

Comprehensive Analysis of the Prognosis of S100 Family Members and Their Relationship with Tumor-Infiltrating Immune Cells in Human Pancreatic Adenocarcinoma

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Abstract

Background: S100 family members(S100s) are small molecular EF hand calcium binding proteins and widely expressed in many tissues and organs. S100s are shown to be biomarkers of disease progression and prognosis in various types of cancers. Nevertheless, the expression patterns, function, prognostic values of S100s and its association with tumor-infiltrating immune cells in Pancreatic Adenocarcinoma(PAAD) patients have not been systematically clarified.

Methods: we explored that the expression and roles of the entire twenty S100s in PAAD patients by using the following public databases: Oncomine, GEPIA, cBioPortal, Metascape, STRING, TIMER and GeneMANIA.

Results: The S100A2/A3/A4/A6/A8/A9/A10/A11/A13/A14/A16/B/P mRNA expression were significantly upregulated in PAAD patients. The mRNA expression of S100A3/A4/A5/A6/A10/A11/A14/A16/Z were significantly negatively related with the tumor stage in PAAD patients. We found that the S100A2/A3/A5/A10/A11/A14/A16 were significantly correlated with poor OS, whereas the increased levels of S100A1/B/G/Z were strongly associated with good OS. We found significant correlations among S100s and Tumor-Infiltrating Immune Cells. Cox proportional risk models revealed that B cells, Dendritic cells and S100A1/A5/A6/A8/A9/A13/A14 were significantly related with outcomes in PAAD patients.

Conclusions: S100A2/A3/A10/A11/A14/A16 may serve as new diagnostic and prognostic biomarkers for PAAD patients and provide new clues for immunotherapy in PAAD patients.

Background

Pancreatic Adenocarcinoma(PAAD) always cause high lethality in patients, and the incidence of PAAD has increased over the past few decades and it is likely to continue to increase further^{1,2}. It is predicted that by 2030, the number of deaths from PAAD will dramatically increase and become the second most common cause of death from cancers³. Hence, there is a need to look for reliable predictive biomarkers and accurate outcomes of PAAD patients.

S100 family members(S100s) are small molecular EF hand calcium binding proteins and that are widely expressed in many tissue organs and play important roles in many biological processes, including Calcium homeostasis, inflammation, proliferation, differentiation, cell migration, apoptosis and so on⁴. Over the past decades, numerous researches have revealed that S100s are shown to be a biomarker for disease progression and prognosis in different types of cancers, for instance, lung cancer⁵, acute myeloid leukemia⁶, melanoma⁷, ovarian cancer^{8,9}, breast cancer¹⁰, and so on. The aberrant expression of S100s and its correlation with prognosis and pathological features have been partially reported in PAAD patients. Several studies have suggested that S100A4 may serve as a possible biomarker, because of its expression was correlated with malignancy, metastasis, invasion and poor OS in PAAD^{11,12}. The higher

expression of S100A2 was associated with progression, and predicts a good response to pancreatectomy in PAAD^{13,14}. S100A6, S100A11 and S100P may also be useful biomarkers for the diagnosis in PAAD patients^{15,16}. However, the functions of other S100s in PAAD have not yet been completely elucidated.

As far as we know, there have not been use systematical bioinformatics analysis to investigate the expression of the entire twenty S100s and associations with tumor immunity in PAAD patients. In recent years, microarray and RNA-seq technology has developed very quickly¹⁷, in our study, we used the different databases to analyze the expression patterns, mutations, functional networks and correlations with tumor stage, outcomes and Tumor-Infiltrating Immune Cells in PAAD patients.

Methods

Oncomine

The Oncomine is an integrated online microarray database¹⁸. In our research, we contrasted the S100s mRNA expression of tumor tissues with that of normal tissues in various cancers, and p value was generated by Student's- t test, with p value <0.01 , fold change ≥ 2 , data type: mRNA, gene bank: 10%.

GEPIA 2

The GEPIA 2 datasets is an easy-to-use online tool and used to analyze the RNA-seq results from the TCGA and GTEx projects, containing 8587 normal and 9736 tumors samples¹⁹. In our research, we performed the S100s mRNA expression analysis, tumor stage analysis, Overall survival(OS), Disease-free survival(DFS) by using GEPIA 2. The threshold of p value was 0.05. The expression and tumor stage analysis were evaluated using the Student's t -test. OS and DFS were evaluated using Kaplan–Meier method.

TCGA and cBioPortal

TCGA database contain RNA-seq data and clinicopathologic information for thirty various cancers²⁰. In our study, we used the cBioPortal^{21,22} to perform the S100s genomics profiles analysis of the Pancreatic Adenocarcinoma(TCGA, Firehose Legacy) dataset, including 186 cases, including mutations, Putative copy-number alterations from GISTIC, mRNA Expression z-Scores (threshold: ± 2.0) and Protein expression Z-scores (threshold: ± 2.0). In addition, we conduct the co-expression analysis and correlation analysis among the genetic mutations of S100s with OS and DFS by using the cBioPortal.

GeneMANIA

The GeneMANIA is a online tool and provides some information, including protein-protein and gene-gene interactions, protein domain similarity, co-localization, co-expression, pathways, and so on²³. In our study, we used GeneMANIA to find neighboring genes which were significantly related with S100s by performing

the pathway enrichment and functional annotation analysis, and then construct a gene-gene interaction network.

STRING

The STRING is a friendly website and used to predict the protein-protein interaction(PPI)²⁴. In our study, we constructed a PPI network of S100s by using STRING.

Metascape

The Metascape is a friendly website for exploring GO enrichment analysis and KEGG enrichment analysis²³. In our research, we analyzed the gene annotation and pathway enrichment analysis of S100s and neighboring genes by using the Metascape. The thresholds of *p* value, Min Overlap and Min Enrichment were limited as 0.01, 3 and 1.5, respectively.

TIMER 2.0

The TIMER 2.0 is a very convenient and practical website for

exploring the correlations between tumor-infiltrating immune cells and their effects on clinical prognosis^{25,26}. In our research, we used the TMIER 2.0 to investigate the relationship of S100s expression with tumor-infiltrating immune cells and clinical outcomes in PAAD patients by Cox proportional hazard model.

Results

The S100s mRNA expression in PAAD patients

We compared the S100s mRNA expression in different types of cancers with normal tissues. S100s, such as S100A1, S100A2, S100A3, S100A4, S100A5, S100A6, S100A7, S100A7A, S100A8, S100A9, S100A10, S100A11, S100A12, S100A13, S100A14, S100A16, S100B, S100G, S100P and S100Z were explored in human multiple cancers in Oncomine(Fig. 1 and Table 1). The databases included 349, 335, 342, 309, 300, 243, 320, 153, 344, 326, 352, 314, 344, 343, 248, 171, 362, 296, 344, 147 unique analyses for S100A1-S100Z, respectively. The mRNA expressions of S100s were significantly altered in a majority of human cancers, including upregulated and downregulated. The mRNA expression of S100A2/A4/A6/A10/A11/A13/A14/A16/P were overexpressed in PAAD patients. The other S100s were not significantly difference in PAAD and normal tissues.

As it shown in Table 1, in Pei's dataset, S100A2/A4/A6/A10/A11/A14/A16/P were overexpressed in PAAD tissue with a fold change of 7.68, 4.857, 9.149, 3.537, 4.953, 6.455, 4.396 and 77.931, respectively. Iacobuzio-Donahue's dataset, S100A2/A10/A11/A13/P were up-regulated in PAAD tissue with a fold change of 3.613, 5.514, 7.198, 2.225 and 24.015, respectively. Logsdon et al. showed that

S100A4/A10/A11/A13/P were increased in PAAD tissue(fold change: 4.442, 7.578, 18.29, 7.197 and 20.309, respectively). Badea et al. shown that the higher levels of S100A4/A6/A10/A11/A13/A16/P were found in PAAD tissue(fold change: 4.374, 5.919, 3.095, 4.43, 2.186, 2.327 and 13.177, respectively). Segara et al. showed that S100A6/A10/A11/A13/P were found higher expressed in PAAD tissue with a fold change of 4.764, 4.299, 7.519, 2.68 and 17.727, respectively. Grutzmann et al. found that the expression of S100A16 (fold change = 2.36) and S100P (fold change = 8.382) were raised in PAAD tissue versus normal tissues. Buchholz et al. reported that S100A16 was over-expressed in PAAD tissue(fold change = 2.162). Ishikawa et al. reported that S100P (fold change = 4.247) was higher expressed in PAAD patients.

Table 1
The significant change of S100s mRNA expression in PAAD patients(Oncomine)

	Type of Pancreatic Cancer vs. Normal	Fold Change	p value	t Test	References	PMID
S100A2	Pancreatic Carcinoma	7.68	2.97E-08	6.379	Pei Pancreas	19732725
	Pancreatic Adenocarcinoma	3.613	6.00E-03	2.936	Iacobuzio-Donahue Pancreas 2	12651607
S100A4	Pancreatic Adenocarcinoma	4.442	6.52E-05	5.738	Logsdon Pancreas	12750293
	Pancreatic Ductal Adenocarcinoma	4.374	1.54E-10	7.614	Badea Pancreas	19260470
	Pancreatic Carcinoma	4.857	3.49E-06	5.569	Pei Pancreas	19732725
S100A6	Pancreatic Carcinoma	9.149	4.98E-12	9.154	Pei Pancreas	19732725
	Pancreatic Ductal Adenocarcinoma	5.919	1.32E-12	9.024	Badea Pancreas	19260470
	Pancreatic Carcinoma	4.764	2.31E-04	4.842	Segara Pancreas	15867264
S100A10	Pancreatic Carcinoma	4.299	8.32E-07	8.149	Segara Pancreas	15867264
	Pancreatic Adenocarcinoma	7.578	2.79E-06	10.075	Logsdon Pancreas	12750293
	Pancreatic Ductal Adenocarcinoma	3.095	1.31E-11	8.377	Badea Pancreas	19260470
	Pancreatic Adenocarcinoma	5.541	7.36E-04	6.074	Iacobuzio-Donahue Pancreas 2	12651607
	Pancreatic Carcinoma	3.537	2.05E-06	6.056	Pei Pancreas	19732725
S100A11	Pancreatic Carcinoma	7.519	7.48E-07	7.646	Segara Pancreas	15867264
	Pancreatic Ductal Adenocarcinoma	4.43	1.19E-14	10.448	Badea Pancreas	19260470
	Pancreatic Adenocarcinoma	18.29	9.25E-05	10.081	Logsdon Pancreas	12750293

	Type of Pancreatic Cancer vs. Normal	Fold Change	<i>p</i> value	<i>t</i> Test	References	PMID
	Pancreatic Adenocarcinoma	7.198	8.54E-04	6.014	Iacobuzio-Donahue Pancreas 2	12651607
	Pancreatic Carcinoma	4.953	6.35E-06	5.827	Pei Pancreas	19732725
S100A13	Pancreatic Carcinoma	2.68	1.85E-05	6.004	Segara Pancreas	15867264
	Pancreatic Adenocarcinoma	7.197	2.00E-03	5.259	Logsdon Pancreas	12750293
	Pancreatic Ductal Adenocarcinoma	2.186	3.32E-10	7.256	Badea Pancreas	19260470
	Pancreatic Adenocarcinoma	2.225	6.00E-03	3.327	Iacobuzio-Donahue Pancreas 2	12651607
S100A14	Pancreatic Carcinoma	6.455	4.85E-07	6.496	Pei Pancreas	19732725
S100A16	Pancreatic Carcinoma vs. Normal	4.396	9.46E-08	7.263	Pei Pancreas	19732725
	Pancreatic Ductal Adenocarcinoma	2.36	7.00E-03	2.678	Grutzmann Pancreas	15548371
	Pancreatic Ductal Adenocarcinoma	2.327	1.05E-09	6.938	Badea Pancreas	19260470
	Pancreatic Ductal Adenocarcinoma	2.162	1.00E-02	2.689	Buchholz Pancreas	16103885
S100P	Pancreatic Adenocarcinoma	24.015	2.13E-08	11.298	Iacobuzio-Donahue Pancreas 2	12651607
	Pancreatic Adenocarcinoma	20.309	4.88E-10	15.495	Logsdon Pancreas	12750293
	Pancreatic Carcinoma	77.931	1.61E-12	10.194	Pei Pancreas	19732725
	Pancreatic Carcinoma	17.727	1.23E-05	6.943	Segara Pancreas	15867264
	Pancreatic Ductal Adenocarcinoma	4.247	3.00E-03	2.944	Ishikawa Pancreas	16053509
	Pancreatic Ductal Adenocarcinoma	13.177	8.53E-13	8.437	Badea Pancreas	19260470

Type of Pancreatic Cancer vs. Normal	Fold Change	<i>p</i> value	<i>t</i> Test	References	PMID
Pancreatic Ductal Adenocarcinoma	8.382	8.00E-03	2.703	Grutzmann Pancreas	15548371

The expression of S100s and their association with the tumor stage in PAAD patients

We compared the S100s mRNA expression of PAAD tissues with control tissues by using GEPIA 2(Fig. 2). We found that the S100A2/A3/A4/A6/A8/A9/A10/A11/A13/A14/A16/B/P mRNA expression were significantly higher in PAAD, however, the other S100s mRNA expression were not significantly changed in PAAD tissues. we also used the GEPIA 2 dataset to analyze the S100s mRNA expression with pathological stage in PAAD patients. We found that the S100A3/A4/A5/A6/A10/A11/A14/A16/P/Z mRNA expression were significantly negatively associated with tumor stage of PAAD patients, whereas other S100s have not significantly difference (Fig. 3).

Prognostic Values of S100s in PAAD patients

Using GEPIA 2, we investigated the prognostic significance of S100s in PAAD patients. Interesting, the analysis revealed that higher expression of S100A2/A3/A5/A10/A11/A14/A16 were correlated with poor OS (Over Survival) significantly, whereas the increased level of S100A1/B/G/Z were strongly associated with good OS(Fig. 4). The other S100s were not correlated with OS in PAAD patients. In addition, the S100A2/A10 /A16 mRNA expression were predicted to have worse DFS(Disease-free survival), whereas the upregulated levels of S100A1/B were correlated with good DFS in PAAD patients(Fig. 5). The OS and DFS of S100A7A was not found in the GEPIA dataset. The other S100s were not correlated with DFS in PAAD patients.

The genetic alteration of S100s in PAAD patients

We explored the genetic alterations and its correlation in PAAD patients(TCGA, Firehose Legacy) by using cBioPortal. S100s were changed in 84 of 186 PAAD patients(45%)(Fig. 6a). The genetic alterations of S100A14/A16/A10/A4 were the top four genes(14%, 11%, 11% and 10%, respectively). Furthermore, the further study found that PAAD patients with genetic alteration had shorter OS and DFS than those with nothing alterations, whereas there was no statistically significant difference(*p*-value was 0.282 and 0.681, Fig. 6b-c).

We also used the cBioPortal dataset to calculate the Pearson's correlation coefficient of S100s with each other and assess whether these genes were correlated with each other(Fig. 7a). The results shown that there was a high correlation between these genes. In addition, we performed a PPI network and explored the potential interaction among S100s by using STRING database(Fig. 7b). As shown in, the PPI network consists of 20 nodes and 74 edges. The results revealed that S100s were directly and indirectly connected to one another and S100A16 was only associated with S100A14, and other S100s displayed

close mutual protein-protein interaction. In addition, we constructed a gene-gene interaction network of S100s and their neighbours genes by using GeneMANIA. The results revealed that 20 genes such as TCHHL1, HRNR, FLG2, RPTN, S100A7L2, FLG, TCHH, CRNN, SNTN, CABP7, CALN1, CABP2, KCNIP4, PVALB, MICU3, MYL5, OCM, EFCAB3, CAPSL and GUCA1A, were mainly correlated with the regulation and function of S100s in PAAD(Fig. 7c).

Functional Enrichment Analysis of S100s in PAAD patients

We used the Metascape to explore the functions of S100s by analyzing GO and KEGG. GO enrichment analysis included biological processes (BP), cellular components (CC) and molecular functions (MF). The analysis shown that the 8 most enriched terms, including calcium ion binding, calcium-dependent protein binding, RAGE receptor binding, S100 protein binding, cornified envelope, leukocyte degranulation, perinuclear region of cytoplasm and response to metal ion (Fig. 8a and Table 2). In addition, we constructed a enriched terms network, colored by *p*-value. Additionally, to further understand the relationship between S100s and PAAD, we constructed PPI Enrichment Analysis. We found that the biological processes were primarily linked to calcium-dependent protein binding, calcium ion binding, protein homodimerization activity, cornified envelope, cornification and keratinization(Fig. 8c-e).

Table 2
The enrichment analysis of S100s in PAAD patients(GeneMANIA)

GO	Category	Description	Count	%	Log10(<i>P</i>)	Log10(<i>q</i>)
GO:0005509	GO Molecular Functions	calcium ion binding	39	97.50	-61.12	-56.76
GO:0048306	GO Molecular Functions	calcium-dependent protein binding	23	57.50	-48.59	-44.53
GO:0050786	GO Molecular Functions	RAGE receptor binding	7	17.50	-18.10	-14.21
GO:0044548	GO Molecular Functions	S100 protein binding	4	10.00	-8.46	-4.70
GO:0001533	GO Cellular Components	cornified envelope	5	12.50	-8.28	-4.62
GO:0043299	GO Biological Processes	leukocyte degranulation	9	22.50	-7.30	-3.84
GO:0048471	GO Cellular Components	perinuclear region of cytoplasm	9	22.50	-6.22	-3.20
GO:0010038	GO Biological Processes	response to metal ion	4	10.00	-2.81	-0.27

The relationship between S100s and Tumor-Infiltrating Immune Cells in PAAD patients

S100s may affect the outcomes of PAAD patients by taking part in immune cells infiltration. Hence, we used TIMER 2.0 Databases to investigate the association between the S100s and Tumor-Infiltrating Immune Cells(Fig. 9). The results showed that there was a positive association between S100A1 and CD4⁺ T cells infiltration, however, there was a negative association between S100A1 and CD8⁺ T cells, Mφ and DCs infiltration. S100A3 and S100A4 were positively related with Neutrophils and DCs infiltration, and S100A3 was also positively associated with CD4⁺ T cells infiltration. S100A5 and S100A6 were negatively related with CD8⁺ T cells and Mφ infiltration, and S100A6 was also negatively associated with Neutrophils and DCs infiltration. S100A7/A10/A11 were negatively correlated with Mφ infiltration. S100A8/A9/A12/B/Z were positively related with CD8⁺ T cells, CD4⁺ T cells, Mφ, Neutrophils and DCs infiltration, and S100A12/B/Z were also positively associated with B cells infiltration. There was a positive relationship between S100A13 and CD4⁺ T cells infiltration, while there was a negative relationship between S100A13 and Mφ infiltration. S100A14 and S100A16 were negatively related with CD4⁺ T cells, Mφ and DCs infiltration, and there was a negative relationship between S100A6 and CD8⁺ T cells infiltration. There was a negative association between S100P and CD4⁺ T cells, Mφ infiltration. However, S100A7A/S100G were not correlated with immune cells infiltration. Additionally, We used Cox proportional risk model to explore the association between S100s and Tumor-Infiltrating Immune Cells. The results revealed that B cells($p = 0.024$), DCs($p = 0.025$), S100A1($p = 0.001$), S100A5($p = 0.026$), S100A6($p = 0.025$), S100A8($p = 0.004$), S100A9($p = 0.017$), S100A13($p = 0.039$), S100A14($p = 0.006$) were obviously related with prognosis of patients with PAAD(Table 3).

Table 3
Cox proportional hazard model of S100s and Tumor-Infiltrating Immune Cells in patients with PAAD(TIMER 2.0).

	coef	HR	95%CI_l	95%CI_u	p-value	sig
B_cell	7.559	1917.085	2.691	1365803.767	0.024	*
CD8_Tcell	3.517	33.678	0.010	110982.584	0.395	
CD4_Tcell	-7.790	0.000	0.000	1.231	0.056	
Macrophage	-0.211	0.810	0.001	509.868	0.949	
Neutrophil	-0.524	0.592	0.000	18554686.963	0.953	
Dendritic	-5.542	0.004	0.000	0.499	0.025	*
S100A1	-0.454	0.635	0.489	0.824	0.001	**
S100A2	0.066	1.068	0.941	1.212	0.307	
S100A3	0.068	1.070	0.737	1.554	0.722	
S100A4	0.081	1.084	0.889	1.323	0.425	
S100A5	-0.556	0.574	0.351	0.937	0.026	*
S100A6	-0.713	0.490	0.263	0.914	0.025	*
S100A7	-0.253	0.776	0.529	1.139	0.195	
S100A7A	0.795	2.215	0.078	63.108	0.642	
S100A8	0.644	1.905	1.224	2.964	0.004	**
S100A9	-0.413	0.662	0.471	0.929	0.017	*
S100A10	-0.038	0.963	0.48	1.932	0.915	
S100A11	0.698	2.010	0.938	4.306	0.073	
S100A12	-0.092	0.912	0.575	1.446	0.696	
S100A13	0.683	1.979	1.035	3.783	0.039	*
S100A14	0.394	1.483	1.122	1.961	0.006	**
S100A16	-0.435	0.647	0.314	1.333	0.238	
S100B	-0.126	0.881	0.639	1.215	0.441	
S100G	0.252	1.286	0.845	1.958	0.241	
S100P	-0.007	0.993	0.819	1.204	0.943	

* indicates $p < 0.05$, ** indicates $p < 0.01$.

	coef	HR	95%CI_l	95%CI_u	p-value	sig
S100Z	-0.667	0.513	0.221	1.195	0.122	
* indicates $p < 0.05$, ** indicates $p < 0.01$.						

Discussion

Accumulative researches indicated that S100s dysfunction plays very important role in many cancers⁴. The functions of S100s in certain cancers has been reported, but comprehensive analysis of the whole twenty S100s in PAAD patients has not been clarified. In our study, we found that the S100A2/A3/A4/A6/A8/A9/A10/A11/A13/A14/A16/B/P mRNA expression were significantly upregulated in PAAD patients. Survival analysis found that the increased levels of S100A2/A3/A5/A10/A11/A14/A16 were significantly correlated with poor OS, whereas the increased levels of S100A1/B/G/Z were strongly correlated with good OS. Our study suggested that S100A2/A3/A10/A11/A14 /A16 could be used as novel prognostic or therapeutic biomarkers.

S100A2 was overexpressed and correlated with poor prognosis and progression in PAAD¹³. Biankin et al. revealed that S100A2 expression is a good predictor of response to pancreatectomy for PAAD²⁷. Bachet et al. showed that the higher expression of S100A2 predicts good DFS and OS in patients receiving adjuvant therapy and should be evaluated as a predictive biomarker in PAAD²⁸. Wen et al. revealed that the S100A2 is downregulated in radioresistant pancreatic cells, suggesting that S100A2 may be involved in radioresistance of PAAD cells²⁹. In our research, we found that the S100A2 was significantly overexpressed in PAAD tissues, and there was a relationship between S100A2 and short OS and DFS in PAAD patients. However, there was no association between S100A2 and the tumor stage in PAAD patients.

S100A3 plays a vital role in some cancers, for instance, ovarian cancer, gastric cancer and colorectal Cancer^{8,30,31}. But the role of S100A3 in PAAD patients has not been investigated. In our research, we first revealed that the S100A3 expression was significantly higher in PAAD patients. Moreover, high S100A3 expression was linked with advanced tumor stage and worse OS in PAAD patients, however, the expression of S100A3 has nothing to do with DFS in PAAD patients. The role and mechanism of S100A3 in PAAD still need to be confirmed by a large number of studies in the future.

S100A10, a plasminogen receptor, was overexpressed in PAAD patients, as a new putative biomarker and could promote the growth and invasion of pancreatic tumor. The expression of S100A10 was driven by methylation of the ~ 400 bp promoter region and oncogenic KRAS^{G12D}. S100A10 promoted the growth of pancreatic tumors and regulated the expression of VEGF and CCND1³². However, the underlying mechanisms are needed to explore further. In our research, the analysis indicated that the S100A10 expression was aberrantly up-regulated in PAAD patients. Moreover, high S100A10 expression was

related with advanced tumor stage. The results indicated that the expression of S100A10 may result in short OS and DFS in PAAD patients.

S100A11 was over-expressed in the early stage of carcinogenesis and down-regulated during the progression of pancreatic cancer³³. Xiao et al. shown that the S100A11 was related with bad prognosis and S100A11 was an independent prognostic indicator of PAAD, and further research confirmed that S100A11 promoted the viability and proliferation of human PAAD PANC-1 cells through the upregulation of the PI3K/AKT pathway^{16,34}. Mitsui et al. found that the secretory S100A11 induced upregulation mobility of PAAD cells by activating the surrounding fibroblasts through the S100A11-RAGE-TPL2-COX2 pathway³⁵. Takamatsu et al. identified that extracellular S100A11 promoted the proliferation of fibroblasts through the RAGE-MyD88-mTOR-p70 S6 kinase pathway³⁶. In this research, the data suggested that the S100A11 expression was aberrantly up-regulated in PAAD patients. Nevertheless, high S100A11 expression was related to advanced tumor stage and shorter OS. However, there was no association between S100A11 and tumor stage in PAAD patients.

S100A14 in PAAD has been rarely reported. In a recently published study, Zhu et al. revealed that S100A14 was significantly over-expressed in PAAD and promoted the progression and gemcitabine resistance³⁷. Our results revealed that the S100A14 expression was significantly over-expressed in PAAD patients. Moreover, the S100A14 expression was related with advanced tumor stage and bad clinical outcomes. However, the S100A14 expression in PAAD patients has no effect on DFS of PAAD patients.

S100A16 is an important member of S100s. In a recent report, Tu et al. used bioinformatics to investigate the S100A16 expression and its prognostic in PAAD patients, and confirmed that S100A16 expression was up-regulated in PAAD patients and affected the prognosis of PAAD patients³⁸. Meanwhile, Experiments by Fang et al. confirmed that S100A16 was overexpressed in PAAD and promoted the metastasis and progression of PAAD through FGF19-mediated Akt and ERK1/2 signaling pathways³⁹. Li et al. reported that S100A16 promoted the metastasis by inducing EMT through up-regulating TWIST1 expression and activating the STAT3 signalling pathway in PAAD⁴⁰. Our results revealed S100A16 expression was significantly over-expressed in PAAD. Moreover, the expression of S100A16 was associated with advanced tumor stage. The above results suggested that the higher S100A10 expression led to short OS and DFS in PAAD patients.

Previous study has focused on tumor Immune Infiltration and its role in PAAD. Ino et al. reported that more CD4⁺ and CD8⁺ T cells infiltration are a prerequisite for longer survival in PAAD patients, and the CD4⁺CD8⁺ T cells status is an independent prognostic factor⁴¹. Jiang et al. revealed that DCs were observed to abundantly infiltrate the tumor tissue, but the function of DCs always were impaired⁴². Di et al. found that the density of Tumor-associated macrophages were correlated with worse outcomes and distant metastasis in PAAD patients⁴³. In our study, the results revealed the S100A3 was positively related with the CD4⁺ T cells, Neutrophils and DCs infiltration. The transcription levels of S100A10/A11 were negatively correlated with Mφ infiltration. There was a negative relationship between the S100A14 and

CD4⁺ T cells, Mφ and DCs infiltration. There was a negative association between the S100A16 and CD8⁺ T cells, CD4⁺ T cells, Mφ and DCs infiltration. The results indicated that S100s may reflect the anti-tumor immunity in PAAD. The Cox proportional risk model revealed that the S100A1/A5/A6/A8/A9/A13/A14 expression was obviously related with the outcomes of PAAD patients.

Nevertheless, our study has some limitations. Because of complex post-transcriptional modifications in cells, the transcriptional levels did not completely reflect the protein levels in PAAD. To further clarify the expression and detailed mechanism of S100s in PAAD, numerous basic experiments are still needed in the future.

Conclusion

In conclusion, we systematically analyzed the S100s expression and its association with outcomes and tumor immune microenvironment in PAAD patients. Our results indicated that the S100A2/A3/A4/A6/A8/A9/A10/A11/A13/A14/A16/B/P mRNA expression were significantly upregulated in PAAD patients. Survival analysis found that the increased levels of S100A2/A3/A5/A10/A11/A14/A16 were significantly correlated with poor OS, whereas the increased levels of S100A1/B/G/Z were strongly correlated with good OS. We found significant correlations among S100s and Tumor-Infiltrating Immune Cells. Additionally, The Cox proportional risk model revealed that B cells, Dendritic cells and S100A1/A5/A6/A8/A9/A13/A14 were obviously related with prognosis of patients with PAAD. Our results reveal that S100s may be a new target for the diagnosis and treatment of PAAD and provide evidence for the clinical application of S100s inhibitors in PAAD patients.

Abbreviations

S100s

S100 family members

PAAD

Pancreatic Adenocarcinoma

GEPIA

Gene Expression Profiling Interactive Analysis

TCGA

The Cancer Genome Atlas

GTE_x

Genotype-tissue Expression

OS

Overall survival

DFS

Disease-free survival

PPI

protein-protein interaction

GO
gene ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
BP
biological processes
CC
cellular components
MF
molecular functions
Dendritic cells
DCs
Macrophages
Mφ.

Declarations

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None

Authors' contributions

MZ and FX contributed to develop the idea and design of this study. YR and BC contributed to analyze the data and write the manuscript. All authors read and approved the manuscript for publication.

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Availability of data and materials

The data used to support the findings of this study are included within the article. All methods were carried out in accordance with relevant guidelines and regulations as publicly available data is used.

Ethics approval and consent to participate

All patients from the TCGA database gave written informed consent to participate in any scientific researches.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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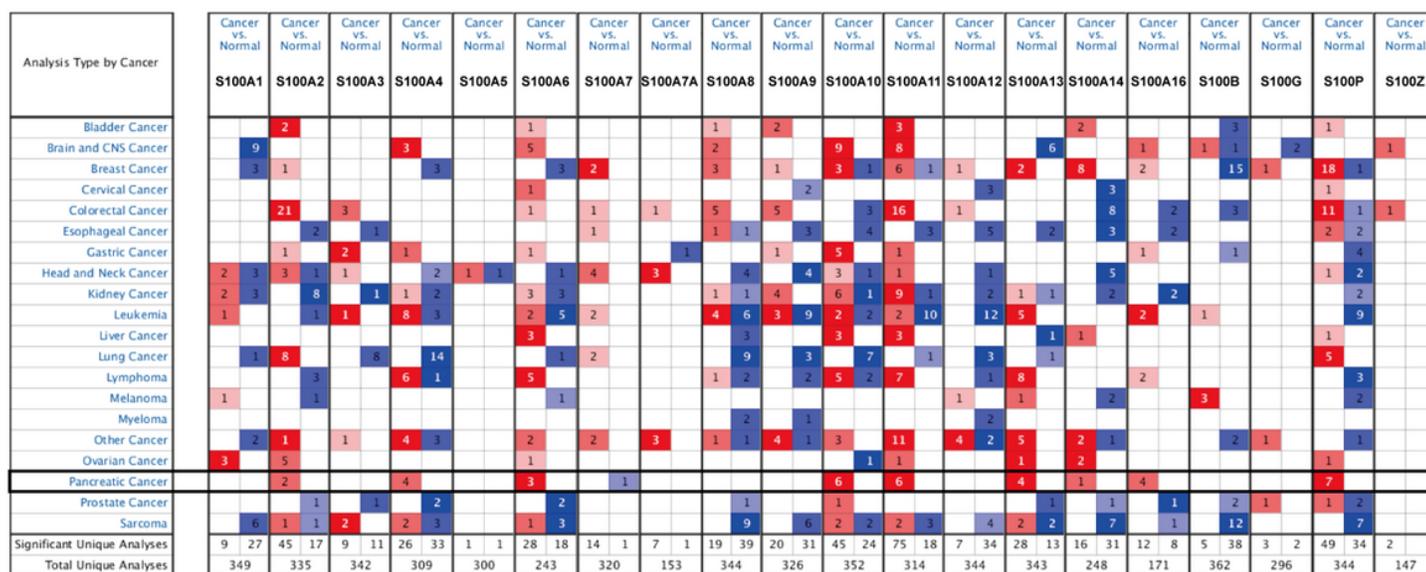
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Figures



Cell color is determined by the best gene rank percentile for the analyses within the cell.

Figure 1

The S100s mRNA expression in various cancers(Oncomine). Numbers represent the number of datasets with the mRNA expression of S100s was up-regulated(red) and down-regulated (blue) significantly. The thresholds were restricted: p -value<0.01, fold change \geq 2, data type: mRNA, gene bank: 10%.

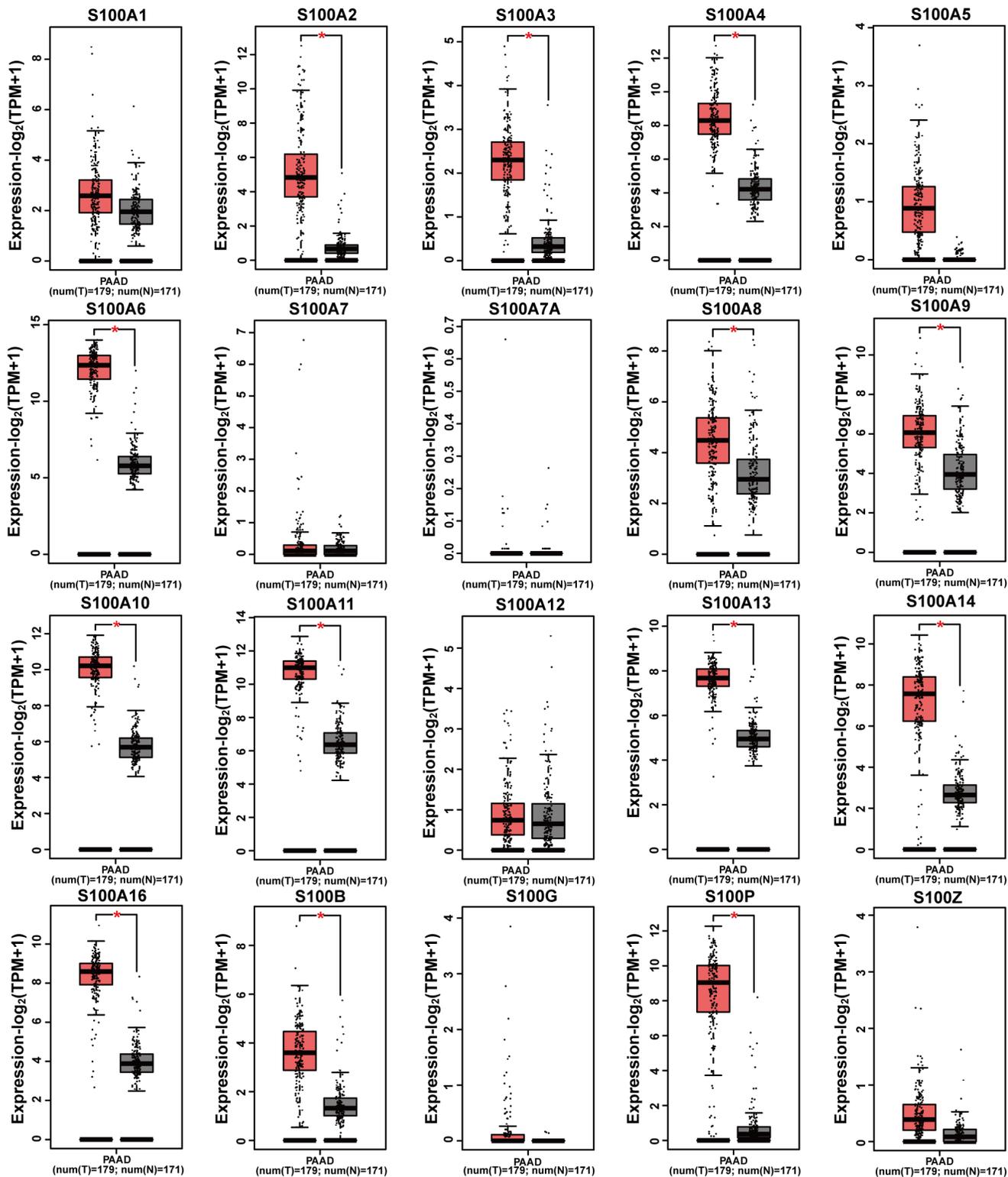


Figure 2

The mRNA expression of S100s in PAAD patients(GEPIA 2). The p -value was set as 0.01, * indicates a statistically significant difference.

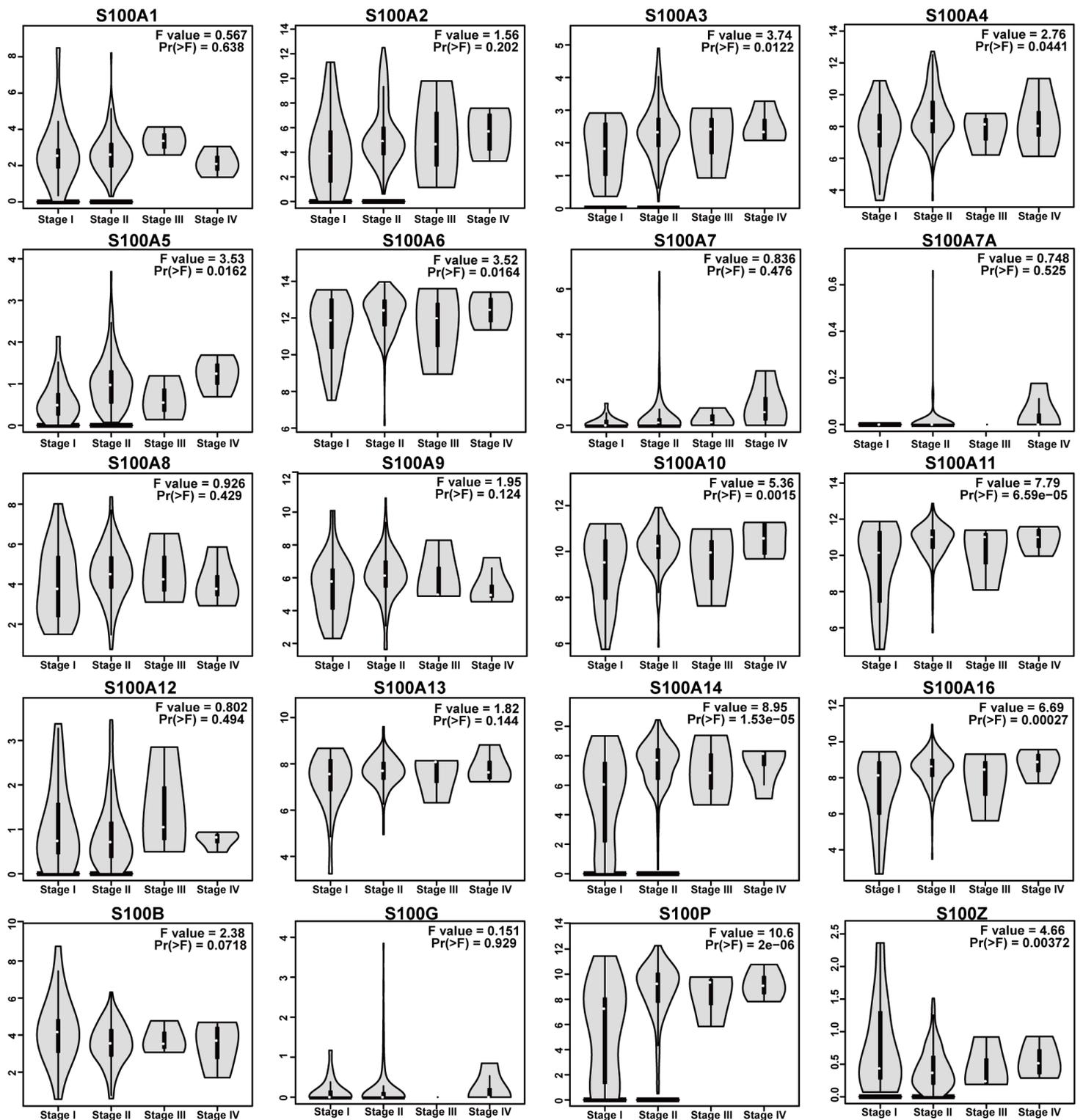


Figure 3

The association between S100s and tumor stage in PAAD patients(GEPIA 2). The p -value < 0.05 was considered to be significant.

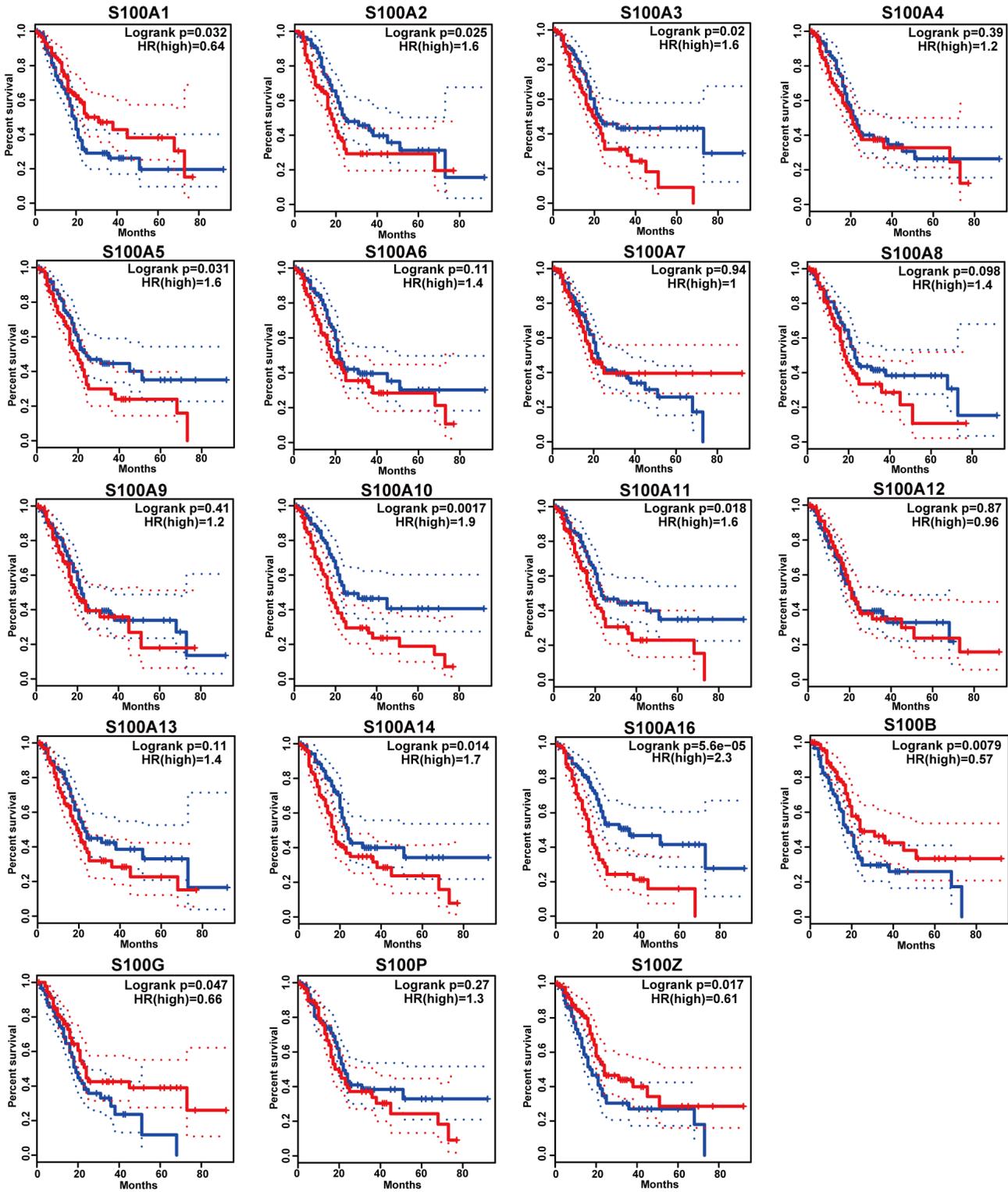


Figure 4

The prognosis value of S100s in patients with PAAD in the OS curve(GEPIA 2).

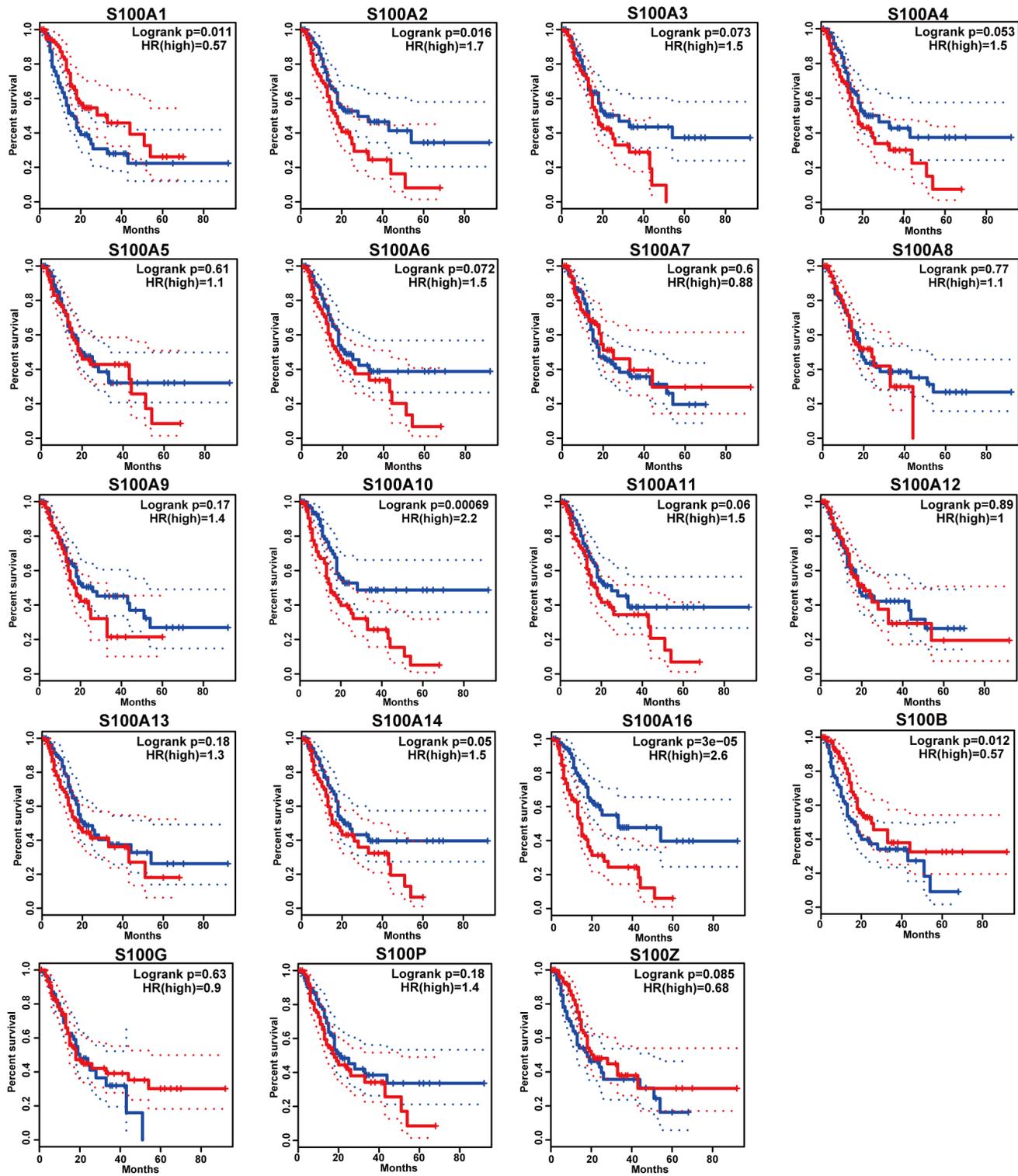


Figure 5

The prognosis value of S100s in patients with PAAD in the DFS curve(GEPIA 2).

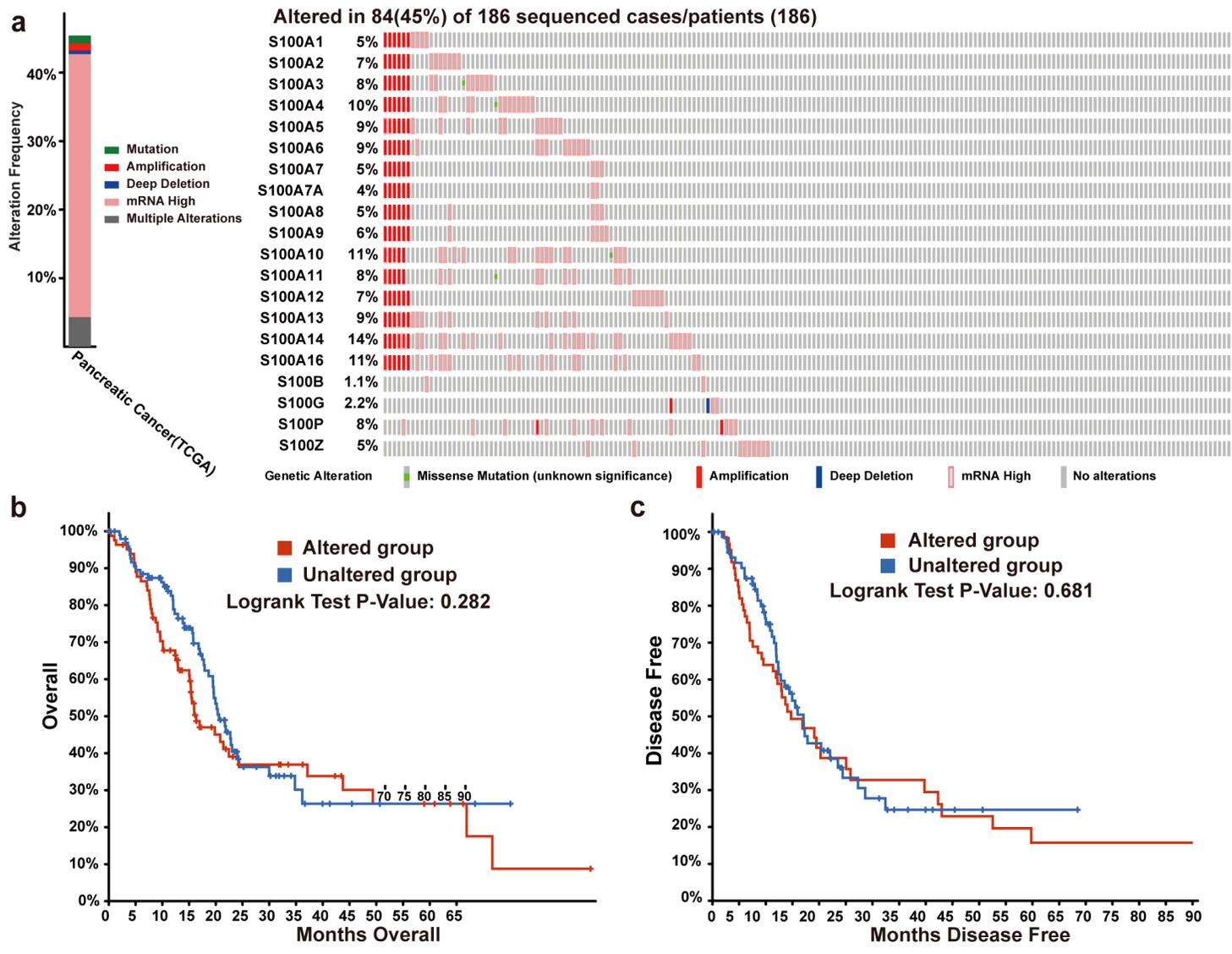


Figure 6

Alteration frequency of S100s and survival analysis in PAAD patients(cBioPortal). (a) Summary of S100s alterations in PAAD patients. OS(b) and DFS(c) in PAAD patients with and without S100s alterations by Kaplan-Meier plots.

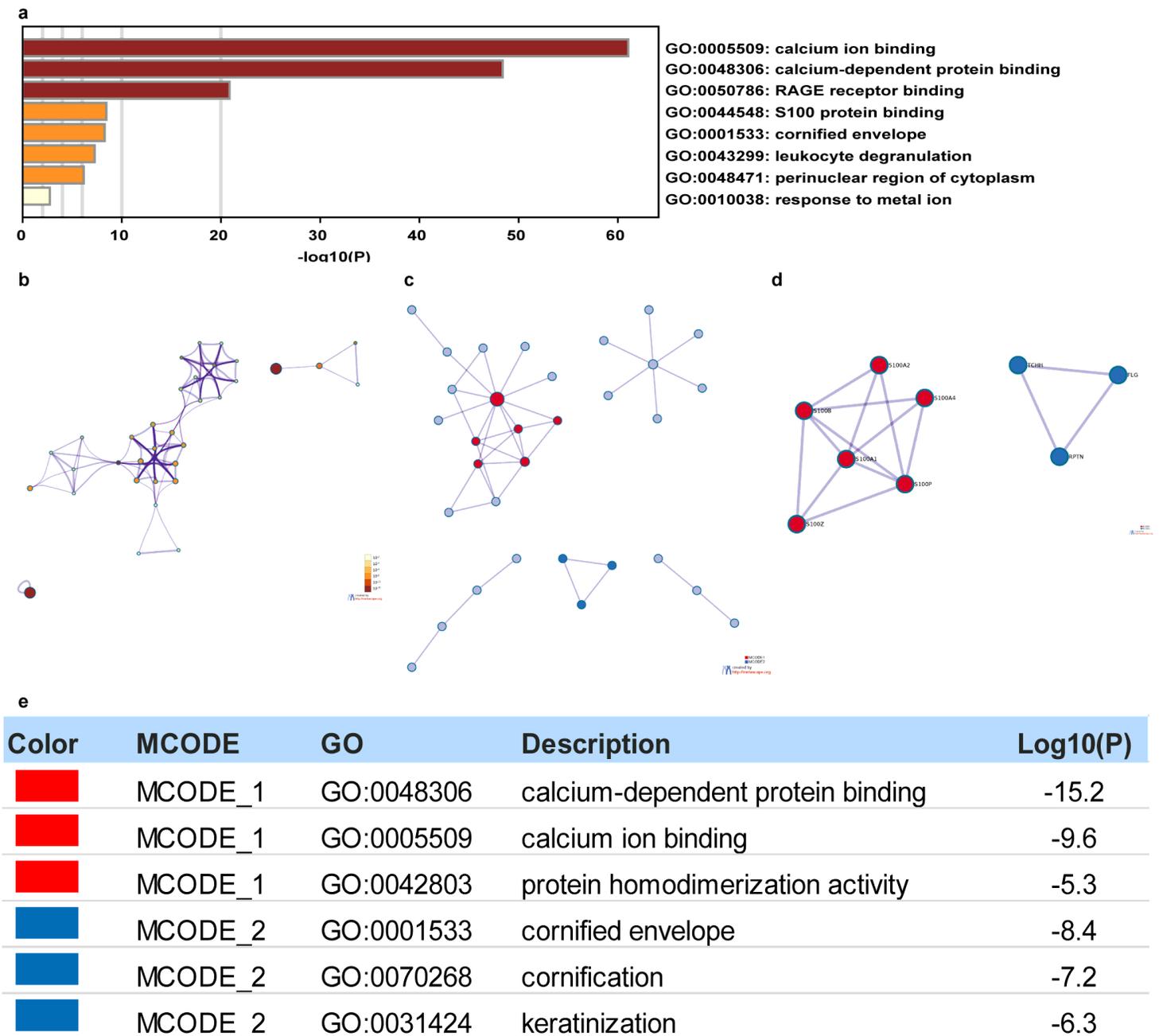


Figure 8

The enrichment analysis of S100s and the co-expression genes in PAAD patients(Matascap). (a) Histogram of the top 8 enriched terms. (b) enriched terms network, colored by p -value. (c-e) PPI network and MCODE components

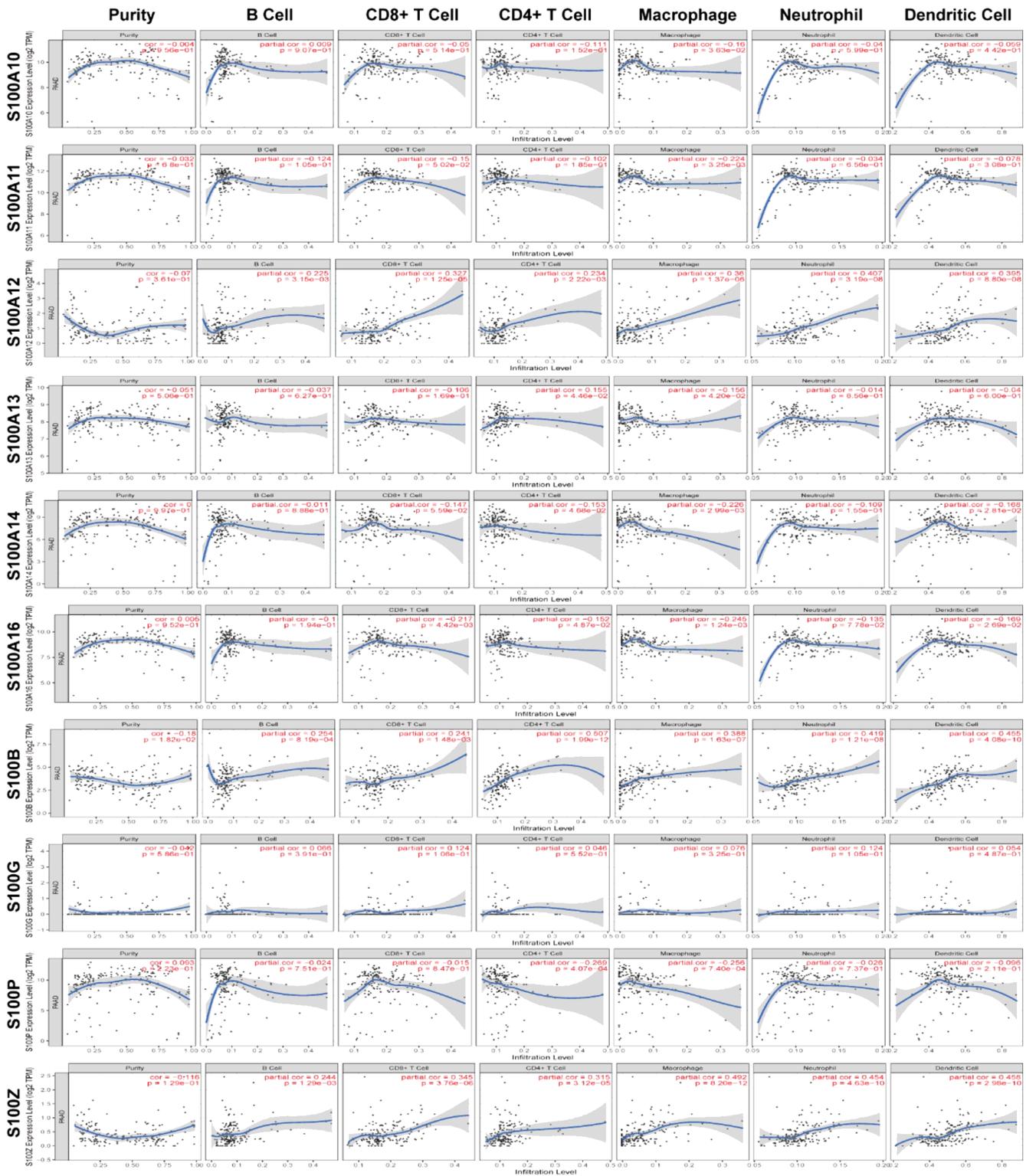


Figure 9

The association between S100s and Tumor-Infiltrating Immune Cells in PAAD patients(TIMER 2.0).