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Original Article

Integrated bioinformatics and immunohistochemical analysis reveal that S100A16 is correlated with mutational burden, immune evasion, and *P53* expression in gastric adenocarcinoma

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Abstract: Introduction: S100A16, a member of the S100 family of calcium-binding proteins, is involved in the progression of several malignancies. However, its specific mechanism of action in gastric cancer is not completely understood. The objective of this study, therefore, was to examine the correlation between the expression of S100A16 and clinicopathological characteristics and to investigate its potential significance in gastric adenocarcinoma. Methods: S100A16 messenger RNA (mRNA) expression in stomach cancer was analyzed using bioinformatics to determine the link(s) between diagnostic utility, prognostic importance, tumor mutational burden (TMB), and immune cell infiltration of S100A16 mRNA in patients with gastric cancer. Results: S100A16 mRNA expression was elevated in gastric adenocarcinoma, particularly in samples with microsatellite instability. S100A16 mRNA demonstrated significant diagnostic efficacy for gastric cancer. There was a strong correlation between S100A16 mRNA expression and TMB, and a negative correlation between S100A16 mRNA levels and Tumor Immune Dysfunction and Exclusion (i.e., "TIDE") score. Immunohistochemistry results revealed significant differences in S100A16 expression across groups with various histological differentiation in gastric cancer. There was a positive association between S100A16 and P53 expression in those with gastric cancer. Conclusions: There was significant upregulation of S100A16 in gastric adenocarcinoma, which was associated with the level of differentiation and expression of P53. S100A16 may play a role in gene mutations, microsatellite status, and TMB in gastric adenocarcinomas. In addition, S100A16 may be associated with the ability of gastric adenocarcinoma cells to evade the immune system.

Keywords: S100A16, gastric adenocarcinoma, bioinformatics, immunohistochemistry

Introduction

Gastric cancer is a prevalent form of malignant neoplasm widely observed across the globe [1], and is currently the fifth most prevalent tumor type, with new cases reported annually. Despite a decline in mortality rates in recent years, gastric cancer remains the fourth leading cause of death among all malignancies [1, 2]. There is a wide range of histological subtypes of gastric cancer, with gastric adenocarcinoma being the predominant type, comprising approximately 90%-95% of all gastric cancers [3]. The primary management of gastric adenocarcinoma involves surgical resection. However, the prognosis of patients in the advanced stages of disease remains poor [4]. Therefore, identification of efficient biomarkers and the implementation of more effective molecular-driven therapeutic strategies have emerged as prominent areas of research investigating the treatment of gastric adenocarcinoma [4, 5].

The S100 protein family is a subset of calcium (Ca^{2+})-binding proteins characterized by their low molecular weight and specificity for distinct tissues or cell types [6]. The S100-binding protein A16 (S100A16) is situated on chromosome 1q21, where most other *S100* genes are also found, a site known for its high rearrangement frequency in tumors, suggesting that S100A16 may have an important effect on tumor pro-

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gression [7]. Recent bioinformatic analyses have highlighted the involvement of S100A16 in the construction of immune-related tumor prognostic models, some of which are strongly associated with gene mutations and tumor mutation burden (TMB) [8, 9]. The wild-type *P53* gene serves as a crucial tumor suppressor gene. Nevertheless, mutations in the *P53* gene have been recognized as a crucial factor facilitating tumor progression [10]. A noteworthy correlation was identified between *P53* expression levels and S100A16 when immunoprecipitation techniques were used on 3T3-L1 preadipocytes [11]. However, the existing body of research examining the association between S100A16 and tumor mutations, as well as P53 expression, in gastric adenocarcinoma, is limited.

The present study analyzed and aimed to predict S100A16 messenger RNA (mRNA) expression in gastric adenocarcinoma and its relationship with clinical features, prognosis, functional enrichment, mutations, and immunity, using bioinformatic methods and multiple databases. Using immunohistochemical techniques, S100A16 protein expression in gastric adenocarcinoma tissues was determined, and the relationship between S100A16 and clinicopathological features was analyzed. The aim was to investigate the correlation between S100A16 and progression of gastric adenocarcinoma, and to provide novel evidence and research concepts for the diagnosis, treatment, and prognosis of gastric adenocarcinoma.

Materials and methods

Bioinformatics analysis

GTEx and TCGA data acquisition: The UCSC Xena data analysis platform (http://xena.ucsc.edu/) was used to screen and retrieve RNA-sequencing (RNA-seq) data from the GTEx database in fpkm format for normal human tissue samples, along with the corresponding tissue site and clinical characteristics data for gastric adenocarcinoma samples. Mutation load data for gastric adenocarcinoma samples were downloaded from the official TCGA website database (https://portal.gdc.cancer.gov/) [12].

GTEx and TCGA data processing: The Ensembl IDs of genes in all RNA-seq data were transformed into gene names using Perl software (www.perl.org). GTEx and TCGA RNA-seq data were numerically converted to standardize the data. Normal gastric tissue data were extracted from GTEx RNA-seq data. Merged GTEx and TCGA gene data were analyzed using R software (R Core Team; R Foundation for Statistical Computing, Vienna, Austria). All TCGA gastric adenocarcinoma mutation load data were processed using Perl software to obtain TMB and mutation frequency data for gastric adenocarcinoma samples, which were merged with the target gene RNA-seq data for mutation correlation analysis.

S100A16 mRNA expression analysis: S100A16 mRNA expression data were extracted from the GTEx and TCGA merged gene data to analyze differences in S100A16 mRNA expression between normal gastric tissues and gastric adenocarcinoma samples. Clinical characteristics, including age, sex, and clinical stage, were integrated with the target gene RNA-seq data. Samples were categorized based on various clinical characteristics to examine variations in S100A16 mRNA expression across different groups.

Analysis of S100A16 mRNA diagnosis and prognosis: The diagnostic utility of S100A16 mRNA expression levels in gastric cancer were evaluated using receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) analysis. Kaplan-Meier survival curves were plotted to analyze the effect of S100A16 on overall patient survival.

S100A16 mRNA functional enrichment analysis: Gene set enrichment analysis (GSEA) is a powerful algorithm that enables the evaluation of functional gene sets for enrichment bias between 2 different state groupings [13]. Gene expression data from the 2 groups - namely, high and low S100A16 expression - were analyzed using GSEA software. Significant enrichment differences in the Kyoto Encyclopedia of Genes and Genomes (i.e., “KEGG”) pathway, Hallmark gene set, and Gene Ontology (GO) gene set were calculated between the 2 groups.

S100A16 mRNA mutation correlation analysis: TMB, defined as the number of mutations per megabase, was calculated for each sample using R software. The Wilcoxon rank-sum test was used to assess differences in TMB between

the S100A16 high- and low-expression groups. The correlation between the continuous variable S100A16 mRNA expression and TMB was evaluated using Spearman's rank correlation analysis. Furthermore, mutation annotation format data for all samples were processed and analyzed using the R package "maftools". This package was used to generate waterfall plots, used to visualize the mutation landscape and display the top 15 most frequently mutated genes in both the S100A16 high and low expression groups, thus enabling a comparative analysis of mutation frequencies.

S100A16 mRNA immune correlation analysis: TIDE (http://tide.dfci.harvard.edu/) is a new algorithm that can predict the effectiveness of immunotherapeutic drugs received by patients to calculate their Tumor Immune Dysfunction and Exclusion (TIDE) score [14]. The TIDE score was computed by inputting complete gene expression data from a database of gastric adenocarcinoma samples. Subsequently, the disparity in TIDE scores between the high and low S100A16 expression groups was examined, and the correlation between S100A16 mRNA and TIDE scores was analyzed. All gastric adenocarcinoma samples were subjected to analysis using the "CIBERSORT" function package in R. To perform the analysis, the infiltration ratios of 22 different types of immune cells were calculated in the samples. Variations in immune cell infiltration ratios between groups with high and low S100A16 expression were examined.

Sample collection

Ninety paraffin-embedded gastric adenocarcinoma and corresponding para-carcinoma tissue specimens, archived in the Department of Pathology of the Affiliated Hospital of Binzhou Medical College (Yantai, China) between March 2014 and November 2021, were collected for this study. Complete clinical and pathological data were available for all patients. The inclusion criteria were as follows: confirmed histopathological diagnosis of gastric adenocarcinoma; and underwent radical gastrectomy as first treatment. The exclusion criteria were as follows: underwent radiotherapy, chemotherapy, or other biologic therapies before surgery; and incomplete clinical or follow-up data. All included patients had undergone radical gastric cancer surgery for the first time and did not undergo radiochemotherapy or biologic treatments before surgery. S100A16 protein expression levels in gastric adenocarcinoma samples were determined using immunohistochemical staining via the EnVision method (Agilent Technologies, Santa Clara, CA, USA). The correlation between S100A16 and PKM2, P53, HER-2 and Ki-67 protein expression was analyzed to determine the relationship between S100A16 expression and the clinicopathological characteristics of patients with gastric adenocarcinoma. The study was approved by the Ethics Committee of the Affiliated Hospital of Binzhou Medical College. Informed consent was obtained from all patients. Finally, research involving human participants was conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry

Immunohistochemical paraffin sections were subjected to routine de-paraffinization using water. Antigen retrieval was performed using EDTA buffer. The sections were then washed 3 times for 5 min each in phosphate-buffered saline (PBS). Slices were then treated with 3% hydrogen peroxide for 15 min to inhibit endogenous catalase activity. After adding the S100A16 antibody (1:1,000 dilution) dropwise, the slices were incubated at 4°C overnight. The sections were then washed 3 times with PBS for 5 min each, followed by addition of a horseradish-peroxidase labelled secondary antibody. DAB (3,3'-diaminobenzidine) chromatography, terminated using running water, was used for color development. Hematoxylin and DAB were used to enhance color, and staining was terminated using running water. Immunohistochemical staining revealed predominant expression of the S100A16 protein in both the cytoplasm and nucleus, with staining exhibiting brownish-yellow granules. Staining intensity was scored and divided into 4 categories as follows: no staining (score = 0); weak staining (score = 1); moderate staining (score = 2); and strong staining (score = 3). Additionally, the range of stained cells was evaluated according to the following categories: 0% (0); 1%-24% (1); 25%-49% (2); 50%-74% (3); and 75%-100% (4). The final result was obtained by multiplying the intensity score by the range of stained cell scores. The resulting scores ranged from 0 (lowest) to 12 (highest). High expression was defined as score ≥ 6, while low expression was defined as a score < 6 [15].

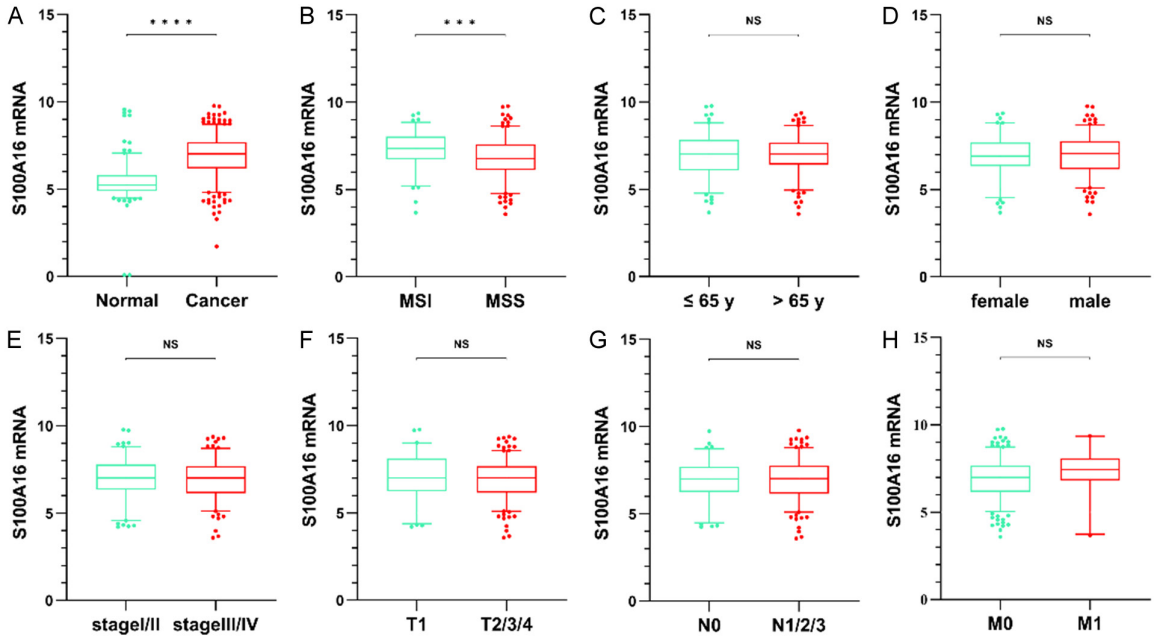

Figure 1. Expression of S100A16 mRNA in different groups of gastric adenocarcinomas. A. Normal gastric tissue and gastric adenocarcinoma tissue; B. Microsatellite status; C. Age; D. Gender; E. Clinical stage; F. T stage; G. N stage. H. M stage. ***$P < 0.001$; ****$P < 0.0001$; NS $P > 0.05$.

Statistical analysis

Statistical analyses and graphical representations were performed using various software programs, including R version 4.2.1, SPSS version 26.0 (IBM Corporation, Armonk, NY, USA), Prism version 8.3.0 (GraphPad Inc., San Diego, CA, USA), and Photoshop CC (Adobe, San Jose, CA, USA). Between-group comparisons were performed using the Student's *t*-test and Wilcoxon test. An AUC > 0.7 was considered to have good diagnostic power. Kaplan-Meier survival analysis and log-rank test were performed, while correlation analysis was performed using Spearman's test. Immunohistochemical expression between the paraneoplastic and tumor tissue groups were compared using the paired chi-squared test. Comparison of rates between groups with different clinicopathological characteristics was performed using Fisher's exact probability approach and the chi-squared test. Differences with $P < 0.05$ were considered to be statistically significant.

Results

S100A16 mRNA expression in gastric adenocarcinoma

By analyzing integrated samples from the GTEx and TCGA databases, there was a notable increase in S100A16 mRNA expression levels in gastric adenocarcinoma tissues compared with those in normal gastric tissues ($P < 0.05$) (**Figure 1A**). Analysis of clinical characteristics revealed that patients who harbored microsatellite instability (MSI) exhibited higher S100A16 mRNA levels ($P < 0.05$) (**Figure 1B**). There were no statistically significant associations with other clinicopathological features ($P > 0.05$) (**Figure 1C-H**).

Diagnostic and prognostic significance of S100A16 mRNA in gastric adenocarcinoma

Analysis of integrated sample data from the GTEx and TCGA databases indicated that S100A16 mRNA expression demonstrated significant diagnostic utility for gastric adenocarcinoma, with an AUC of 0.8533 ($P < 0.05$) (**Figure 2A**). Furthermore, the high and low S100A16 expression groups exhibited no significant difference in total survival according to Kaplan-Meier survival analysis ($P > 0.05$) (**Figure 2B**).

GSEA of S100A16 mRNA in gastric adenocarcinoma

GSEA revealed that the S100A16 high-expression group exhibited significant enrichment in several KEGG pathways and gene sets. Specifically, the top 4 KEGG pathways that ful-

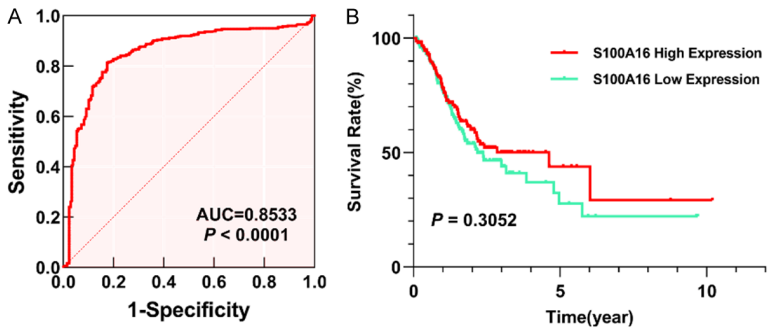

Figure 2. The diagnostic and prognostic value of S100A16 mRNA in gastric adenocarcinoma. A. ROC curve of S100A16 mRNA; B. Kaplan-Meier survival curves of S100A16 mRNA.

filled the statistical criteria were terpene skeleton biosynthesis, pyrimidine metabolism, aminoacyl tRNA biosynthesis, and base excision repair ($P < 0.01$) (**Figure 3A**). Additionally, the top 5 hallmark gene sets enriched in the S100A16 high-expression group included cholesterol homeostasis, the P53 pathway, glycolysis, mTORC1 signaling, and late estrogen response ($P < 0.01$) (**Figure 3B**). Furthermore, the GO molecular function gene set enriched in the S100A16 high-expression group included small nucleolar RNA (snoRNA) binding, catalytic activity for RNA, transfer RNA (tRNA) binding, nucleotide transferase activity, and catalytic activity for tRNA ($P < 0.01$) (**Figure 3C**). The GO biological process gene set enriched in the S100A16 high-expression group included ribosomal RNA metabolism, ribosomal biosynthesis, non-coding RNA (ncRNA) processes, mitochondrial membrane structure, and ribonucleoprotein complex biosynthesis ($P < 0.01$) (**Figure 3D**). Additionally, the gene sets for GO cellular components enriched in the S100A16 high-expression group included the mitochondrial matrix, inner mitochondrial membrane intrinsic components, organelle envelope lumen, nucleoid, and precatalytic spliceosome ($P < 0.01$) (**Figure 3E**).

Results of mutation-related analysis of S100A16 mRNA

Analysis revealed a statistically significant increase in TMB in the S100A16 high-expression group compared with that in the low-expression group ($P < 0.05$) (**Figure 4A**). Consistent with this, Spearman's correlation analysis revealed a significant positive correlation between S100A16 mRNA expression and TMB ($r = 0.2684$, $P < 0.05$) (**Figure 4B**). Visualization of the mutational landscape via waterfall plots highlighted the top 15 most frequently mutated genes in each group, including key players such as titin (TTN), tumor protein P53 (TP53), and mucin 16 (MUC16) (**Figure 4C**, **4D**). Notably, the overall proportion of samples with ≥ 1 gene alteration(s) was significantly higher in the S100A16 high-expression group (94.63%) than that in the low expression group (83.22%) ($P < 0.05$), further underscoring the link between S100A16 and a hypermutated genomic state.

Results of immune correlation analysis of S100A16 mRNA

In this study, the low S100A16 mRNA expression group had a significantly higher TIDE score than the high-expression group ($P < 0.05$) (**Figure 5A**). An inverse correlation was found between S100A16 mRNA expression and TIDE score ($r = -2112$, $P < 0.05$) (**Figure 5B**). Analysis of the immune cell infiltration proportions revealed that, among the 22 immune cells examined, regulatory T cells (Tregs) exhibited a significantly higher proportion of infiltration in the S100A16 low-expression group than that in the high-expression group. Conversely, activated mast cells and neutrophils demonstrated a significantly higher proportion of infiltration in the S100A16 high-expression group ($P < 0.05$) (**Figure 5C**).

Expression of S100A16 protein in gastric adenocarcinoma

Immunohistochemical staining revealed that the S100A16 protein was predominantly expressed in the cytoplasm and nucleus, exhibiting brownish-yellow granules (**Figure 6**). Among 90 patients with gastric adenocarcinoma, 58 (64.4%) exhibited high S100A16 expression, which was significantly higher than that observed in paraneoplastic tissues (34.4% [31/90]). This difference was statistically significant ($P < 0.05$) (**Table 1**).

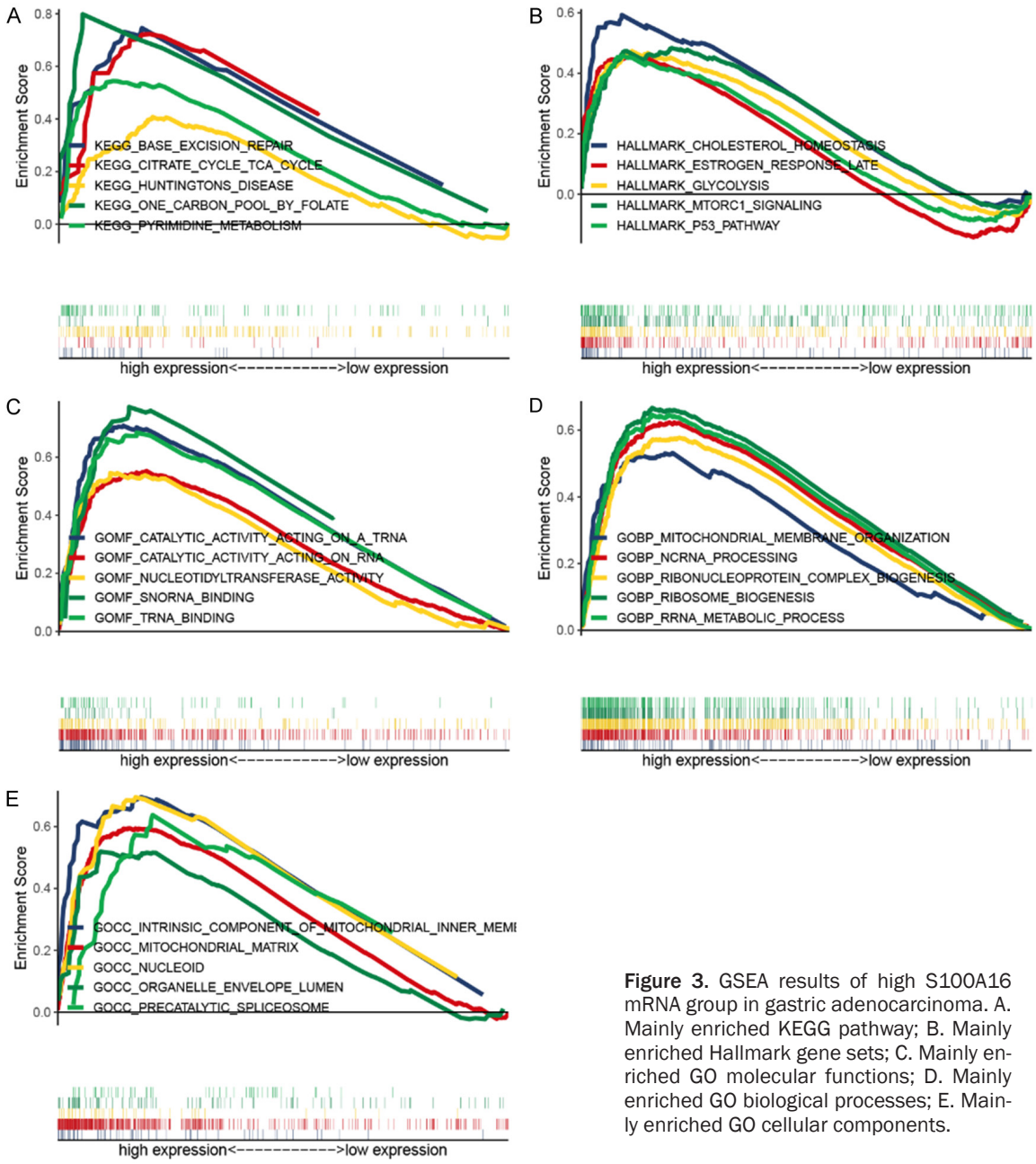

Figure 3. GSEA results of high S100A16 mRNA group in gastric adenocarcinoma. A. Mainly enriched KEGG pathway; B. Mainly enriched Hallmark gene sets; C. Mainly enriched GO molecular functions; D. Mainly enriched GO biological processes; E. Mainly enriched GO cellular components.

Correlation between S100A16 protein expression and P53, HER-2 and Ki-67

Among 90 gastric adenocarcinoma tissue samples, there were 7 cases of positive and 83 cases of negative HER-2 interpretation results; there were 75 cases of positive and 15 cases of negative P53 interpretation results; there were 72 cases of > 50% and 18 cases ≤ 50% Ki-67 proliferation index interpretation results. The P53 protein was mostly expressed in the nucleus and stained as brown-yellow granules (**Figure 7**). Spearman correlation analysis revealed a positive association between the expression of S100A16 and P53 in gastric cancer tissues (r = 0.228, $P < 0.05$); however, there was no notable association between the expression of S100A16 protein and the expression of HER-2 and Ki-67 ($P > 0.05$) (**Table 2**).

Relationship between S100A16 expression and clinicopathological features of gastric adenocarcinoma

Expression of the S100A16 protein was different in gastric adenocarcinoma groups with dif-

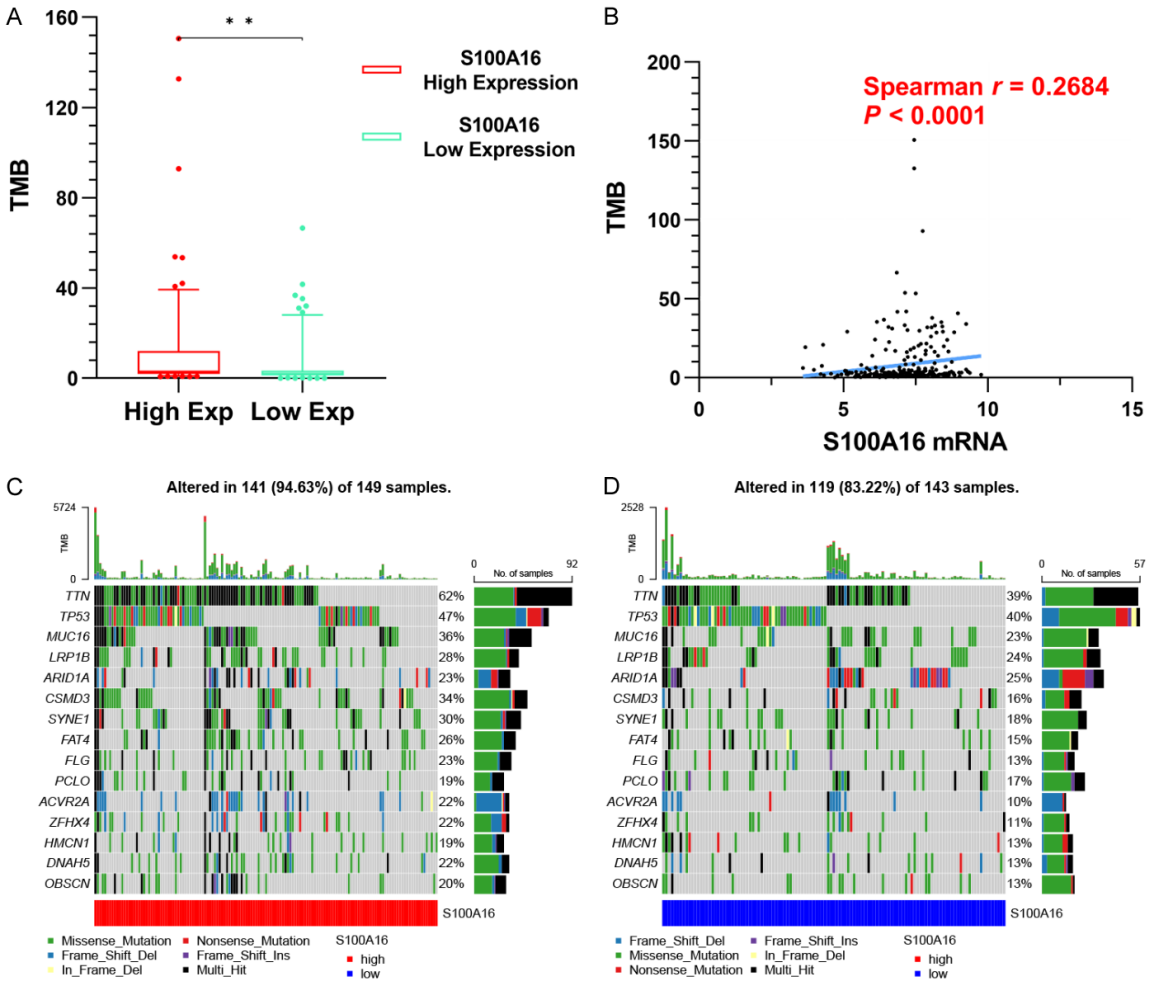

Figure 4. Mutational-related analysis results of S100A16 mRNA in gastric adenocarcinoma. A. The difference of TMB between high and low S100A16 mRNA groups; B. Correlation between S100A16 mRNA and TMB; C. Waterfall plot of mutation frequency of high S100A16 mRNA group; D. Waterfall plot of mutation frequency of low S100A16 mRNA group.

ferent histological differentiation ($P < 0.05$). There were no notable variations in clinicopathological features of S100A16 among the different groups ($P > 0.05$) (**Table 3**).

Discussion

Gastric cancer is a prevalent neoplasm worldwide and poses a significant risk to human well-being. Presently, the primary method of preventing gastric adenocarcinoma is to reduce exposure to risk factors and perform early screening. Nevertheless, the highly invasive and heterogeneous characteristics of gastric adenocarcinoma make it one of the most serious health problems worldwide [16]. As such, the identification of more effective and accurate biomarkers for diagnosis and treatment is of great value in improving the prognosis of patients with gastric adenocarcinomas.

The S100 protein family is classified under the EF family of chiral calcium-binding proteins and constitutes the largest subfamily, with 25 identified members [17]. These proteins regulate various cellular functions including inflammation, proliferation, and energy consumption [18]. Abnormal expression of these family members has been linked to the progression and prognosis of various types of tumors [17]. Lu et al. [19] reported that S100A7 was overexpressed in esophageal squamous cell carcinoma tissues and serum samples, and S100A7 promoted M2-type macrophage infiltration and angiogenesis in esophageal squamous cell carcinoma. Research investigating leukemia has

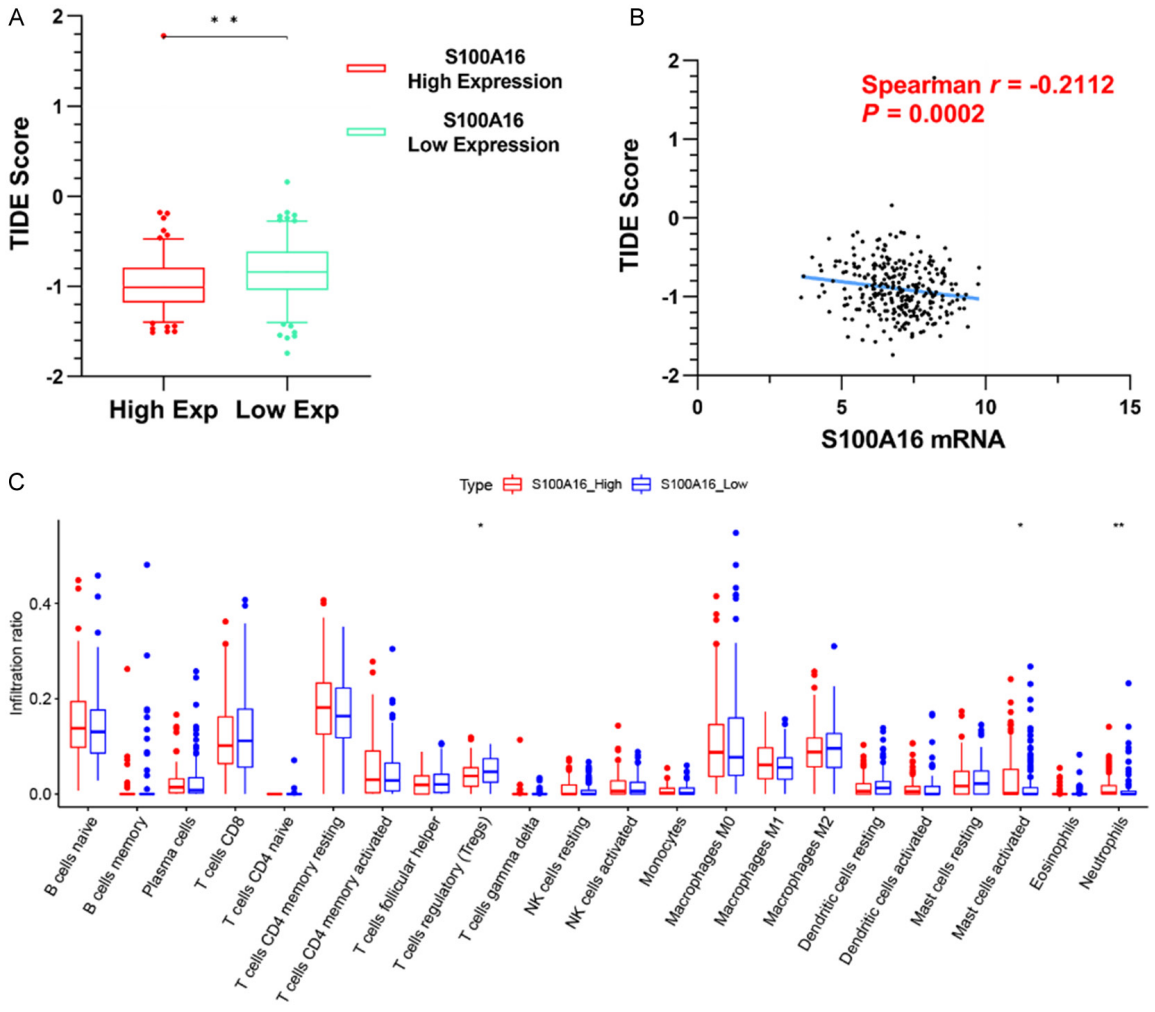

Figure 5. Immune-related analysis results of S100A16 mRNA in gastric adenocarcinoma. A. The difference in TIDE score between high and low S100A16 mRNA groups; B. Correlation between S100A16 mRNA and TIDE score; C. The difference in immune cell infiltration ratio between high and low S100A16 mRNA groups.

revealed that S100A8/A9 may enhance the growth and proliferation of leukemia cells by releasing pro-inflammatory cytokines and/or regulating the immune system [20]. However, only a few members of this protein family, such as S100A8/A9, have been extensively studied and described, and the roles of other members may be underestimated.

S100A16 is a newly discovered member of the S100 protein family that is highly conserved in mammals [21]. The biological function of S100A16 is mainly described as participating in the formation of lipids, but is also involved in various processes such as inflammation, glucose and lipid metabolism, and cell differentiation [22-24]. One study reported that S100A16 is located in a region of chromosome 1q21 that is highly prone to rearrangement in tumors [25]. S100A16 is closely associated with the progression of various malignancies, including colorectal, bladder, and stomach cancers [15, 26, 27]. Sun et al. [15] found that S100A16 has low expression in colorectal cancer and is associated with a poor prognosis. In studies investigating bladder cancer, S100A16 was shown to be regulated by Snail, an epithelial-mesenchymal transition (EMT)-related regulator, and overexpressed in tumor cells [26]. Jiang et al. [27] found that S100A16 was overexpressed in gastric cancer and was associated with the degree of differentiation. However, the mechanism underlying the involvement of S100A16 in gastric adenocarcinoma has not been fully described and requires further study.

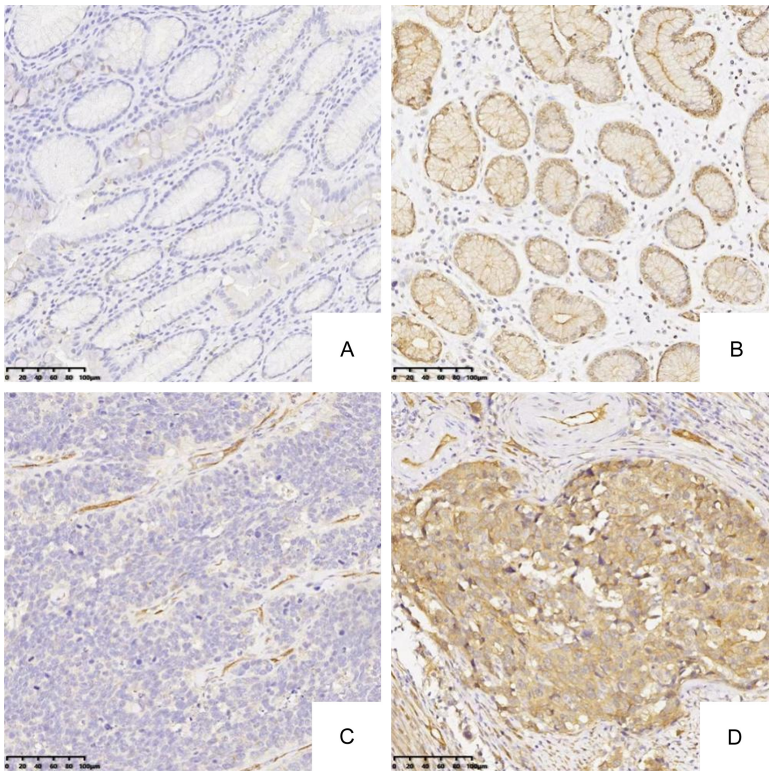

Figure 6. Expressions of S100A16 in para cancer and gastric adenocarcinoma tissues (EnVision, 200×). A. Low expression of S100A16 in para cancer tissue; B. High expression of S100A16 in para cancer tissue; C. Low expression of S100A16 in gastric adenocarcinoma; D. High expression of S100A16 in gastric adenocarcinoma.

Table 1. Expressions of S100A16 in gastric adenocarcinoma and paracancer tissue

S100A16 in paracancer tissue	S100A16 in gastric adenocarcinoma		all	*P*
	High	Low		
High	14	17	31	0.001*
Low	44	15	59	
All	58	32	90	

*$P < 0.05$.

In the present study, RNA-seq data from normal gastric tissue housed in the GTEx database were collated and normalized to the TCGA data to compensate for the shortage of normal tissue samples in the TCGA database. Analysis revealed that S100A16 mRNA expression in gastric cancer tissues was markedly elevated compared with that in normal gastric tissues. Additionally, immunohistochemistry results revealed that the S100A16 protein was abundantly expressed in gastric adenocarcinoma tissue(s), which was consistent with the findings of earlier studies investigating S100A16 in gastric adenocarcinomas [27-29].

In this study, S100A16 protein was correlated with the degree of histological differentiation of gastric adenocarcinoma. S100A16 has been associated with EMT in multiple tumor studies [28, 30, 31]. Landeros et al. [32] found that poorly differentiated diffuse gastric adenocarcinoma was closely associated with the EMT process at the molecular level, and that the EMT process also affects tumor progression and metastasis. Results of analysis indicated that there was no discernible variation in S100A16 protein expression levels among the various N-stage groups. The correlation between S100A16 expression and EMT in gastric adenocarcinoma needs to be further verified by expanding the sample size.

The present study investigated the link between S100A16 and immunohistochemical markers, particularly P53, using correlation analysis. Our findings demonstrated a favorable correlation between P53 expression and S100A16 expression in gastric cancer. Studies have shown that immunohistochemical results for P53, especially its strong expression, can be used as an alternative marker to predict *P53* mutation(s) [33]. Tomiyama et al. [34] found that overexpression of S100A16 led to P53 degradation, and inhibition of S100A16 could restore P53 protein expression in the Yumoto cervical squamous cell line. However, studies directly investigating the correlation between S100A16 and P53 in gastric adenocarcinomas are lacking. In this investigation, we hypothesized that overexpression of S100A16 in gastric adenocarcinoma was positively correlated with *P53* gene mutation(s).

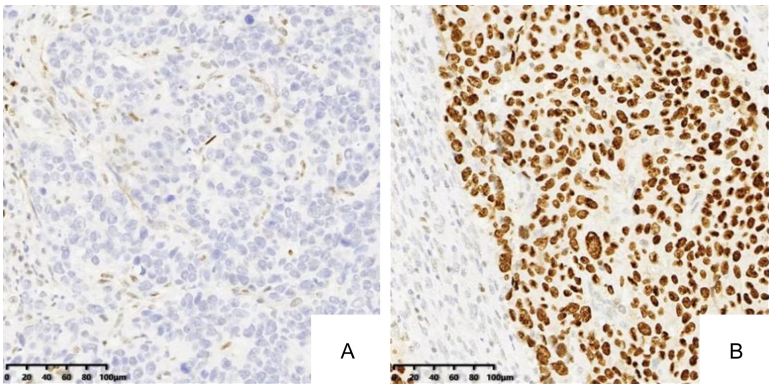

Figure 7. Expressions of P53 in gastric adenocarcinoma tissues (EnVision, 200×). A. Negative expression of P53 in gastric adenocarcinoma; B. Positive expression of P53 in gastric adenocarcinoma.

In addition, the AUC was almost 0.9, indicating that S100A16 mRNA, as an observational indicator, has ideal diagnostic utility for gastric adenocarcinoma. Chen et al. [35] found that, compared with patients with diffuse gastric adenocarcinoma, those with enteric gastric adenocarcinoma with high and moderate differentiation experienced better five-year overall survival rates. However, in a bioinformatics study, the overall survival of the high- and low-S100A16 expression groups did not differ significantly.

To further explore signal transduction pathways through which S100A16 may affect the progression of gastric adenocarcinoma, functional set enrichment analysis of S100A16 expression was performed. GSEA results revealed that the P53 pathways were mainly concentrated in the hallmark gene set in the S100A16 high-expression group. It has been further proposed that S100A16 may influence *P53* mutations, which in turn may contribute to the progression of gastric adenocarcinoma when combined with the examination of clinicopathological characteristics.

Because patients with gastric adenocarcinoma and MSI status may exhibit higher S100A16 mRNA levels, and S100A16 may be closely related to the mismatch repair function of gastric adenocarcinoma, we performed a mutation-related analysis of S100A16. In gene sequencing, the TMB represents the number of mutations per megabase of DNA (muts/Mb). The total number of mutations in a tumor can be measured using TMB, and tumors with high mutations are believed to carry a richer antigen load [36]. In predicting the efficacy of immunotherapy, TMB can be used as one of the favorable features for comprehensive evaluation of immunotherapy efficacy [37]. The results indicated a significant difference in TMB between the S100A16 high- and low-expression groups. Furthermore, a direct relationship was found between elevated S100A16 mRNA expression in gastric cancer and increased TMB. This observation is consistent with the results reported for S100A16 in other tumor types [27, 28]. In the present study, the expression S100A16 exhibited a specific degree of correlation with TMB in gastric adenocarcinoma.

Among the 15 genes examined in this study, TTN was the most frequently mutated, with the highest difference in mutation frequency between the high- and low-expression groups. Specifically, the S100A16 high-expression group exhibited a 23% higher TTN mutation frequency than the low-expression group. Jia et al. [38] found that TTN mutations were common in solid tumors. Additionally, TTN mutations are positively correlated with higher TMB and objective response rates to immune checkpoint blockade (ICB) treatment. TP53 exhibited the second highest mutation frequency, with a 7% difference in mutation frequency between the 2 groups. This finding was consistent with the GSEA results. Collectively, these findings suggest that S100A16 overexpression may influence mutations in *TTN*, *P53*, and other genes, which, in turn, contribute to tumor progression by affecting TMB.

Given the significant advances in immunotherapy in recent years and the relationship between ICB therapy, TMB, and MSI status, we performed an immunologically relevant analysis of S100A16 expression in gastric adenocarcinoma. TIDE score calculation revealed that samples belonging to the S100A16 high-expression group had lower TIDE scores. This suggests that samples from the S100A16 low-expression group were more prone to immune escape when undergoing immunotherapy. Additionally, these samples may be less effective in respond-

Table 2. Correlation between S100A16 and PKM2, HER-2, P53 and Ki-67 protein expression

Immunohistochemical index		N	S100A16 expression		*r*	*P*
			High expression	Low expression		
PKM2	High expression	66	43	23	0.024	0.819
	Low expression	24	15	9		
HER-2	Positive	7	4	3	0.044	0.678
	Negative	83	54	29		
P53	Positive	75	52	23	0.228	0.030*
	Negative	15	6	9		
Ki-67	> 50%	72	47	25	0.035	0.745
	≤ 50%	18	11	7		

*$P < 0.05$.

Table 3. The relationship between S100A16 expression and clinicopathological features

Clinicopathologic feature		n	S100A16 expression		*P*
			High expression	Low expression	
Sex	Male	57	38	19	0.563
	Female	33	20	13	
Age	≤ 65 y	44	27	17	0.550
	> 65 y	46	31	15	
Tumor length (cm)	≤ 4.5 cm	42	28	14	0.680
	> 4.5 cm	48	30	18	
Histological differentiation	High	5	1	4	0.044*
	Medium	43	26	17	
	Low	42	31	11	
T stage	T1-2	23	14	9	0.678
	T3-4	67	44	23	
N stage	N0	26	13	13	0.068
	N1-3	64	45	19	

*$P < 0.05$.

ing to immune-related therapies. When examining the degree of immune cell infiltration, we observed that only Tregs, activated mast cells, and neutrophils differed in their degree of infiltration between the S100A16 high- and low-expression groups. However, most studies have been limited to quantifying the total number of immune cells and have not identified their specific subtypes. This is also due to the limitations of bioinformatics analysis, and this study lacked effective experimental verification. Therefore, many problems remain to be solved to explore the effect of S100A16 on the immune microenvironment of gastric adenocarcinoma. Apolipoprotein B mRNA editing catalytic polypeptide-like 3B (APOBEC3B) is a key driving factor inducing multiple tumor mutations [39]. Driscoll et al. [40] found that inducing the overexpression of APOBEC3B in tumors can enhance the TMB of tumors, and better immunotherapy efficacy can be obtained when combined with ICB. Based on the research ideas and conclusions reported by Driscoll et al. [40], combined with the relationship between S100A16 and the prognosis and mutation of gastric adenocarcinoma in this study, we found that, although S100A16 was closely related to poor histological differentiation and P53 expression in gastric adenocarcinoma, the relationship between its expression, MSI status, and TMB suggests that the recognizable antigen load of immune cells in gastric adenocarcinoma overexpressing S100A16 may be upregulated. This may explain why S100A16 overexpression did not significantly affect the overall survival of patients with gastric adenocarcinoma.

In this study, P53 expression and the degree of differentiation were associated with high

S100A16 expression in gastric adenocarcinomas. S100A16 may be involved in gene mutations, microsatellite status, and TMB affecting gastric adenocarcinoma, and is related to the immune escape potential of gastric adenocarcinoma, providing a theoretical basis for the selection of immunotherapy for gastric adenocarcinoma.

The present study had some limitations. Some content can guide the development of relevant experiments to some extent due to the predictive results of living information analysis; however, no experimental verification was performed in this study. Future studies investigating S100A16 overexpression and progression of gastric adenocarcinoma, TMB, and immunotherapy may provide a more solid scientific basis supporting the participation of S100A16 in the molecular mechanisms of gastric adenocarcinoma and provide new potential biomarkers for the treatment and prognosis of patients with gastric adenocarcinoma.

Conclusion

Results of the present study demonstrated that S100A16 was significantly upregulated in gastric adenocarcinoma and that its expression level was positively correlated with the degree of histological differentiation and P53 protein expression. S100A16 was also significantly associated with the TMB and exhibited elevated expression in MBI-high status, suggesting its potential involvement in the gene mutation process in gastric adenocarcinoma. Furthermore, low S100A16 expression was linked to a higher TIDE score, indicating its possible role in tumor immune evasion. These findings reveal the multifaceted roles of S100A16 in the pathogenesis and progression of gastric adenocarcinoma, particularly in the accumulation of mutations and regulation of the immune microenvironment. This study identified S100A16 as a potential biomarker for immunotherapy and the prognostic evaluation of gastric adenocarcinoma, providing a novel theoretical basis. Future functional experiments are warranted to validate the specific molecular mechanisms underlying the progression of gastric adenocarcinoma.

Disclosure of conflict of interest

None.

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