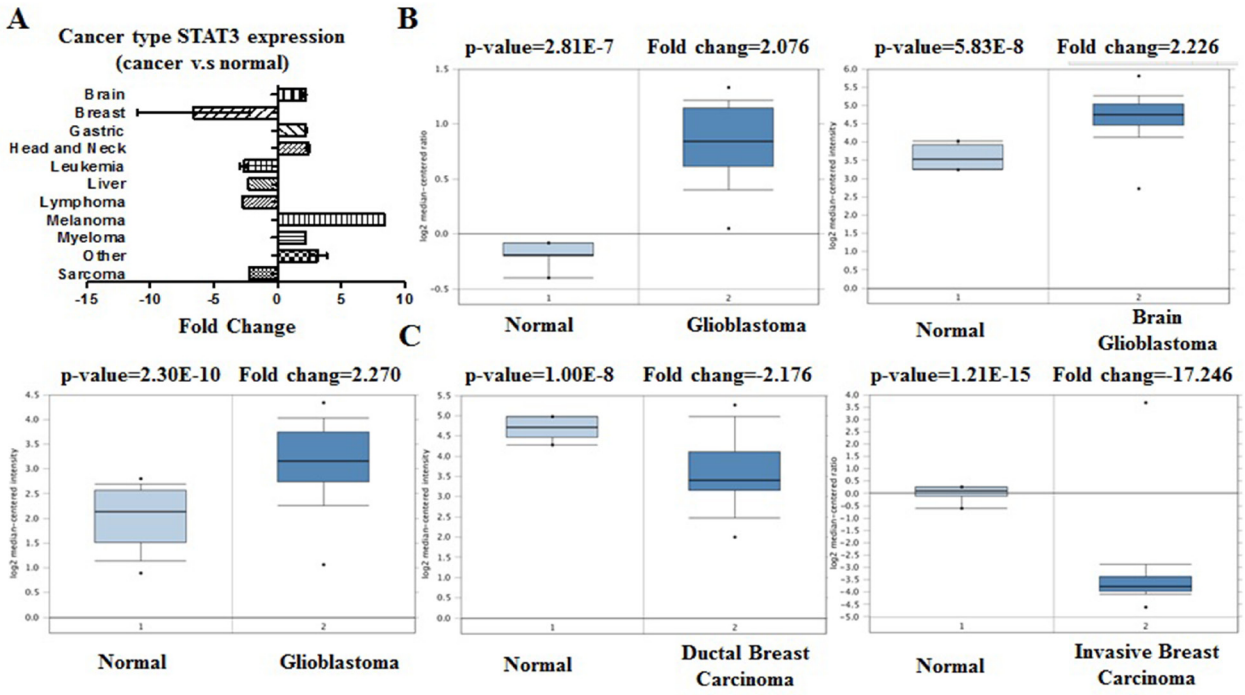

Figure 2: STAT3 analysis in different cancer types (Oncomine database). The box plot comparing specific STAT3 expression in normal (left plot) and cancer tissue (right plot) was derived from Oncomine database. The fold change of STAT3 in various types of cancers was identified from our analyses in Table 1 and expressed as the forest plot (**A**). The analysis was shown in glioblastoma carcinoma relative to normal breast (**B**), in breast carcinoma relative to normal pancreatic (**C**).

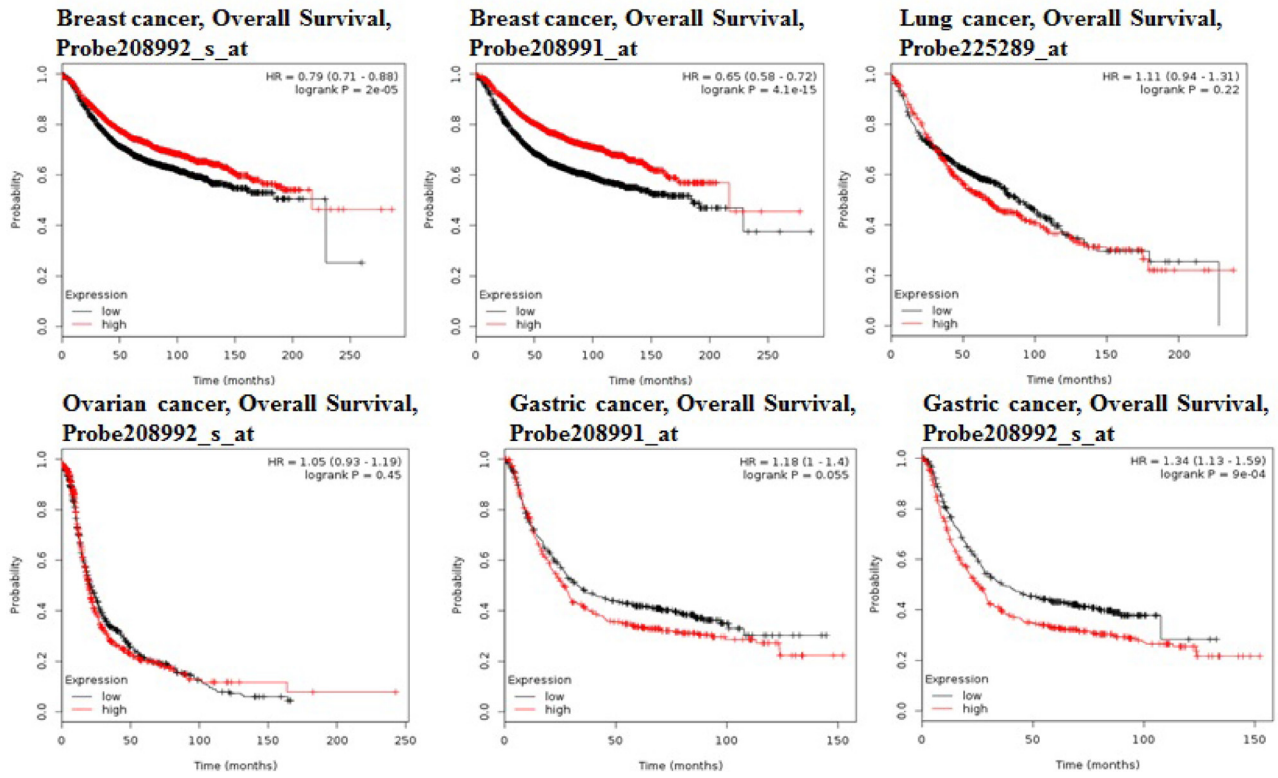

Figure 3: STAT3 genes in Breast, Ovarian, Gastric and Lung cancer (Kaplan–Meier Plotter). The survival curve comparing the patient with high (red) and low (black) expression in breast, ovarian, gastric and lung cancer was plotted from KaplanMeier plotter database.

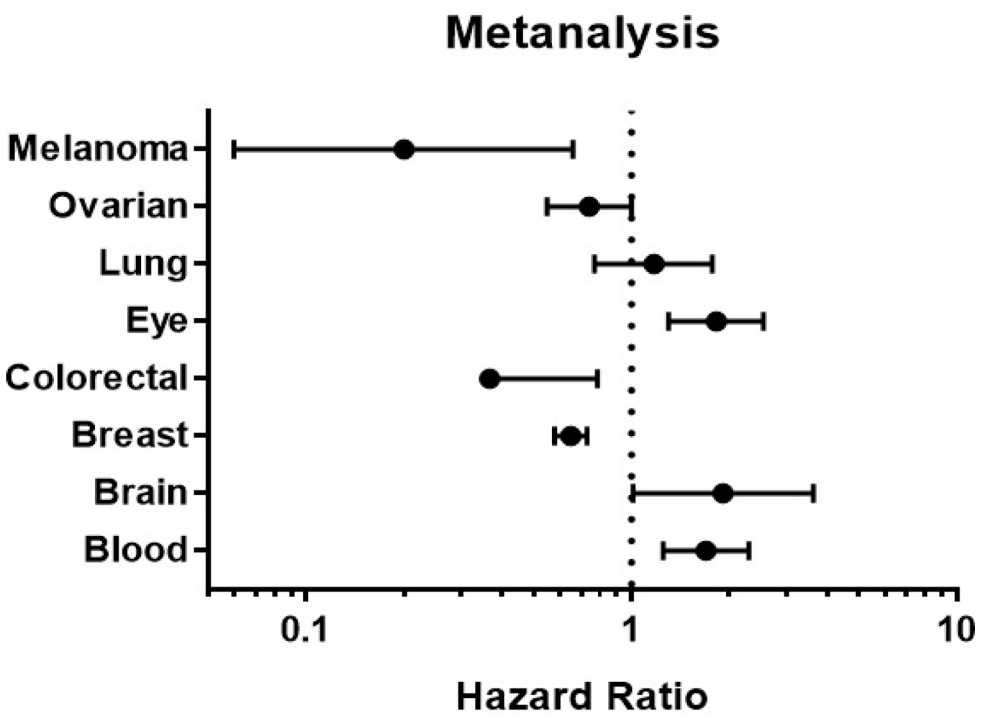

Figure 4: STAT3 genes in different cancer types (PrognoScan database). The statistically significant hazard ratio in various types of cancers was identified from our analyses in Table 2 and expressed as the forest plot. The analysis of survival curve was identified as the threshold of cox p-value < 0.05.

Table 2: The association of STAT3 expression and the survival in cancer patients

Cancer type	Dataset	Endpoint	Probe id	*N*	Cox *p*-value	Hr
Blood	GSE12417-GPL97	Overall Survival	243213_at	163	0.000744	1.69
Brain	GSE4271-GPL97	Overall Survival	243213_at	77	0.047646	1.91
Breast	GSE3143	Overall Survival	39708_at	158	0.031082	0.47
	GSE7849	Disease Free Survival	39708_at	76	0.017328	4.53
	GSE7849	Disease Free Survival	289_at	76	0.014144	4.92
	GSE12276	Relapse Free Survival	208991_at	204	0.067027	0.75
	GSE6532-GPL570	Relapse Free Survival	208991_at	87	0.015187	0.54
	GSE6532-GPL570	Distant Metastasis Free Survival	208992_s_at	87	0.012369	0.49
	GSE6532-GPL570	Distant Metastasis Free Survival	208991_at	87	0.015187	0.54
	GSE6532-GPL570	Relapse Free Survival	225289_at	87	0.046274	0.52
	GSE6532-GPL570	Distant Metastasis Free Survival	225289_at	87	0.046274	0.52
	GSE6532-GPL570	Relapse Free Survival	208992_s_at	87	0.012369	0.49
	GSE9195	Relapse Free Survival	208991_at	77	0.021717	3.80
	GSE9195	Relapse Free Survival	243213_at	77	0.018195	8.75
	GSE9195	Distant Metastasis Free Survival	243213_at	77	0.035491	9.33
	GSE12093	Distant Metastasis Free Survival	208992_s_at	136	0.016456	0.59
	GSE12093	Distant Metastasis Free Survival	208991_at	136	0.005146	0.27
	GSE11121	Distant Metastasis Free Survival	208992_s_at	200	0.020347	0.44
	GSE9893	Overall Survival	21668	155	0.017780	0.69
	GSE2034	Distant Metastasis Free Survival	208992_s_at	286	0.015785	0.78
	GSE2034	Distant Metastasis Free Survival	208991_at	286	0.003215	0.53
	GSE2990	Relapse Free Survival	208991_at	62	0.037864	0.55
	GSE2990	Relapse Free Survival	208992_s_at	62	0.010824	0.33
Colorectal	GSE17537	Disease Free Survival	225289_at	55	0.027763	0.21
Eye	GSE22138	Distant Metastasis Free Survival	208992_s_at	63	0.005425	2.00
	GSE22138	Distant Metastasis Free Survival	208991_at	63	0.035231	1.66
Lung	GSE31210	Relapse Free Survival	208992_s_at	204	0.001112	4.53
	GSE31210	Relapse Free Survival	225289_at	204	0.017076	4.72
	GSE31210	Relapse Free Survival	243213_at	204	0.034308	0.54
	GSE31210	Overall Survival	208992_s_at	204	0.047705	3.43
	GSE8894	Relapse Free Survival	243213_at	138	0.002854	0.02
Ovarian	GSE9891	Overall Survival	208991_at	278	0.049049	0.74
Skin	GSE19234	Overall Survival	208991_at	38	0.008049	0.20

varied from 10–53% for individual genes (CCND1, 27%; EGFR, 21%; IL6, 25%; JAK1, 18%; JAK3, 23%; MAPK1, 10%; MYC, 53%; SOCS3, 27%; SRC, 22%; and STAT3, 21%), the MYC gene was amplified predominantly in the NEPC type (Figure 9, Table 4).

In order to discover whether each gene pair has a significant correlation, the portal performs a Fisher's exact test. The mutual exclusivity panel analysis revealed that the co-occurrent alternations of STAT3 and CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC

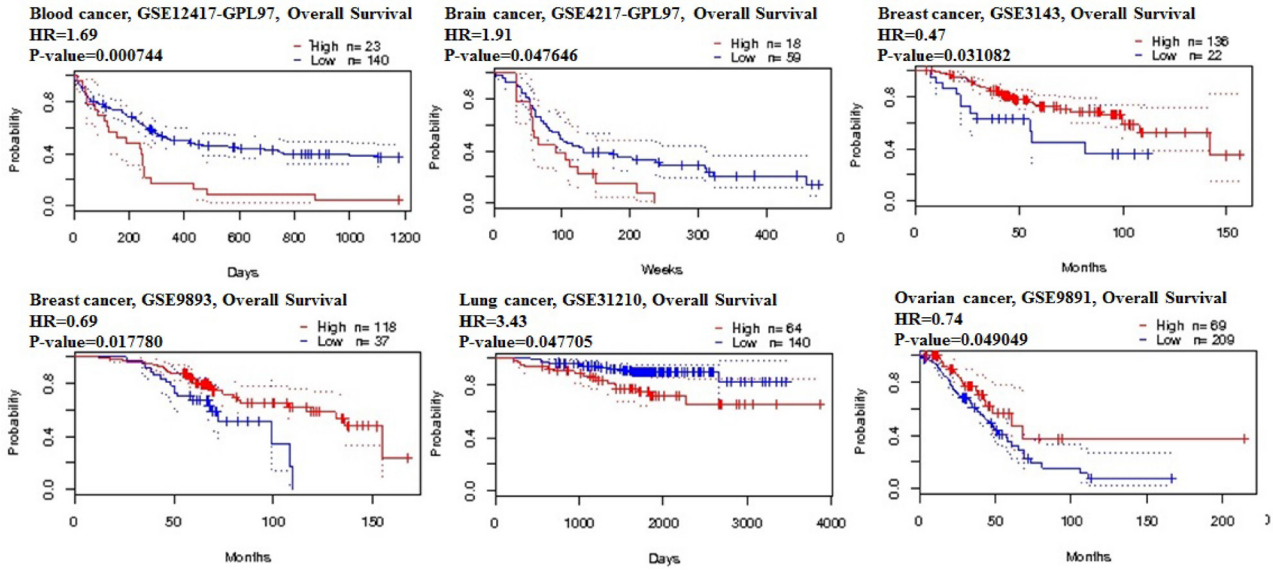

Figure 5: STAT3 genes in blood, brain, lung and ovarian cancer types (PrognoScan database). The survival curve comparing the patient with high (red) and low (black) expression was plotted from PrognoScan database. The survival curve comparing the patient with high (red) and low (black) expression in blood cancer, brain cancer, lung cancer and ovarian cancer was plotted from PrognoScan database as the threshold of cox *p*-value < 0.05.

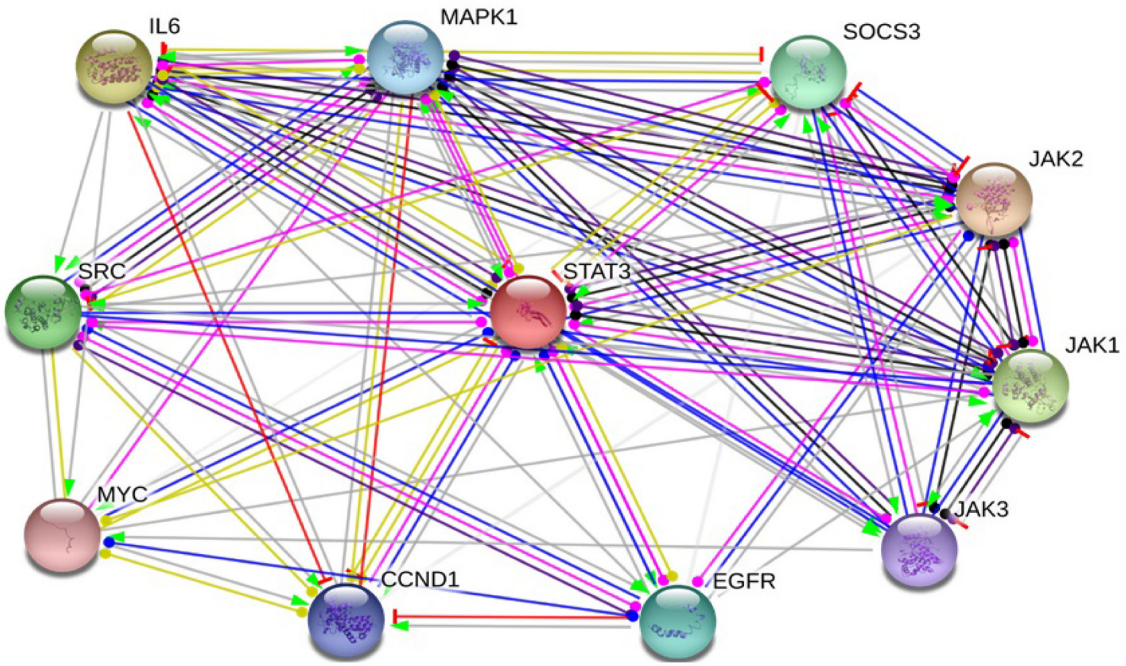

Figure 6: Identifcation of known and predicted structural proteins essential for STAT3 function. Interacting nodes are displayed in colored circles using String, v10.0. Predicted functional partners of STAT3 are shown based upon peer reviewed published data and curated database entries. [STRING v.10 (http://string-db.org)].

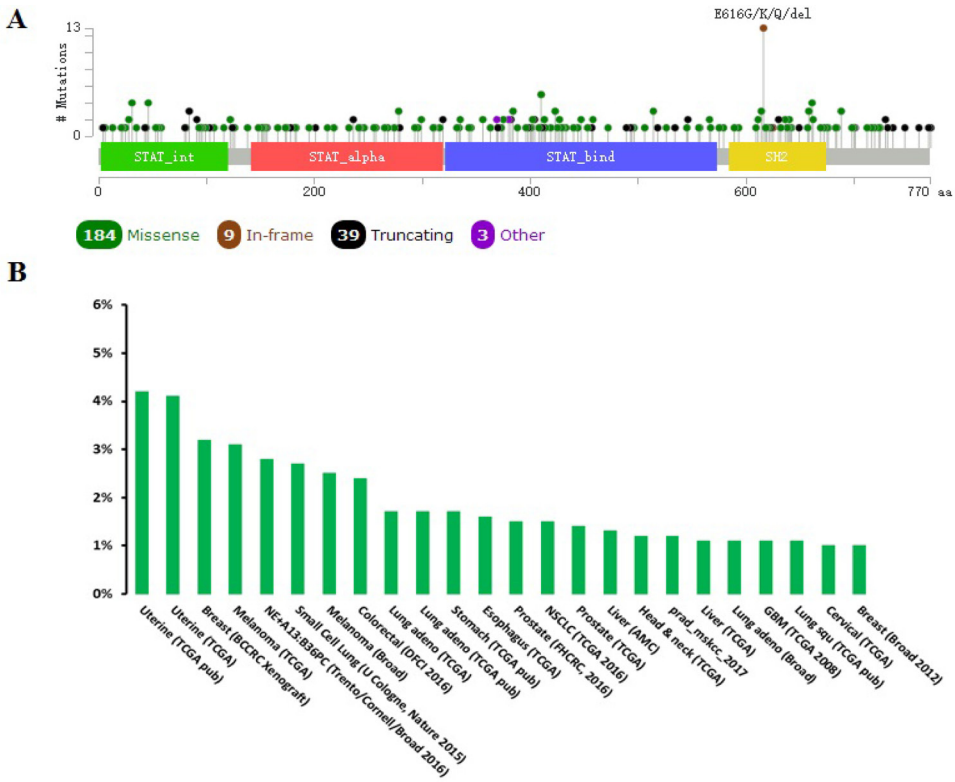

Figure 7: Mutation diagram of STAT3 in different cancer types across protein domains. A total of 300 mutation sites were detected and located between amino acids 0 and 770. STAT3 mutation mainly occurred in uterine cancer and existed in a hotspot in SH2 domain.

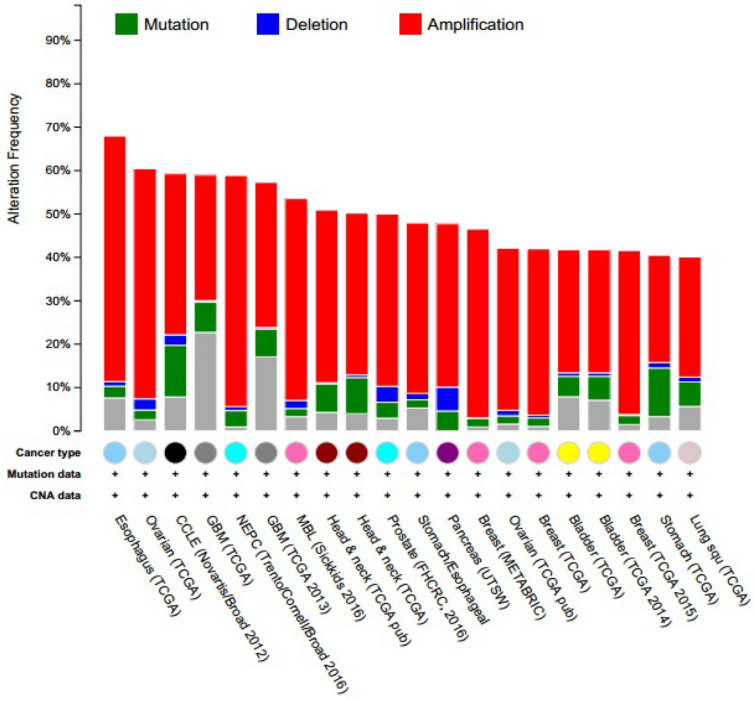

Figure 8: Copy number alteration of STAT3 genes and cancer subtypes. The alteration frequency of a ten-gene signature (CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC, STAT3) was determined using the cBioPortal (http://www.cbioportal.org). Only cancer types containing >100 samples and an alteration frequency of >40% are shown. The alteration frequency included deletions (blue), amplifcation (red), multiple alterations (grey) or mutation (green). The total number of samples for each cancer type are indicated by the numbers at the top of each column.

Table 3: Cross-cancer alteration summary for CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC, STAT3

Cancer	Data source	*N*	Frequency (%)	Multiple alterations (%, *N*)	Amplification (%, *N*)	Mutation (%, *N*)	Deletion (%, *N*)
Esophagus	TCGA	184	67.9%	7.6% (14)	56.5% (104)	2.7% (5)	1.1% (2)
Ovarian	TCGA	311	60.5%	2.6% (8)	53.1% (165)	2.3% (7)	2.6% (8)
CCLE	Novartis/Broad 2012	881	59.3%	7.8% (69)	37.1% (327)	11.9% (105)	2.4% (21)
GBM	TCGA	273	59%	22.7% (62)	28.9% (79)	7% (19)	0.4% (1)
NEPC	Trento/Cornell/ Broad 2016	107	58.9%	0.9% (1)	53.3% (57)	3.7% (4)	0.9% (1)
Glioblastoma	TCGA 2013	281	57.3%	117.1% (48)	33.5% (94)	6.4% (18)	0.4% (1)
MBL	Sickkids 2016	213	53.3%	3.3% (7)	46.5% (99)	1.9% (4)	1.9% (4)
Head & neck	TCGA pub	279	50.9%	4.3% (12)	39.8% (111)	4.3% (12)	0.4% (1)
Head & neck	TCGA	504	50.2%	4% (20)	37.3% (188)	8.3% (42)	0.6% (3)
Prostate	FHCRC, 2016	136	50%	2.9% (4)	39.7% (54)	3.7% (5)	3.7% (5)
Stomach/ Esophageal	TCGA	265	47.9%	5.3% (14)	39.2% (104)	1.9% (5)	1.5% (4)
Pancreas	USTW	109	47.7%	0% (0)	37.6% (41)	4.6% (5)	5.5% (6)
Breast	METABRIC	2051	46.5%	0.9% (19)	43.5% (892)	2% (41)	0.1% (2)
Ovarian	TCGA pub	316	42.1%	1.6% (5)	37.3% (118)	1.9% (6)	1.3% (4)
Breast	TCGA	963	42%	1% (10)	3813% (369)	2% (19)	0.6% (6)
Bladder	TCGA	127	41.7%	7.9% (10)	28.3% (36)	4.7% (6)	0.8% (1)
Bladder	TCGA	127	41.7%	7.1% (9)	28.3% (36)	5.5% (7)	0.8% (1)
Breast	TCGA 2015	816	41.5%	1.5% (12)	37.7% (308)	2.1% (17)	0.2% (2)
Stomach	TCGA	393	40.5%	3.3% (13)	24.7% (97)	11.2% (44)	1.3% (5)
Lung squ	TCGA	177	40.1%	5.6% (10)	27.7% (49)	5.6% (10)	1.1% (2)

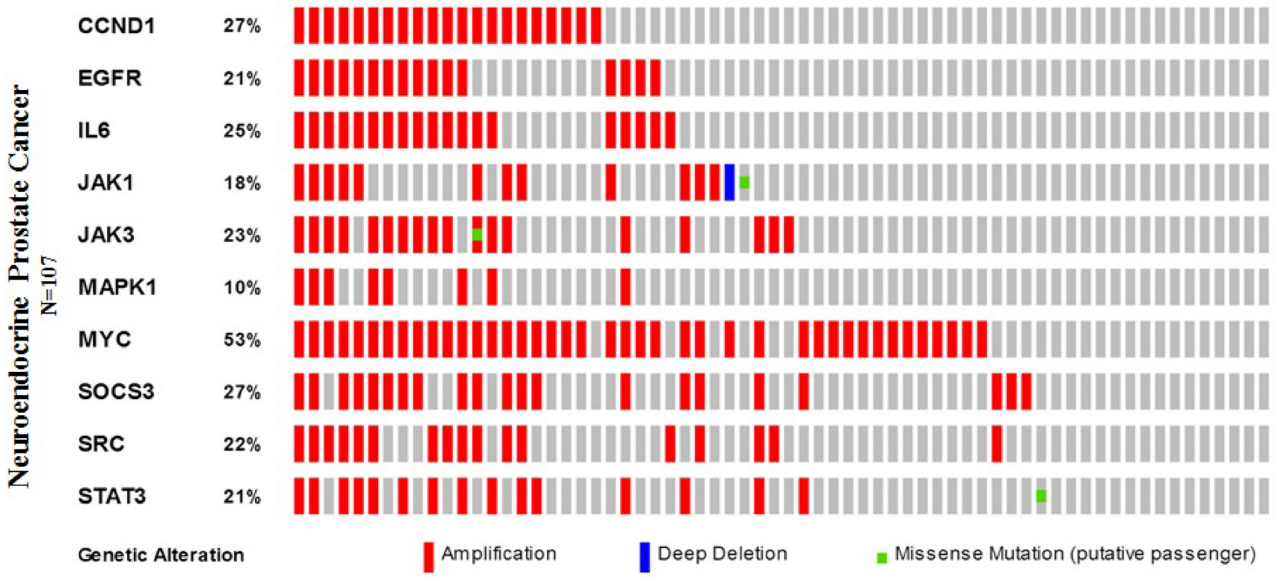

Figure 9: Neuroendocrine prostate cancer types frequently amplify STAT3. We used the Oncoprint feature of the cBioPortal (http://www.cbioportal.org) to determine the copy number alteration frequency of each individual gene (CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC, and STAT3) in STAT3 within selected cancer subtypes.

Table 4: The percentages of alterations in CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC, STAT3

Cancer	CCND1	EGFR	IL6	JAK1	JAK3	MAPK1	MYC	SOCS3	SRC	STAT3
Esophagus	36%	16%	9%	3%	5%	1.6%	27%	3%	3%	4%
Ovarian	8%	3%	4%	4%	11%	5%	41%	6%	4%	1.9%
CCLE	12%	15%	7%	8%	5%	7%	24%	8%	5%	6%
GBM	0.4%	55%	0.7%	1.1%	1.5%	1.8%	1.8%	0.4%	0.7%	1.1%
NEPC	27%	21%	25%	18%	23%	10%	53%	27%	22%	21%
Glioblastoma	0.4%	53%	0.7%	1.1%	1.1%	1.4%	1.8%	0.7%	0.7%	0.7%
MBL	25%	8%	2.8%	4%	2.8%	1.9%	19%	6%	5%	2.3%
Head & neck	28%	14%	1.4%	1.8%	1.8%	4%	13%	0.4%	1.8%	1.4%
Head & neck	25%	14%	2.2%	2.4%	2.2%	4%	13%	0.2	1.8%	1.6%
Prostate	13%	4%	6%	6%	6%	1.9%	41%	1.9%	7%	7%
Stomach/Esophageal	12%	10%	6%	2.3%	5%	1.9%	23%	4%	5%	5%
Pancreas	9%	1.8%	0.9%	6%	8%	7%	13%	10%	6%	6%
Breast	17%	4%	2.8%	3%	1.9%	1.1%	27%	6%	2.7%	1.4%
Ovarian	4%	2.2%	1.9%	1.6%	6%	2.2%	31%	2.8%	1.6%	0.6%
Breast	16%	2.7%	1.9%	2.6%	2.4%	1%	22%	6%	2.5%	2.6
Bladder	13%	11%	6%	5%	2.4%	4%	12%	4%	3%	1.6%
Bladder	13%	9%	6%	5%	3%	4%	12%	4%	3%	1.6%
Breast	16%	2.6%	1.5%	2.7%	2.6%	1.3%	21%	6%	2.8%	3%
Stomach	8%	10%	3%	6%	3%	2.3%	15%	2%	4%	2.8
Lung squ	12%	10%	5%	3%	4%	5%	10%	4%	2.3%	2.8%

has statistically significant. Functional plotting of the corresponding mRNA level associated with the genetic status of STAT3 revealed that deletion of STAT3 was associated with increased mRNA expression (Figure 10).

The cBioPortal analysis program identified 12 types of human cancer with significant CNAs in the chosen genes' signature (STAT3, CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, and SRC). The STAT3 signature was created such as to represent the structures and functions of STAT3. The CNAs of specific structural components of the STAT3 in tumors may be potential targets to prevent metastatic spread. Network view of STAT3 and other chosen genes in neuroendocrine prostate cancer was presented in Figure 11. The query genes, STAT3, CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, and SRC were depicted with a thick border and neighbor genes were distributing around them.

DISCUSSION

STAT3 has been proved to participate in the generation and development of various cancers [38]. Moreover, numbers of researches have shown that STAT3-targetd therapy can effectively inhibit tumor development [12]. However, the exact role of STAT3 overexpression in human tumors is till controversial. In order to have the compelling analysis, in the current research, we performed the analyses depend on numerous genes expression with clearly defined parameters between cancer and normal tissues. In Oncomine analysis, STAT3 was found to be unregulated in brain and CNS, gastric, head and neck, melanoma, myeloma cancer, but deregulated in breast, leukemia, liver, lymphoma, and sarcoma cancer.

To gain further insights into the role of a prognostic marker, we next investigated the association of STAT3 expression and OS in various cancers, the prognostic value of STAT3 mRNA expression was assessed using the Kaplan-Meier Plotter and PrognoScan. Overall, high levels of STAT3 gene expression result in low survival in ovarian, lung, blood, and brain cancer, however, which is not clear in breast cancer. Therefore, to assess the tumorigenic or tumor suppressor role of STAT3 in breast cancer, many previous studies have demonstrated that the protein expression was significantly up-regulated in breast cancer tissues compared with their matched normal breast tissues [39–41]. Furthermore, the expression of STAT3 in tumor tissues was significantly associated with a tumor, lymph node metastasis, and TNM stage in breast patients [42]. In addition, Kaplan-Meier analysis demonstrated that the overall survial rate in breast cancer patients with high STAT3 levels was significantly lower than that in those with low STAT3 levels [41].

Somatically acquired genetic, epigenetic, transcriptomic, and proteomic alternations are the major

four factors in tumorigenesis [4]. The somatic loss-of-function or gain-of-function alterations are happened in specific genomic regions, which could indicate their potential inhibitory or carcinogenic roles, respectively [43]. Therefore, we used cBioPortal to identify human cancers discovered significant CAN in the STAT3-gene signature. STAT3 mutation mainly occurred in uterine cancer and existed in a hotspot in SH2 domain. From the lowest to highest dominance hierarchy, the ratio of alteration ranged over 40.1–67.9%. The particularly interest constituted

Gene A	Gene B	p-Value	Log Odds Ratio	Association
STAT3	CCND1	<0.001	2.207	Tendency towards co-occurrence Significant
STAT3	EGFR	<0.001	2.328	Tendency towards co-occurrence Significant
STAT3	IL6	<0.001	2.434	Tendency towards co-occurrence Significant
STAT3	JAK3	<0.001	2.242	Tendency towards co-occurrence Significant
STAT3	MYC	<0.001	2.416	Tendency towards co-occurrence Significant
STAT3	SOCS3	<0.001	2.970	Tendency towards co-occurrence Significant
STAT3	SRC	<0.001	1.914	Tendency towards co-occurrence Significant
STAT3	MAPK1	0.002	2.404	Tendency towards co-occurrence Significant
STAT3	JAK1	0.030	1.361	Tendency towards co-occurrence Significant

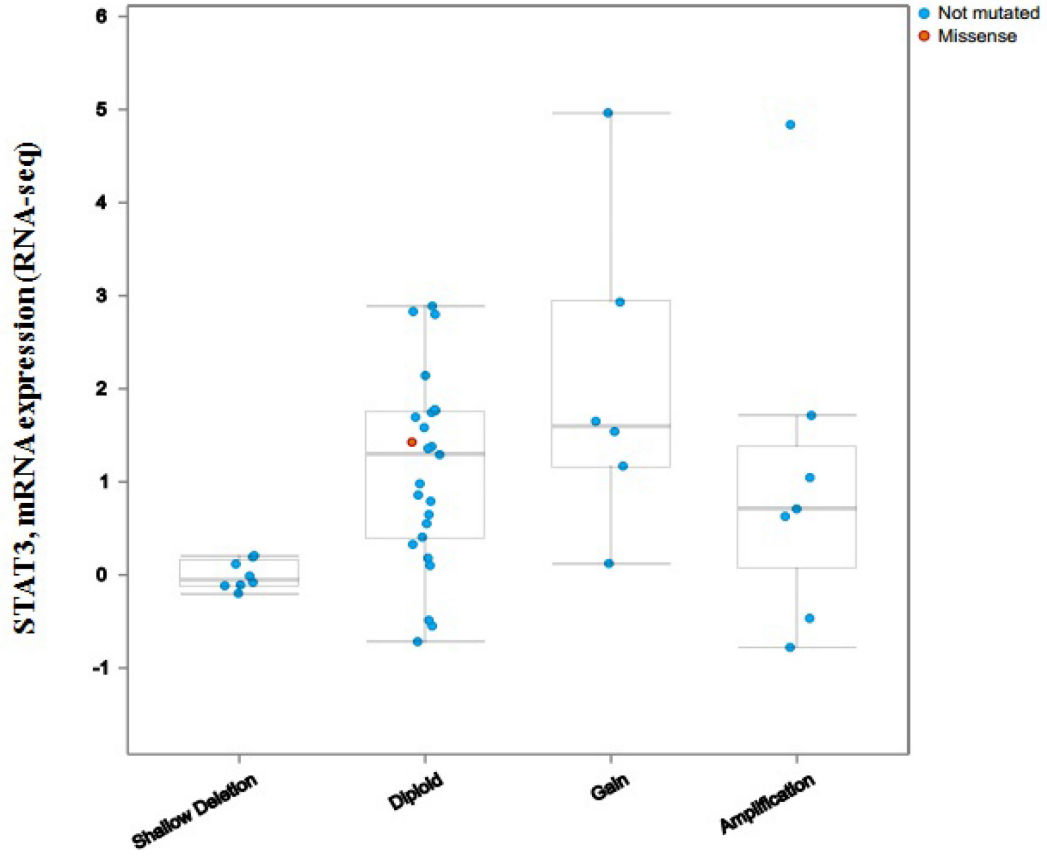

Figure 10: The mutual exclusivity panel analysis revealed that the co-occurrent alternations of STAT3 and CCND1, EGFR, IL6, JAK1, JAK3, MAPK1, MYC, SOCS3, SRC has statistically significant. The *P* values are determined by a Fisher's exact test, $P < 0.05$ (http://www.cbioportal.org/index.do?session_id=59847ef8498e5df2e2937e6b&show_samples=false&).

the predominant pattern of amplification occurring in neuroendocrine prostate cancer (NEPC).

Subsequently, we performed cBioPortal to interactive analysis and visualize the altered networks of STAT3. From the network analysis, we can discover more information about the mechanisms of interaction among the different genes [5]. Figure 11 displayed that the Network view of the STAT3 neighborhood in neuroendocrine prostate cancer, those results were better to comprehend the molecular mechanisms of STAT3 underlying cancer. After an extensive literature review on previous related studies, STAT3 is proven to involve in various tumors by impacting target genes or signal pathway, which is consistent with our bioinformatics analyses [6, 44–49]. As the previous studies revealed that STAT3 transactivates proliferative genes (*cMyc* and *CyclinD1*), prosurvival genes (*Bcl-xl* and *Survivin*) and invasive genes (*VEG-f* and *Klf-8*), leading to fast-growing tumors with highly metastatic capability [50]. Yuanyan Li *et al*. recently demonstrated that BMX can promote cell proliferation through STAT3 signaling pathways in cervical cancer cells [51], meanwhile Zhongde Zhang *et al*. revealed that STAT3 could bind promoter region of TXNDC17 for regulating its expression and mediating Taxol resistance via enhancing autophagy in human colorectal cancer cells [52]. Along with the mechanistic insights, identification of the cell context-dependent functions for STAT3 may help ultimately develop therapeutic strategies targeting STAT3.

In the present study, we used portals to systemically analyze the expression and prognostic value of STAT3 in cancer development, which contributes to a better understanding of molecular etiology and epidemiology of cancer, and ultimately accelerates the transformation of genomic knowledge into clinical practice. Our finding demonstrates that STAT3 might serve as a diagnostic and therapeutic target for certain types of cancer, including lung, ovarian, gastric, blood and brain cancers. However, the deep mechanism of these results remains unclear, further researches need to be performed in the future.

MATERIALS AND METHODS

Oncomine database analysis

Oncomine database (https://www.oncomine.org/resource/login.html), an online database consisting of previously published and open-access microarray data, was performed to identify the transcription level of STAT3 gene in various types of cancers [53, 54]. The

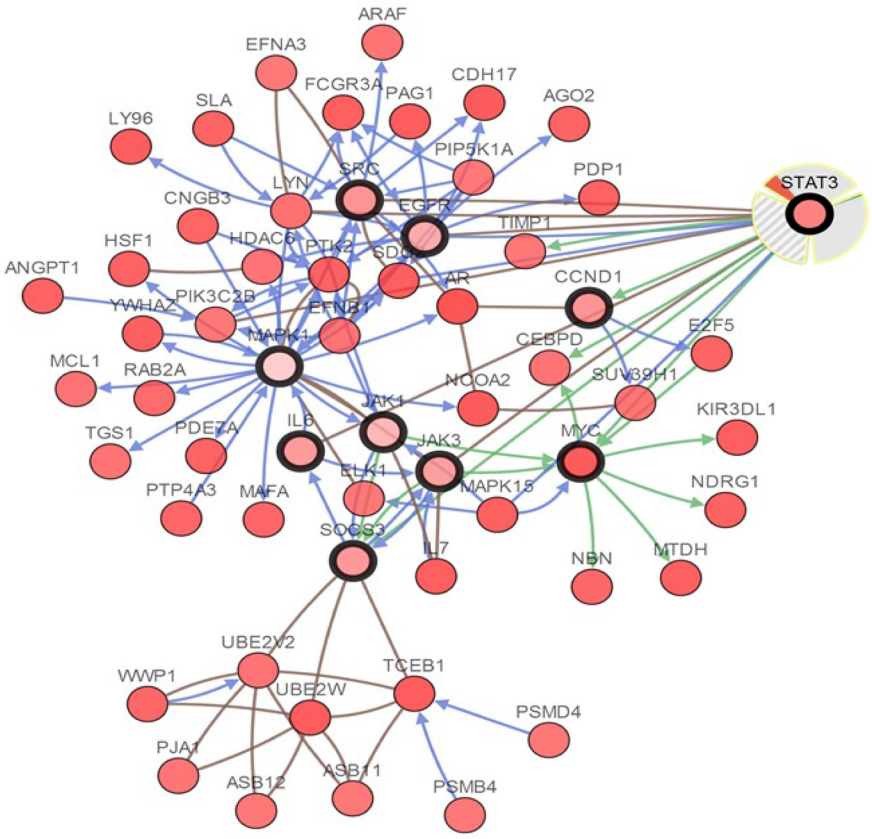

Figure 11: Interactive analysis and visualize the altered networks of STAT3 (cBio Cancer Genomics Portal). Darker red indicates increased frequency of alteration (defned by mutation, copy number amplifcation, or homozygous deletion) in Neuroendocrine Prostate Cancer.

mRNA expression of STAT3 in clinical cancer tissue was compared with that in normal control, using a Students' *t*-test to generate a *p* value. The parameters *p*-value < IE-4, fold change >2, and gene ranking in the top 10% were used to obtain the most significant STAT3 probes. Heat map was used to define the co-expression profiles of STAT3 gene in different types of cancers.

cBioPortal database analysis

The cBioPortal for Cancer genomics is an open-access resource (http://www.cbioportal.org/) [55, 56], providing visualization and analyzing tool for more than 5,000 tumor samples from 105 cancer studies in TCGA pipeline. The search interface combined with customized data storage enabled researchers to interactively explore genetic alterations across samples from other cancer studies and specific genes. The term "STAT3" was searched in cBioPortal database and a cross-cancer summary was obtained for it. The search parameters included alterations (amplification, deep deletion, missense mutations), copy-number variance (CNV) from GISTIC and RNA seq data with the default setting. OS and DFS were calculated on the basis of cBioPortal's online instruction.

Kaplan–Meier plotter database analysis

Kaplan-Meier Plotter (http://kmplot.com/analysis/) is an online database of published microarray datasets that assess the effect of 54,675 genes on survival using 10,461 cancer samples (5,143breast, 1,816 ovarian, 2,437 lungs and 1,065 gastric cancer) [57]. We performed the Kaplan-Meier plotter to assess the prognostic value of STAT3 expression in patients with breast, gastric, ovarian and lung cancer. The hazard ratio (HR) with 95% confidence intervals (CI) and log rank *p*-value was also computed.

Prognoscan database analysis

PrognoScan (http://www.prognoscan.org/) is a comprehensive online platform for assessing potential tumor biomarkers and therapeutic targets. We used the PrognoScan platform to validate the prognostic value of STAT3 expression in patients with various types of cancers. The threshold was adjusted to cox *p*-value < 0.05.

Identifying the protein components of STAT3 axis

We utilized the STRING analysis tool (http://www.string-db.org/), a database of known and predicted protein interacting, to determine interacting proteins using STAT3 as the query [58].

Statistical analysis

All statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA). Survival curves were plotted using the cBioPortal and Kaplan–Meier plots. All results are displayed with *P* values from a long-rank test. Similarly, with Oncomine, heatmaps. A *P* values of < 0.05 were considered to be statistically significant.

CONFLICTS OF INTEREST

None.

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