Identification of STAT5A as drug screening biomarker and immune checkpoint inhibitor in stomach adenocarcinoma via comprehensive bioinformatics analysis

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Research

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Abstract

Background: Gastric cancer is one of the fatal diseases and the third leading cause of cancer-related deaths worldwide. Limited drugs or therapeutic methods could be applied for these patients, and their prognosis was poor median overall survival (OS) of about 10 months. Increasing evidences demonstrated STAT gene family as biomarkers for prognosis, immunotherapy and drug screening.

Methods: The drug screening biomarker and immune checkpoint inhibitor among STAT family were explored with several online bioinformatics tools.

Results: The level STAT1/3/5A were significantly increased in STAD tissues compared with normal tissues. Methylation could downregulate STAT family expression, except STAT4. STAT1/5A/5B/6 may functioned as biomarkers for the prognosis of STAD patients. amplification and mRNA high were the most common genetic alteration form, genetic alterations could affect the disease-free survival but not overall survival of STAD patients. STAT family were involved in the activation of apoptosis pathway, EMT pathway, and hormone ER pathways, and the inhibition of cell cycle pathway, and DNA damage response pathways. Moreover, STAT5A and STAT5B were related with the drug sensitivity. Immune infiltration revealed that STAT5A level showed significant correlation with the abundance of immune cells (CD8+ T cells, CD4+ T cells, Macrophage, Neutrphils and Dendritic cells) and the level immune biomarkers. Somatic copy number alterations of STAT5A could significantly inhibit immune cell infiltration.

Conclusions: STAT5A act as drug screening biomarker and immune checkpoint inhibitor in STAD.

1. Introduction

Gastric cancer is one of the fatal diseases and the third leading cause of cancer-related deaths worldwide, with nearly 1 million people initially diagnosed with gastric cancer each year (1). Gastric adenocarcinoma (stomach adenocarcinoma, STAD) were the most common subtypes of gastric cancer, ranking over 95% of all the gastric cancer cases. About half of gastric cancer patients are present with eventually relapse (2). Moreover, 2/3 of gastric cancer patients are already in advanced stage (3). However, limited drugs or therapeutic methods could be applied for these patients, and their prognosis was poor median overall survival (OS) of about 10 months (4). These sobering data illustrate a critical need of therapeutic targets in gastric cancer.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway functions as a significant downstream mediator, involved in the regulation of cytokines and immune response (5). Activation JAK/STAT signaling and STATs mutation are found to be widely existed in the pathogenesis and progression of malignancies (6). For example, STAT3 and STAT5A activation have been highlighted in the promotion of tumorigenesis and cancer stem cell (7). Moreover, increasing evidences demonstrated JAK and STAT gene family as biomarkers for prognosis, immunotherapy and drug screening, such as JAK2 as drug targets in myeloproliferative neoplasms(8) and STAT3 as therapeutic target for breast cancer (9). As far as we know, the role of STATs in STAD remain a mystery.

Thus, our study is the first study STATs function exploration in STAD. We performed a bioinformatic analysis to clarify STATs expression level and their role in the pathological stage, prognosis, cancer hallmarks. Besides, STAT5 is selected for further analysis about its association with immune infiltration and drug sensitivity. Our results identified STAT5A as drug screening biomarker and immune checkpoint inhibitor in STAD.

2. Materials And Methods

2.1. Oncomine

Oncomine is a bioinformatics portal helping individual researchers gain more insights for gene expression, drug identification and biological functions (10). In our study, STATs mRNA level dataset in STAD were extracted from Oncomine. And the threshold is 0.05 for P-value, 2 for old-change, and Top 10% for Gene rank.

2.2 UALCAN

UALCAN is a bioinformatics portal helping individual researchers gain more insights for gene expression and its correlation with prognosis (11). In our study, relative STATs level in tumor tissue and normal tissues extracted from UALCAN. And the threshold is 0.05 for P-value

2.3 GEPIA

GEPIA is a bioinformatics portal helping individual researchers gain more insights for gene expression and its correlation with pathological stage (12). In our study, correlation between STAT expression and pathological stage was extracted from GEPIA.

2.4 The Kaplan Meier plotter

The Kaplan Meier plotter (KM plotter) is a bioinformatics portal helping individual researchers gain more insights for survival analysis (13). In our study, the significance of STATs on the first progression (PF), overall survival (OS) and post progression survival (PPS) was evaluated with KM plotter. And the medium value of STATs expression was the cut-off for splitting patients.

2.5 cbioportal

cbioportal is a bioinformatics portal helping individual researchers gain more insights for multidimensional cancer genomics (14). In our study, STATs expression genetic alteration and its association with the disease-free survival (DFS) and OS was evaluated with cbioportal.

2.6 GSCALite

GSCALite is a bioinformatics portal helping individual researchers gain more insights gene expression, Methylation, cancer-related signaling activity, drug sensitivity (15). In our study, the correlation between

STATs expression and Methylation, cancer-related signaling activity as well as drug sensitivity were evaluated with GSCALite.

2.7 TIMER

TIMER is a bioinformatics portal helping individual researchers gain more insights for tumor-immune infiltrations (16). In our study, the correlation between STAT5A expression and the immune cell infiltrates as well as immune gene biomarkers were evaluated with TIMER.

3. Results

3.1 Defining STAT family in STAD and normal tissue STAD.

To explore the function of STAT family in STAD, we first check their expression with Oncomine. And seven members of STAT family were identified in mammal (Fig. 1). Based on the data of Oncomine, the mRNA level of STAT1/3/5A/5B was significantly increased in STAD tissues (Table 1). A total of two datasets suggested that STAT1 was upregulated in gastric intestinal type adenocarcinoma (*P* = 6.96E-15) and gastric mixed adenocarcinoma (*P* = 1.34E-04), with a fold Change of 2.703, and 2.449, respectively (17). As for STAT3 expression, three datasets revealed upregulation of STAT3 STAD (18). According to the dataset of Mariarosaria, STAT5A (fold change = 2.563, *P* = 4.53E-04) and STAT5B (fold change = 2.89, *P* = 5.59E-045) were upregulated in Gastric Mixed Adenocarcinoma compared with normal gastric tissue.

Table 1
The mRNA levels of STAT family in STAD.

TLR	Type	Fold Change	P value	t-test	Reference
STAT1	Gastric Intestinal Type Adenocarcinoma	2.703	6.96E-15	9.751	PMID:12925757
	Gastric Mixed Adenocarcinoma	2.449	1.34E-04	5.291	PMID:12925757
STAT3	Gastric Mixed Adenocarcinoma	2.190	6.45E-06	7.834	PMID:19081245
	Diffuse Gastric Adenocarcinoma	2.096	4.08E-04	5.117	PMID:19081245
	Gastric Intestinal Type Adenocarcinoma	2.252	2.26E-10	7.653	PMID:19081245
STAT5A	Gastric Mixed Adenocarcinoma	2.563	4.53E-04	5.012	PMID:19081245
STAT5B	Gastric Mixed Adenocarcinoma	2.895	5.59E-04	5.077	PMID:19081245

UALCAN was used to further detect STAT family expression in STAD. As a consequence, the TCGA STAD dataset suggested that the level of STAT1(*P* < 1E-12), STAT2 (*P* = 1.62E-12), STAT3 (*P* = 1.78E-08), STAT4 (*P* = 1.90E-07), STAT5A(*P* = 7.50E-08) and STAT6 (*P* = 0.0084) were increased in STAD (Fig. 2A). However, no significance of STAT5B expression was obtained between tumor tissues and normal tissues.

We then analyzed the correlation between STAT family expression and pathological stage as well as methylation. As shown in Fig. 2B, the expression of STAT2, STAT4, and STAT6 were significantly correlated with pathological stage. More specifically, with the development of STAD, the expression of STAT2, STAT4, and STAT6 gradually increased (Fig. 2B). Moreover, methylation analysis revealed that methylation could downregulate STAT family expression, except STAT4 (Fig. 2C)

3.2 The prognostic value of STAT family in STAD.

We then evaluated the prognostic value of STAT family in STAD. In overall survival, we revealed that STAD patients with high level of STAT1(HR = 0.7, 95%CI:0.59–0.83, *P* = 5E-05) and low level of STAT5A(HR = 1.25, 95%CI:1.06–1.48, *P* = 0.0094), STAT5B(HR = 1.54, 95%CI:1.3–1.83, *P* = 6.8E-07), and STAT6(HR = 1.28, 95%CI:1.08–1.52, *P* = 0.0042) (Fig. 3A). Moreover, low STAT1(HR = 0.6, 95%CI:0.48–0.75, *P* = 4.9E-06) level and high level of STAT4(HR = 1.36, 95%CI:1.09–1.7, *P* = 0.0056), STAT5A(HR = 1.53, 95%CI:1.22–1.91, *P* = 0.00016), STAT5B(HR = 2.37, 95%CI:1.88–2.99, *P* = 4.2E-14), and STAT6(HR = 1.36, 95%CI:1.09–1.69, *P* = 0.0063) were significantly associated with a worse PPS (Fig. 3B). As for PF analysis, the data suggested a worse PF in STAD patients with low STAT1(HR = 0.71, 95%CI:0.58–0.87, *P* = 0.00091) level and high STAT5B (HR = 1.61, 95%CI:1.31–1.97, *P* = 4.4E-06) level. (Fig. 3C)Therefore, STAT1/5A/5B/6 may functioned as biomarkers for the prognosis of STAD patients.

3.3 STAT family associates with the cancer hallmarks in STAD.

In order to evaluate the potential effects of disruption of STAT family in STAD patients, we performed genetic alteration, cancer-related pathway and STAT family. We found that STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6 were altered in 8%, 6%, 6%, 7%, 11%, 8%, 11% of the queried STAD samples, respectively (Fig. 4A). Moreover, amplification and mRNA high were the most common alteration forms (Fig. 4A). Interestingly, these genetic alterations could affect the disease-free survival (Fig. 4B, P = 0.0117) but not overall survival (Fig. 4C, P = 0.306). we also analysis the role of STAT family in famous cancer-related pathway activity, which revealed that STAT family were involved in the activation of apoptosis pathway, EMT pathway, and hormone ER pathways (Fig. 4D). Besides, STAT family were involved in the inhibition of cell cycle pathway, and DNA damage response pathways (Fig. 4D).

3.4 Enrichment analysis of STAT family in STAD.

In order to further clarify the function of STAT family in STAD, we performed enrichment analysis of STAT family in STAD. GO enrichment analysis revealed that the functions of STAT family in STAD were mainly associated with JAK-STAT cascade, interleukin-9-mediated signaling pathway, and interleukin-21-

mediated signaling pathway (Fig. 5A-5B). Enrichment analysis of KEGG pathway suggested that the significant association between STAT family and hepatitis B, Th1 and Th2 cell differentiation as well as Th17 cell differentiation (Fig. 5C-5D). MCODE was extracted and revealed the involvement of STAT family in JAK-STAT cascade, STAT cascade and cellular response to interleukin-9 (Fig. 5E-5F).

3.5 Drug sensitivity analysis of STAT family in STAD.

Previous results suggested that STAT family may play an important part in the tumorigenesis and progression of STAD, and some of STAT family may act as the therapeutic targets in STAD. An important way to develop drug therapy targets for cancer is to evaluate the association between genes and existing drug targets. Thus, we analyzed the correlation of STAT family expression and 481 small molecules or drugs from Therapeutics Response Portal (CTRP) as well as 265 small molecules or drugs from Genomics of Drug Sensitivity in Cancer (GDSC). Based on the results of GDSC, sensitivity (negative correlation) is associated with the expression of STAT5A and STAT5B (Fig. 6). Interestingly, similar results were obtained for CTRP. STAT5A and STAT5B were related with the drug sensitivity (negative correlation) (Fig. 7). The results may suggest that STAT5A and STAT5B is potential biomarkers for drug screening.

3.6 Immune infiltration of STAT5A expression in STAD.

Increasing evidences demonstrated that JAK/STAT pathway is crucial in the regulation of the immune response, and some of STAT family could act as the immune checkpoint inhibitor for various diseases, including cancers (5, 7, 19). Above result suggested STAT5A played an important part in the tumorigenesis and progression of STAD and acted as a potential biomarker for drug screening in STAD. Therefore, STAT5A were selected for further analysis its potential as the immune checkpoint inhibitor for STAD. We first evaluated the correlation between STAT5A level and immune cell infiltration, which revealed that STAT5A level showed significant correlation with the abundance of 5 immune cells (all $P<$ 0.001, Fig. 8A), including CD8 + T cells (Cor = 0.419), CD4 + T cells(Cor = 0.35), Macrophage (Cor = 0.354), Neutrphils (Cor = 0.436) and Dendritic cells(Cor = 0.543). Moreover, somatic copy number alterations of STAT5A could significantly inhibit immune cell infiltration (Fig. 8B). In order to further clarify the significant role in immune infiltration, we also evaluated the correlation between STAT5A level and immune biomarkers, which have been widely reported (6, 20–22). As expected, strong correlations were obtained between STAT5A level and immune biomarkers in STAD (Table 2). We revealed positive correlation between STAT5A expression and the level of all the biomarkers of CD8 + T cell (CD8A, CD8B), T cell (CD3D, CD3E, CD2), B cell (CD19, CD79A), Monocyte (CD86, CD115), TAM (CCL2, CD68, IL10). Most of the biomarkers of M1 Macrophage, M2 Macrophage, Neutrophils and Natural killer cell were positively associated STAT5A expression. Moreover, the expression of all the biomarkers of Dendritic cell (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, CD1C, NRP1, ITGAX), Th1 (TBX21, STAT4, STAT1, IFNG, TNF), Th2(GATA3, STAT6, IL13) and Tfh (BCL6, IL21) presented positive correlations with STAT5A level in STAD. Those STAD patients with high STAT5A level were also present with high level of FOXP3, CCR8,

STAT5B, TGFB1, PD-1, CTLA4, LAG3, TIM-3, and GZM). These evidences demonstrated STAT5A as immune checkpoint inhibitor for STAD immunological therapy.

Table 2
The association between STAT5A and immune biomarkers in STAD.

Immune cells	Biomarkers	Correlation	P-value
CD8 + T cell	CD8A	0.479	***
	CD8B	0.259	***
T cell (general)	CD3D	0.424	***
	CD3E	0.454	***
	CD2	0.455	***
B cell	CD19	0.356	***
	CD79A	0.362	***
Monocyte	CD86	0.416	***
	CD115(CSF1R)	0.488	***
TAM	CCL2	0.228	***
	CD68	0.296	***
	IL10	0.411	***
M1 Macrophage	INOS (NOS2)	0.104	*
	IRF5	0.257	***
	COX2(PTGS2)	0.044	0.392
M2 Macrophage	CD163	0.452	***
	VSIG4	0.376	***
	MS4A4A	0.452	***
Neutrophils	CD66b (CEACAM8)	-0.041	0.429
	CD11b (ITGAM)	0.548	***
	CCR7	0.42	***

Immune cells	Biomarkers	Correlation	P-value
Natural killer cell	KIR2DL1	0.147	**
	KIR2DL3	0.129	*
	KIR2DL4	0.186	***
	KIR3DL1	0.202	**
	KIR3DL2	0.285	***
	KIR3DL3	0.064	0.214
	KIR2DS4	0.094	0.0674
Dendritic cell	HLA-DPB1	0.454	***
	HLA-DQB1	0.349	***
	HLA-DRA	0.45	***
	HLA-DPA1	0.463	***
	BDCA-1(CD1C)	0.356	***
	BDCA-4(NRP1)	0.306	***
	CD11c (ITGAX)	0.466	***
Th1	T-bet (TBX21)	0.497	***
	STAT4	0.452	***
	STAT1	0.226	***
	IFN-g (IFNG)	0.267	***
	TNF-a (TNF)	0.165	**
Th2	GATA3	0.321	***
	STAT6	0.251	***
	STAT5A	-	-
	IL13	0.138	***
Tfh	BCL6	0.235	***
	IL21	0.27	***
Th17	STAT3	0.43	***
	IL17A	-0.106	0.04

Immune cells	Biomarkers	Correlation	P-value
Treg	FOXP3	0.419	***
	CCR8	0.481	***
	STAT5B	0.533	***
	TGFb (TGFB1)	0.357	***
T cell exhaustion	PD-1 (PDCD1)	0.448	***
	CTLA4	0.308	***
	LAG3	0.364	***
	TIM-3 (HAVCR2)	0.467	***
	GZMB	0.227	***

4. Discussion

STAT family, cytokine signaling regulator, play a vital function in various biological progress, such as tumorigenesis, angiopoiesis, proliferation, and metastasis (23, 24). JAK/STAT Pathway inhibitors have demonstrated efficacy in the treatment of some disease (25). Though the significance of JAK/STAT signaling pathway in cancers have been widely reported, limited study has been performed about JAK/STAT signaling pathway as the drug screening biomarker or immune checkpoint inhibitor in cancers, including STAD. Thus, our study was performed to evaluated the expression and clinical significance of STAT family, and their potential as drug screening biomarkers or immune checkpoint inhibitors in STAD.

We found that all the STAT family was upregulated in STAD tissues compared with normal tissues. Moreover, the level of STAT2/4/6 were associated with the pathological stage of STAD patients. Our study also found that STAT5A/5B/6 may function as biomarkers for the prognosis of STAD patients and correlate with poor survival. In fact, some of STAT family have been reported in gastric cancer. For example, Pan et al. suggested that STAT3 was upregulated in gastric cancer and related to advanced TNM stage and poor prognosis (26). Another review demonstrated STAT3 inhibitors as a potential drug and therapeutic target in gastric cancer (27). In other types of cancers, STAT family were also suggested as biomarkers for diagnosis or prognosis, such as STAT1 as a prognostic biomarker in ovarian cancer (28).

Another important finding of our study is that the expression alterations of STAT family are involved in the activity of several cancer-related pathways. In our study, the data highlight the inhibitory effects of cell cycle pathway and DNA damage response pathways, and the activatory effects of apoptosis pathway, EMT pathway, and hormone ER pathways. In accordance with the result of Li et al, the data in the current study further confirmed the close connection between STAT family and cell cycle arrest (29). Moreover, Lan et al. also observed the vital function of JAK/STAT signaling pathway in tumor cell

apoptosis (30). STAT family-associated EMT pathway has been highlighted in tumorigenesis and progression, including cancer growth, metastasis and drug resistance (31, 32). Therefore, STAT family may affect the tumorigenesis and progression of STAD by regulating these pathways.

In order to find out the drug screening biomarkers or the therapeutic targets among STAT family, we then evaluated the correlation of STAT family and drug sensitivity. the Pearson correlation coefficients of transcript levels and AUCs was used and normalized based on Fisher's Z transformation based on CTRP and GDSC. And STAT5A and STAT5B were related with the drug sensitivity (negative correlation), demonstrating STAT5A and STAT5B is potential biomarkers for drug screening.

In order to confirm STAT5A as the biomarker for drug screening or therapeutic target in STAD, we then explore the role of STAT5A in immune infiltration in STAD. These immune cells and biomarkers played a significant role in the pathogenesis and progress of type of cancers, including STAD. For example, CD4+/CD8 + T cells was found to be as prognostic biomarkers in gastric cancer, and affected tumor progression and patients' survival (33). CTLA-4 and PD-1, the immune checkpoints of gastric cancer, were associated with locally advanced and metastasis (34, 35). In our study, STAT5A level showed significant correlation with the abundance of immune cells (CD8 + T cells, CD4 + T cells, Macrophage, Neutrphils and Dendritic cells) and the level immune biomarkers. Somatic copy number alterations of STAT5A could significantly inhibit immune cell infiltration. These evidences suggested that STAT5A served as an immune checkpoint inhibitor in STAD. These evidences also confirmed the potential of STAT5A as drug screening biomarker in STAD.

To sum up, this study was performed with several bioinformatics tool to clarify the expression, and clinical significance of STAT family in STAD. The results identified STAT5A as drug screening biomarker and immune checkpoint inhibitor in STAD.

5. Conclusions

In summary, This paper is the first to report that STAT5A as drug screening biomarker and immune checkpoint inhibitor in stomach adenocarcinoma by bioinformatics. In order to find the potential of STAT5A which may be involved in the progress of STAD. We intend to clarify STATs expression level and their role in the pathological stage, prognosis, cancer hallmarks. Besides, STAT5 is selected for further analysis about its association with immune infiltration and drug sensitivity. Nevertheless, further molecular biological explorations are required to verify the function of the STAT5A in STAD. We believe this paper may be of particular interest to readers because this study will enrich our knowledge of prognosis, immunotherapy and drug screening, and provide new opportunities for targeted therapies in stomach adenocarcinoma.

Declarations

6. **Funding**

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7. **Competing interests**

The authors declare that they have no competing interests.

8.Authors' contributions

Lin Tao and wen tao zhang were responsible for the design of the study, data analysis work and the writing the manuscript. shuang wu and jian wen wen were responsible for the edit of the manuscript. All authors read and approved the final manuscript.

9. **Availability of data and materials**

The analyzed data sets generated during the study are available from the corresponding author on.

10. **Ethics approval and consent to participate**

Not applicable.

11. **Acknowledgements.**

Not applicable.

12. **Declaration of consent**

The authors declare that consent for publication in the journal.

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Figures

Figure 1

STAT family level in STAD. The number in the figure was the numbers of datasets with statistically significant ($p<0.01$) mRNA over-expression (red) or down-expression (blue) of STATs, which was obtain with the P-value of 0.05 and fold change of 2.

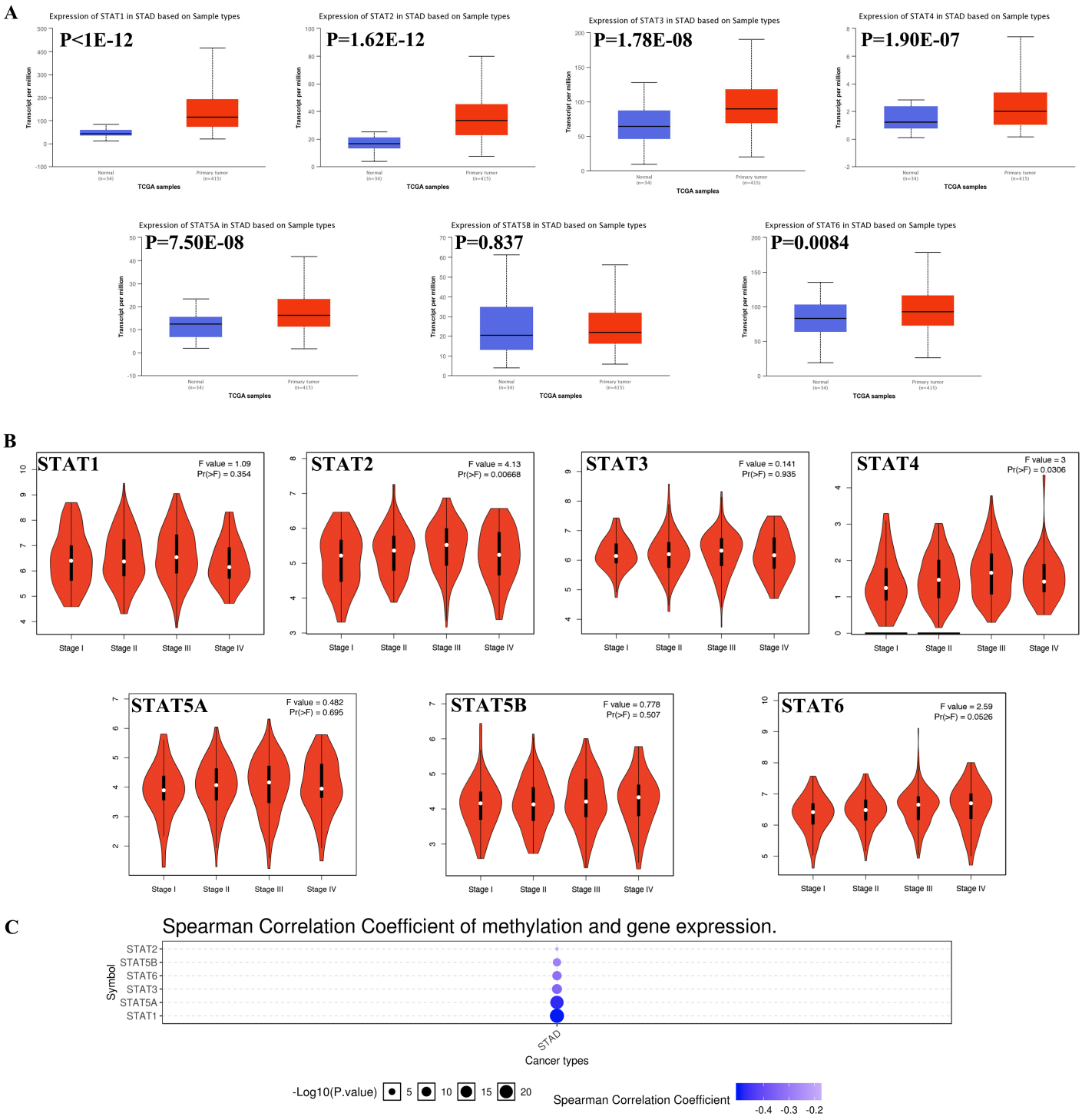


Figure 2

STAT family level in STAD. (A) The relative STAT family level in STAD tissues and normal tissues. (B) the correlation STAT family level and pathological stage. (C) the correlation STAT family level and methylation.

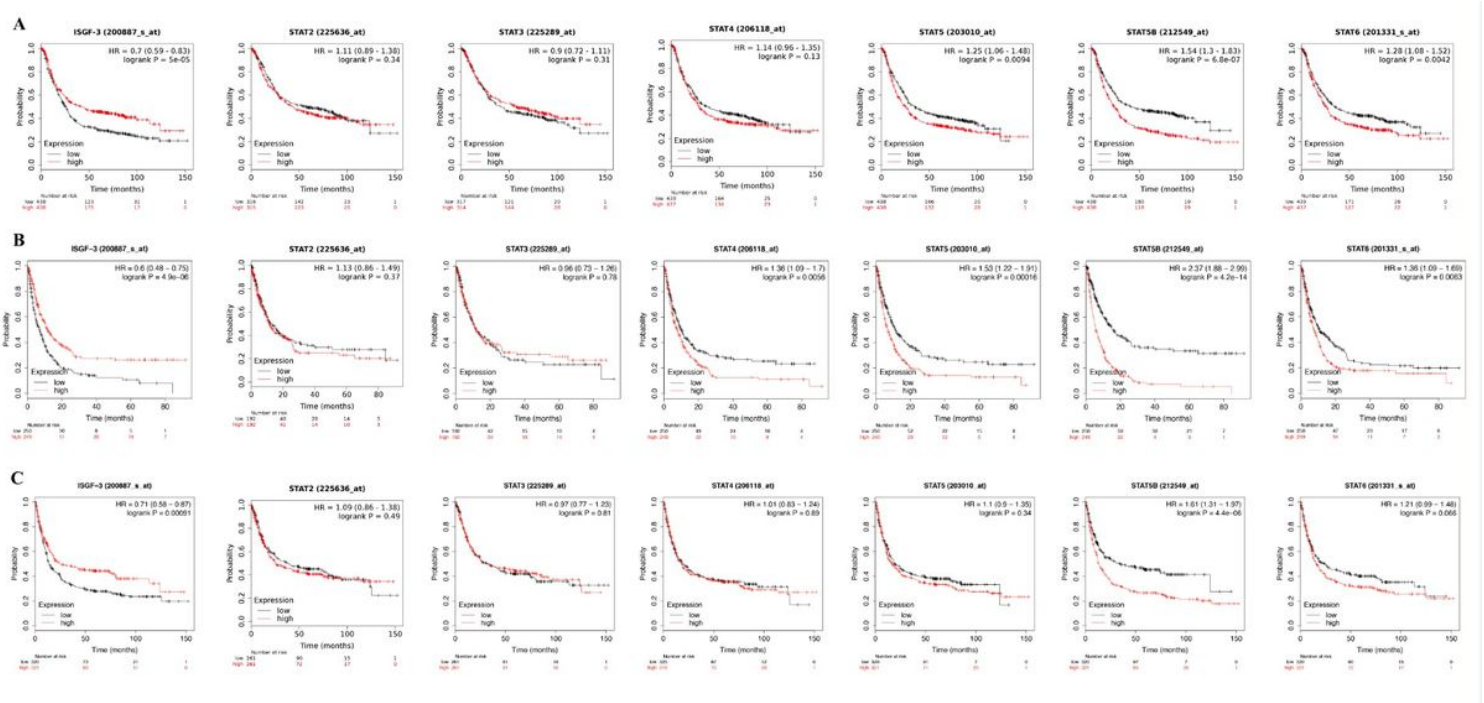


Figure 3

The prognostic value of STAT family in STAD. (A) the role of STAT family in the overall survival of STAD. (B) the role of STAT family in the post progression survival of STAD. (C) the role of STAT family in the first progression of STAD.

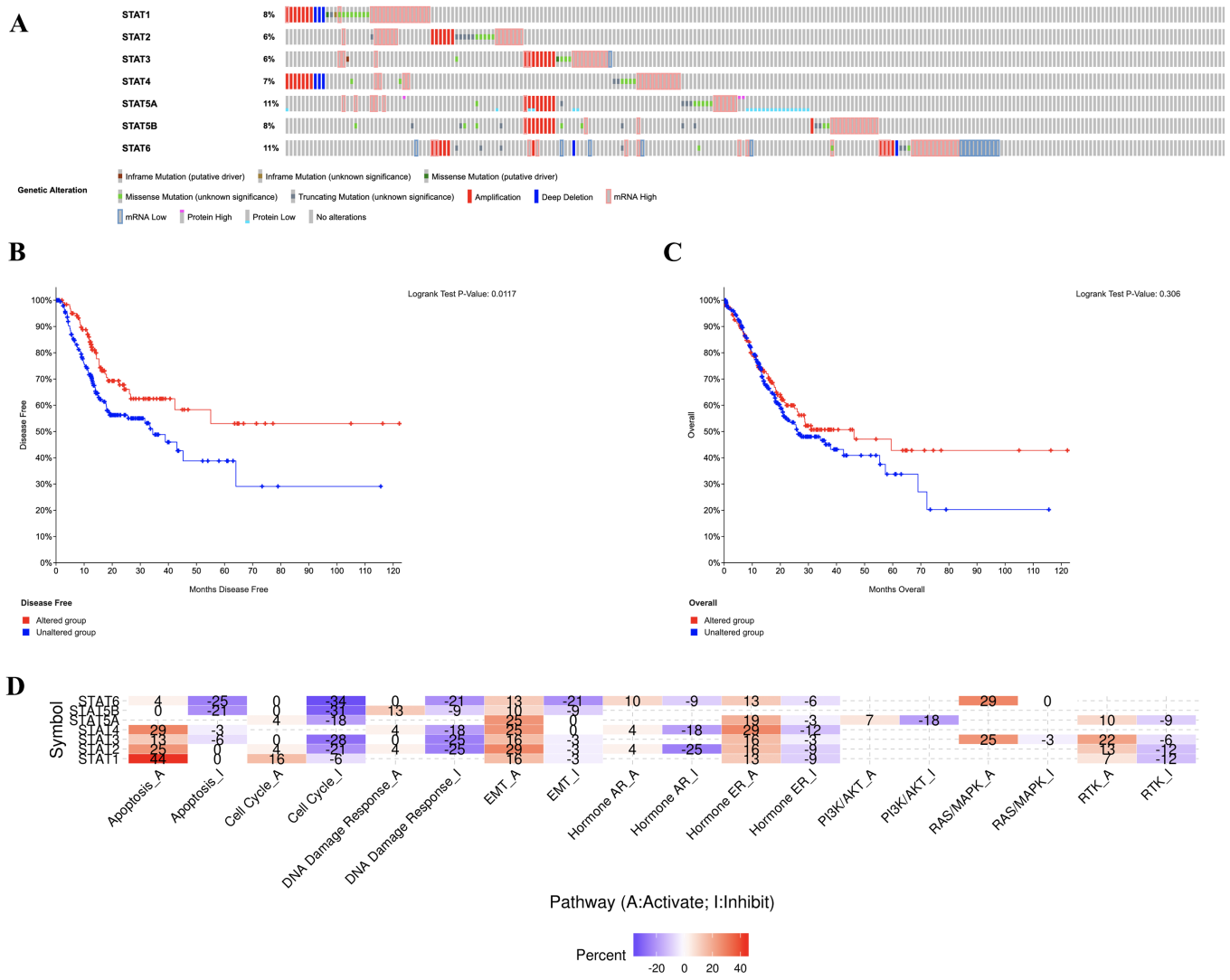
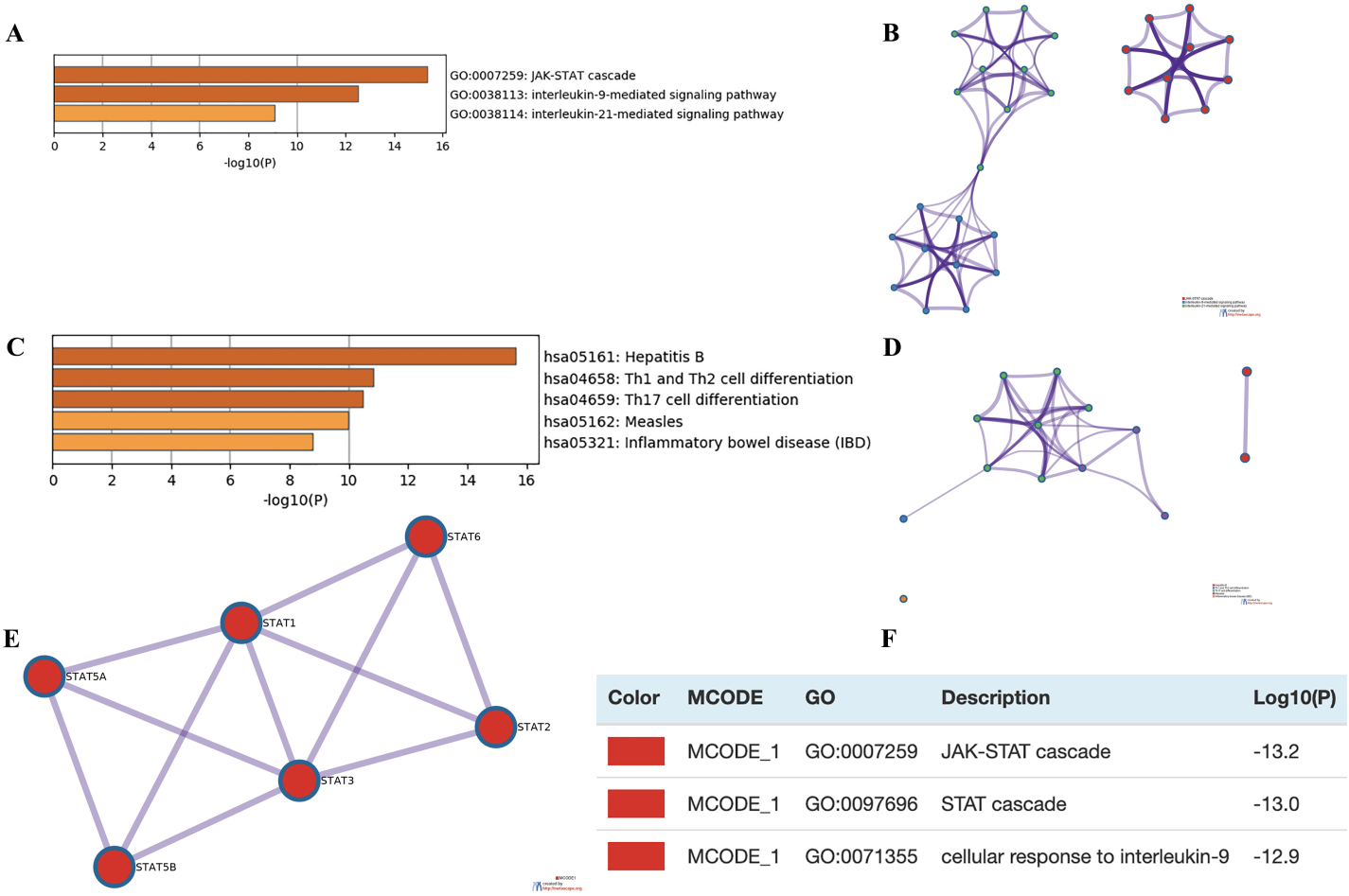


Figure 4

The cancer hallmarks analysis of STAT family in STAD. (A) Summary of alterations of STAT family in STAD. (B-C) Kaplan–Meier plots comparing disease-free survival and overall survival in cases with/without STAT family alterations. (D) the role of STAT family in the famous cancer-related pathway.



Color	MCODE	GO	Description	Log10(P)
	MCODE_1	GO:0007259	JAK-STAT cascade	-13.2
	MCODE_1	GO:0097696	STAT cascade	-13.0
	MCODE_1	GO:0071355	cellular response to interleukin-9	-12.9

Figure 5

The enrichment analysis of STAT family in STAD. (A-B) Heatmap and Network of Gene Ontology (GO) enriched terms colored by p-values. (C-D) Heatmap and Network of Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched terms colored by p-values. (E-F) Network and enriched items of MCODE analysis.

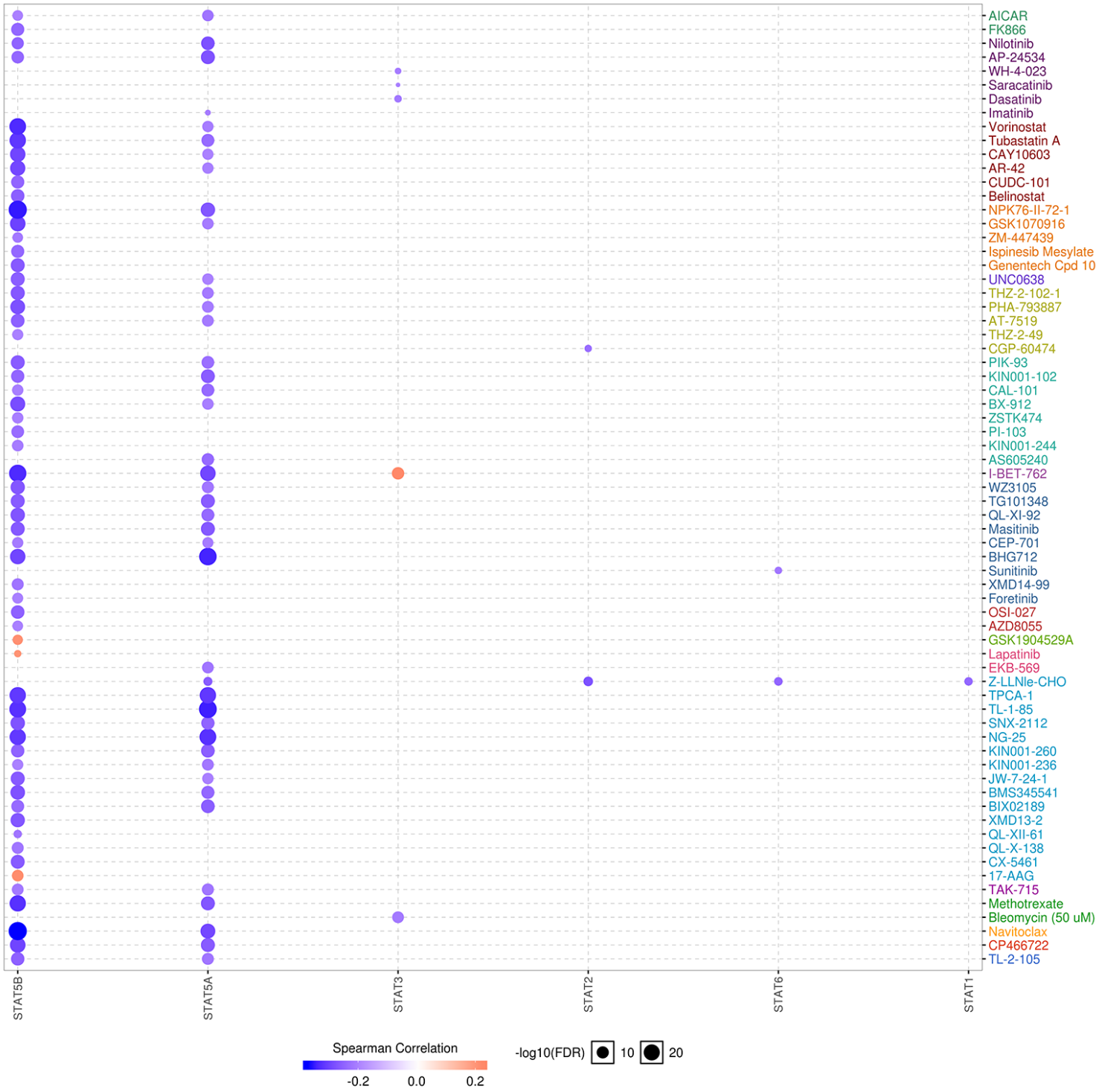


Figure 6

The drug resistance analysis of STATs based on GDSC IC50 drug data. The Spearman correlation represent the gene expression correlates with the drug. The positive correlation means that the gene high expression is resistant to the drug, vise verse.

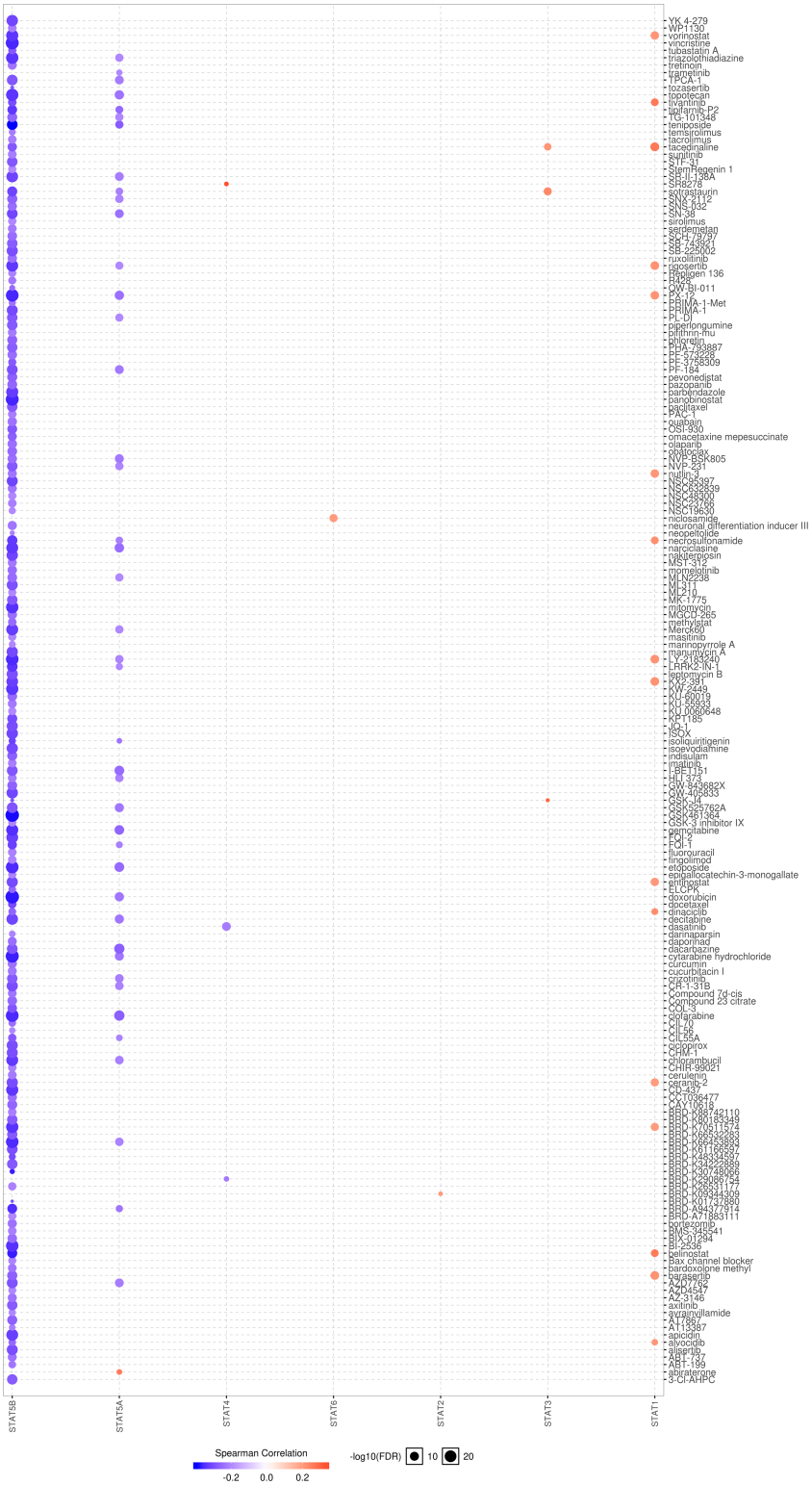


Figure 7

The drug resistance analysis of STATs based on CTRP IC50 drug data. The Spearman correlation represent the gene expression correlates with the drug. The positive correlation means that the gene high expression is resistant to the drug, vise verse.

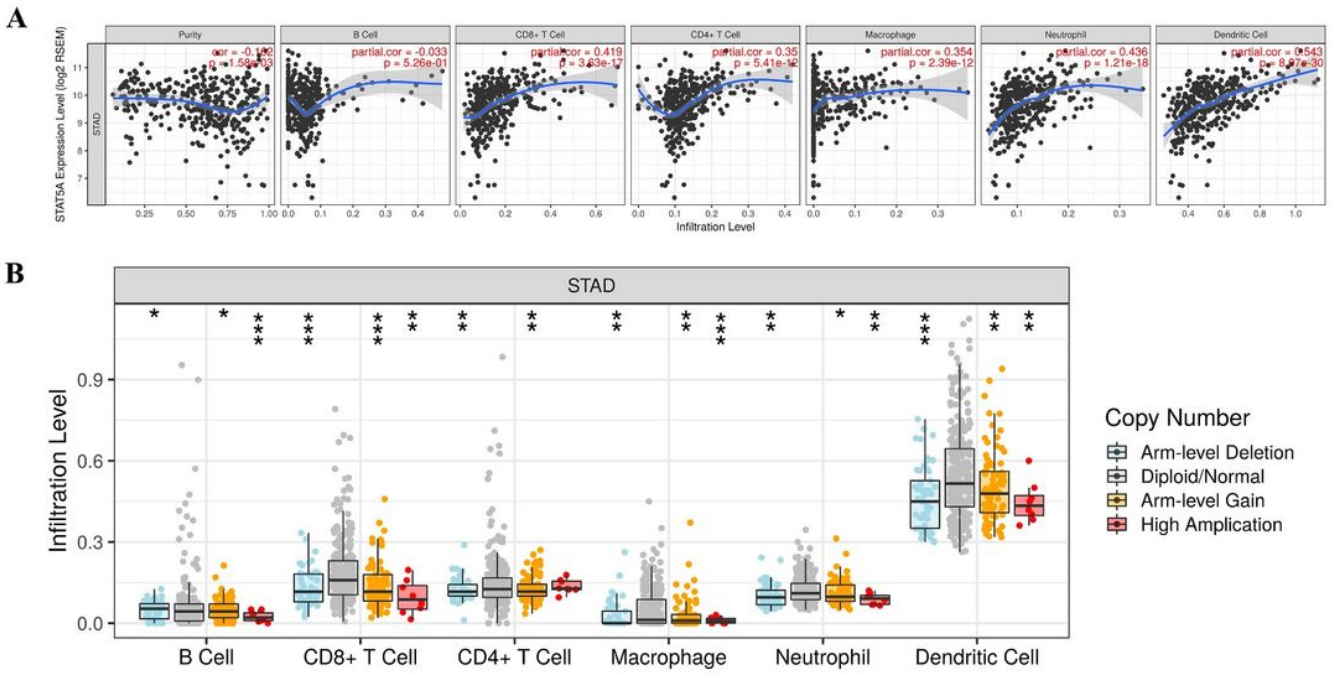


Figure 8

Immune infiltration STATs in STAD. (A) The correlation between STAT5A and the abundance of different immune cell level in STAD. (B) The correlation between copy number alteration of STAT5A and immune cell infiltration in STAD.