Influence of Tumor Mutational Burden on Immune-infiltrating Cells in Stomach Adenocarcinoma

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Research Article

Keywords: stomach adenocarcinoma, tumor mutational burden, tumor-infiltrating immune cells, differentially expressed genes, survival analysis

Posted Date: December 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-122577/v1>

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Abstract

Background: Gastric cancer (GC) is one of the most common and fatal cancers worldwide. Tumor mutational burden (TMB) is described as a novel powerful signature to predict response to immunotherapy in gastric cancer. Nevertheless, tumor-infiltrating immune cells (TIICs) in different TMB GC remains unknown.

Results: (1) The most common missense were single nucleotide polymorphisms and C>T. Furthermore, titin (TTN), tumor protein p53 (TP53), and mucin 16 (MUC16) had a higher rate of mutations in STAD. (2) TMB-high and -low groups were not associated with survival, T stage, M stage, and grade of patients with stomach adenocarcinoma (STAD) ($P_{\text{survival}}=0.086$, $P_T=0.331$, $P_M=0.804$, $P_{\text{grade}}=0.695$). However, TMB groupings were related with N stage and sex ($P_N=0.003$, $P_{\text{sex}}=0.003$). (3) There were intricate differences in immune infiltration cells (eg, T cells, monocytes, and mast cells) between the TMB-high and TMB-low groups. (4) A total of 458 genes were identified as DEGs; Dynein cytoplasmic 1 intermediate chain 1 (DYNC111), matrix metalloproteinase 13 (MMP13) and zinc finger and BTB domain containing 16 (ZBTB16) were correlated with overall survival (OS). (5) DEGs were mainly involved in the regulation of the extracellular matrix-receptor interaction, and the Wnt and PI3K-AKT signaling pathways.

Conclusion: The present study provides a comprehensive and systematic analysis of TMB and its clinical significance, and reveals the complicated differences of 22 TIICs between TMB-high and TMB-low groups in STAD.

Introduction

Gastric cancer (GC), one of the most common cancers, has distinct differences in incidence rates depending on the geographic regions and adenocarcinoma occupies the majority part. In particular, higher rates of new cases are reported in East Asia[1]. In addition, GC is the third most common type of cancer and second most common cause of cancer-related death in China[2]. GC, a malignant tumor with high incidence of somatic mutations, appeared to benefit from immunotherapy[3]. With the progress of many research studies and clinical trials, programmed death-1 (PD-1) blockade-based immunotherapy has been applied to patients with advanced gastric carcinoma (AGC) and achieved satisfactory clinical therapeutic effects[3, 4]. According to the KEYNOTE-012 and KEYBOTE-059 trials, pembrolizumab showed a 22% objective response rate (ORR) in patients with programmed death-ligand 1 (PD-L1)-positive AGC (n = 36)[5, 6]. Nivolumab also exhibited good efficacy (11.2% ORR) in patients with chemo-refractory PD-L1 non-selected AGC[7]. However, the economic cost of immunotherapy is high; hence, not all patients with GC can benefit from this treatment. Therefore, it is necessary to identify new biomarkers for predicting the effect of immunotherapy.

Tumor mutational burden (TMB) refers to the number of somatic mutations per 1 million bases, excluding single nucleotide polymorphisms (SNP), germline, copy number variation, and structural variation[8, 9]. TMB has been shown to enhance clinical response to immunotherapy, including patients

with melanoma and non-small cell lung cancer[10]. Additionally, in GC, TMB-high patients responded significantly better to immunotherapy than TMB-low patients (NCT02915432)[3]. The reason is the difference in the immune microenvironment between the two groups, especially the complicated and unknown infiltration of immune cells[11, 12].

Tumor inflammatory response plays an important role in the growth, invasion, and metastasis of tumor cells. Several studies have shown that tumor-infiltrating immune cells (TIICs) can help hosts resist tumor cells and promote the development of solid tumors[13]. Immune infiltration in patients with GC is also closely associated with clinical prognosis[14–16]. Nevertheless, the effect of immunotherapy on patients with GC classified into different TIIC subgroups remains unknown. Hence, further investigation is warranted.

CIBERSORT is a gene expression matrix-based deconvolution algorithm. It has been developed as a new system biology tool, which can identify the diversity characteristics of tumor-infiltrating immune cells; this tool has been used in multiple research studies on tumors[17, 18]. In this study, the gene expression data and the Masked Somatic Mutation data of patients with GC were collected and analyzed. The TIICs subsets of GC were quantified using CIBERSORT and revealed the genomic phenotypes and heterogeneity of immune cell infiltration in patients with GC between the TMB-high and TMB-low groups. We completed the gene ontology (GO) and KOBAS-Kyoto Encyclopedia of Genes and Genomes (KEGG) gene enrichment analysis of DEGs and drew protein-protein interaction (PPI) network for further GC molecular research.

Results

Mutations in gastric adenocarcinoma

We evaluated the variation in each The Cancer Genome Atlas (TCGA) gastric adenocarcinoma sample to gain insight into the factors associated with gastric adenocarcinoma mutagenesis. The results showed that missense mutations, SNP, and C > T mutation were the most common mutations (Fig. 2). The highest mutation frequency was 5,612. The waterfall diagram revealed the integration status of somatic mutations in TCGA gastric adenocarcinoma. The results showed that the frequency of somatic mutations of titin (TTN), tumor protein p53 (TP53), mucin 16 (MUC16), AT-rich interaction domain 1A (ARID1A), LDL receptor related protein 1B (LRP1B), spectrin repeat containing nuclear envelope protein 1 (SYNE1), and filaggrin (FLG) was higher.

TMB and clinicopathological features

TMB was correlated with clinicopathological features, including ages, sex, and N stage ($P_{\text{ages}}=0.0006$, $P_{\text{sex}}=0.003$, $P_N<0.003$). The TMB-high group was characterized by advanced age, greater proportion of female patients, and higher N stage (Table 1). Univariate and multivariate Cox regression analyses showed that the TMB grouping had no effect on prognosis ($P > 0.05$) (Table 2).

Table 1
TMB and clinicopathological features

		TMB-high group	TMB-low group	P-value
		N = 80	N = 310	
Age		71.08 ± 9.72	66.66 ± 10.28	0.0006
Sex	Male	39	206	0.003
	Female	41	104	
Grade	G3	50	180	0.695
	G2 and G1	30	123	
	Unknown	0	7	
Stage	Stage I	15	38	0.104
	Stage II	25	92	
	Stage III	25	138	
	Stage IV	8	30	
	Unknown	7	12	
T	T1 and T2	24	77	0.263
	T3 and T4	52	228	
	Unknown	4	5	
N	N0 and N1	56	169	0.003
	N2 and N3	19	135	
	Unknown	5	6	
M	M0	72	22	0.804
	M1	5	279	
	Unknown	3	9	
Abbreviations: TMB, tumor mutational burden				

Table 2
Univariate and multivariate Cox regression analyses

Overall survival Cox models (N = 390)						
	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.02	1.01–1.04	0.01	1.42	1.02–1.06	< 0.01
Male versus female	0.99	0.70–1.42	0.97	0.94	0.63–1.40	0.76
Grade	1.18	0.86–1.63	0.31	1.23	0.85–1.77	0.27
Stage	1.69	1.36–2.11	< 0.01	1.36	0.88–2.12	0.16
T	1.31	1.06–1.62	0.01	1.21	0.89–1.66	0.22
N	1.33	1.14–1.56	< 0.01	1.10	0.86–1.40	0.44
M	2.68	1.56–4.60	< 0.01	2.30	1.02–5.18	0.04
TMB-high versus TMB-low	0.67	0.43–1.03	0.09	1.27	0.47–3.40	0.63
Abbreviations: CI, confidence interval; HR, hazard ratio; TMB, tumor mutational burden						

Association of TMB and TIICs in gastric adenocarcinoma

Subsequently, the proportion of 22 TIICs in all samples was determined. We selected the samples (n = 215) with mutation information and $P < 0.05$. The violin plot, which describes the difference of 22 TIICs between the TMB-high group and TMB-low group in gastric adenocarcinoma, was produced using the “vioplot” package in R (Fig. 3). Differences in the abundance of 22 TIICs between the two groups showed that samples in the TMB-high group had a significant increase in the abundance of activated memory CD4 + T cells ($P < 0.001$), T cells follicular helper ($P < 0.001$), T cells gamma delta ($P = 0.007$), resting natural killer cells ($P = 0.049$), macrophages M1 ($P < 0.001$), eosinophils ($P = 0.049$), and neutrophils ($P = 0.003$). Moreover, they had a significant decrease in the abundance of naïve B cells ($P = 0.045$), T regulatory (Treg) cells ($P < 0.001$), monocytes ($P < 0.001$), and resting mast cells ($P = 0.011$). The difference in TIICs between the two groups implies that they may play an important role in the immunotherapy of gastric adenocarcinoma.

Function enrichment analysis of DEGs

Based on the cutoff threshold, a total of 458 DEGs (62 upregulated and 396 downregulated RNAs in the TMB-high group) were selected from both TMB-high and TMB-low groups. A heatmap was constructed to display the distinctive pattern of DEGs expressed in these two groups (Fig. 4). GO enrichment analysis was performed to elucidate the biological functions, and KEGG enrichment analysis was conducted to speculate the biological pathway of DEGs. Figure 5 shows the GO terms associated with extracellular structure organization, organic hydroxy compound transport, extracellular matrix (ECM), proteinaceous

ECM, organic acid transmembrane transporter activity, and carboxylic acid transmembrane transporter activity. As shown in Fig. 6, KEGG pathway analysis revealed that the DEGs were mainly involved in 'ECM-receptor interaction', 'Wnt signaling pathway', 'protein digestion and absorption', and the 'PI3K-AKT signaling pathway'.

Survival analysis of DEGs

A total of 3 genes (DYNC11, MMP13, ZBTB16) were associated with survival, and these survival-related genes were reconfirmed by Kaplan Meier-plotter (KM plotter, <http://kmplot.com/analysis/>) ($P < 0.05$, Fig. 7).

PPI network of DEGs

The PPI networks of the DEGs were constructed and visualized using the STRING online database with minimum required interaction score > 0.95 (Fig. 8A). We selected the top 30 DEGs with the highest connectivity to complete the histogram (Fig. 8B).

Discussion

Nearly half of new GC cases annually are reported in China, and half of Chinese patients are diagnosed at an advanced stage of the disease[2]. The safety and efficacy of anti-PD-1 therapy in Chinese patients with AGC as salvage monotherapy or in combination with XELOX (oxaliplatin and capecitabine) at the first-line setting has been previously reported. The identification of reliable predictive markers for the OS of patients with GC receiving immunotherapy is the current research hotspot[23]. Somatic missense mutations strongly contribute to the generation of novel tumor epitopes[22]. The presence of more neoantigens on the tumor surface facilitates their recognition by immune cells, thus increasing the likelihood of immune response induction and potentially enhancing the efficacy of immunotherapy[24]. Furthermore, according to a clinical study, TMB-high patients responded to immunotherapy significantly better than TMB-low patients[3]. Although the development of immunotherapies has provided a new basis for the treatment of patients with GC, their potential mechanisms remain unclear. Thus, further investigations should be performed to develop the corresponding targeted therapy and immunotherapy[25–27]. Moreover, the increase in TMB, leads to more novel tumor epitopes, which may influence the TIICs. TIICs can promote and inhibit tumor growth, and studies have suggested that TIICs have great potential for the prediction of clinical outcome and therapeutic response among individual patients. The density and distribution of TIICs may play a role in predicting the survival of patients with GC[28]. Previous studies revealed that TIICs, such as mature T cells, dendritic cells, and memory T cells with increased infiltration, are associated with good prognosis, whereas immunosuppressive Treg cells are linked to poor prognosis[29, 30]. Some studies suggest that M1 macrophages can inhibit the growth of GC, while M2 macrophages play an important role in tumor progression, promoting a pro-angiogenic and immunosuppressive signal in the tumor[31]. However, most research studies applied immunohistochemical methods to evaluate TIICs and identify the TIIC subgroup with a single surface

marker; these methods are ineffective in identifying closely related cell types. Furthermore, the distribution of multiple immune cells and their relative proportions are rarely studied. In addition, differences in TIICs between the TMB-high and TMB-low groups of patients with GC have been rarely reported. We propose that the differences in TIICs between the two GC groups may influence tumor immunotherapy and the OS of patients. Some meta-analyses showed that the expression levels of CD3, CD4, and CD8 were positively correlated with overall survival; however, higher FOXP3 expression was correlated with reduced overall survival[32, 33].

A combination of CIBERSOFT algorithms can overcome the shortcomings of traditional immunohistochemical methods and accurately determine the relative proportions of different TIICs[34]. In this study, we used CIBERSORT to describe the proportion of 22 immune cell subsets between the TMB-high and TMB-low groups. The results showed that the TMB-high group had a significant increase in some immune cells. With elevated TMB, more neoantigens may occur, and affect TIICs in the immune microenvironment[35]. According to the multivariate analysis, CD8 + T cells, activated memory CD4 + T cells, and neutrophils were associated with better OS. In contrast, monocytes, macrophages M2, and eosinophils were associated with worse OS[36]. Whether these complex differences in TIICs between the two groups could be potential targets for immunotherapy warrants further investigations.

In the present study, we analyzed mutations in gastric adenocarcinoma samples. The results showed that missense mutations, SNP, and C > T mutation were the most common mutations. The three most frequently mutated genes were TTN, TP53, and MUC16. TP53 is an established tumor suppressor gene reported to regulate the cell cycle, thus inhibiting the development of cancerous cells. TTN is the largest described protein and constitutes the third most abundant type of filament in both cardiac and skeletal human muscle. TTN is emerging as a major gene in human inherited disease[37]. MUC16 is frequently mutated in multiple types of human cancer, particularly GC[38]. In addition, MUC16 can play an important role in tumorigenicity and acquired resistance to therapy[39].

The potential biological functions of DEGs in gastric adenocarcinoma were analyzed. The functions of DEGs were mainly associated with extracellular structure organization, organic hydroxy compound transport, ECM, proteinaceous ECM, organic acid transmembrane transporter activity, and carboxylic acid transmembrane transporter activity. These genes were mainly involved in 'ECM-receptor interaction', 'Wnt signaling pathway', 'protein digestion and absorption', and the 'PI3K-AKT signaling pathway'. The pathway of 'ECM-receptor interaction' has been reported in numerous types of cancer; the involved genes conferred the occurrence and progression of tumors and influenced cell phenotype[40–42]. The Wnt signaling pathway is mainly responsible for the development and homeostasis of cells and tissues via regulation of their endogenous stem cells, and could be used as a new therapeutic target to eliminate cancer stem cells[43, 44]. Activation of the PI3K/AKT pathway plays a critical role in frequent molecular alterations and the initiation and progression of cancer[45–47].

In this study, we found that 4 survival-related DEGs can influence the survival of gastric adenocarcinoma. These genes may become new targets for immunotherapy. It has been reported that DYNC111 could

promote the proliferation and migration of GC by upregulating the expression of interleukin 6 (IL6)[48]. Matrix metalloproteinase 13 (MMP13) was highly expressed in GC tissues and related to invasion, metastasis, tumor stage, and worse survival[49]. Moreover, PD-1 ligand expression and CD3 + T cells are regulated by MMP13, which should be taken into account when using PD-1/PD-L1 blocking immunotherapies[50]. Zinc finger and BTB domain-containing 16 (ZBTB16) is a new biomarker for the primitive neuroectodermal tumor element or Ewing sarcoma[51]. Whether these genes could influence immune cell infiltration and be novel latent targets for immunotherapy warrants further investigation.

Conclusion

The present study provides an integrated and systematic analysis of TIICs between TMB-high and TMB-low groups of patients with gastric adenocarcinoma. Our findings also revealed some DEGs between the two groups, as well as their significance in prognosis.

Methods

Gene expression datasets and Masked Somatic Mutation data

TCGA is a cancer genomics program providing publicly available data that contribute to cutting-edge cancer studies (<https://portal.gdc.cancer.gov>). RNA-sequencing gene expression HTSeq-FPKM data and associated clinicopathological data of patients with gastric adenocarcinoma were downloaded from the TCGA database on 25 April, 2020[19]. In total, the samples included 343 cancer tissues and 30 adjacent tissues. The Masked Somatic Mutation data (varscan. Somatic. Maf) were obtained, analyzed, and visualized using the 'maftools' in the R package. The TMB data of 433 cancer tissues were calculated. A total of 390 TMB samples had corresponding clinical information and were included in the statistical analysis. The flow diagram of this study is shown in Fig. 1.

TIICs quantification via the CIBERSORT algorithm

The CIBERSORT algorithm was used to determine the immune-related signature from 547 immune genes markers, and quantify the relative fraction of each immune cell type. This method was employed to infer their relative proportions of 22 immune cells. The proportions of 22 TIIC in gastric adenocarcinoma was obtained using the 'CIBERSORT' R package, with a cut-off P-value < 0.05. Samples in the top 20% of TMB (TMB > 12) were defined as TMB-high, while the remaining samples (TMB < 12) were defined as TMB-low; the latter has been associated with better overall survival (OS) under immunotherapy[3]. We used the Wilcoxon test to analyze the differences in immune cell abundance between patients with high-TMB and low-TMB gastric adenocarcinoma, which were visualized using the 'vioplot' R package.

Differential mRNA analysis

The 'limma' R package was utilized to normalize and identify TMB-related DEGs between patients with TMB-high and TMB-low gastric adenocarcinoma[20]. All DEGs with false discovery rate < 0.05 and |log2 fold change| >0.5 were exported, and the 'pheatmap' R package was used to perform hierarchical clustering.

Functional enrichment analysis and protein-protein interaction (PPI) network analysis of DEGs

The gene ontology (GO) pathway enrichment analysis and KOBAS-Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of DEGs were performed using 'clusterProfiler, org.Hs.eg.db, plot, ggplot2' in the R package[21]. The PPI networks of DEGs were constructed and visualized using the STRING online database with minimum required interaction score > 0.95.

Survival analysis

The survival package in R was used to assess the prognostic value of DEGs in patients with gastric adenocarcinoma. Survival curves were plotted by the Kaplan-Meier method and were compared using the log-rank test based on the median cutoff DEGs. Prognostic value of survival-related key genes in gastric cancer patients were reconfirmed by Kaplan Meier-plotter (KM plotter, <http://kmplot.com/analysis/>). A flow diagram of the DEGs analysis is shown in Fig. 1.

Statistical analysis

R3.5.2, Bioconductor (<https://www.bioconductor.org/>) and SPSS version 26 (IBM Corp., Armonk, NY, USA) were used for statistical analysis[22]. The Kaplan-Meier analysis were completed using the survival R package. The relationships of clinical pathologic features between the TMB-high and TMB-low groups were examined using Mann–Whitney *U* and chi-squared tests. P-values < 0.05 denoted statistically significant differences.

Abbreviations

DEGs

Differentially expressed genes (DEGs)

GC

gastric cancer

STAD

stomach adenocarcinoma

TMB

tumor mutational burden

SNP

single nucleotide polymorphisms

TTN

titin

TP53

tumor protein p53

MUC16

mucin 16

DYNC1I1

Dynein cytoplasmic 1 intermediate chain 1

MMP13

matrix metalloproteinase 13

ZBTB16

zinc finger and BTB domain containing 16

OS

overall survival

PD-1

programmed death-1

AGC

advanced gastric carcinoma

ORR

objective response rate

PPI

protein-protein interaction

GO

gene ontology

KEGG

KOBAS-Kyoto Encyclopedia of Genes and Genomes

TCGA

The Cancer Genome Atlas

Declarations

Acknowledgements

Not applicable.

Funding

The authors declare that there are no sources of funding to be acknowledged.

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Contributions

Zongxian Zhao and Shilong Li performed the data analysis and assisted in writing the manuscript. Shuliang Li and Daohan Wang designed the study and assisted in writing the manuscript. Huaxiang Yang assisted in polishing the language. Weihua Fu read and approved the final manuscript.

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Ethics Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability Of Data And Materials

All data sets used in this study are available from TCGA (<https://portal.gdc.cancer.gov/>) and Kaplan Meier-plotter (KM plotter, <http://kmplot.com/analysis/>).

References

1. McGuire S: **World Cancer Report 2014**. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. *Adv Nutr* 2016, **7**(2):418–419.

2. Feng RM, Zong YN, Cao SM, Xu RH: **Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics?** *Cancer Commun (Lond)* 2019, **39**(1):22.
3. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, Yuan XL, Chen Y, Yang SJ, Shi JH *et al*: **Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432.** *Ann Oncol* 2019, **30**(9):1479–1486.
4. Kim JM, Chen DS: **Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure).** *Ann Oncol* 2016, **27**(8):1492–1504.
5. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, Sun W, Jalal SI, Shah MA, Metges JP *et al*: **Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial.** *JAMA Oncol* 2018, **4**(5):e180013.
6. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B *et al*: **Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial.** *The Lancet Oncology* 2016, **17**(6):717–726.
7. Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK *et al*: **Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial.** *Lancet* 2017, **390**(10111):2461–2471.
8. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A *et al*: **Tumor mutational load predicts survival after immunotherapy across multiple cancer types.** *Nat Genet* 2019, **51**(2):202–206.
9. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM *et al*: **Erratum: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients.** *Nat Med* 2017, **23**(8):1004.
10. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, Rittmeyer A, Fehrenbacher L, Otto G, Malboeuf C *et al*: **Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab.** *Nat Med* 2018, **24**(9):1441–1448.
11. Mao M, Yu Q, Huang R, Lu Y, Wang Z, Liao L: **Stromal score as a prognostic factor in primary gastric cancer and close association with tumor immune microenvironment.** *Cancer Med* 2020, **9**(14):4980–4990.
12. Ren Q, Zhu P, Zhang H, Ye T, Liu D, Gong Z, Xia X: **Identification and validation of stromal-tumor microenvironment-based subtypes tightly associated with PD-1/PD-L1 immunotherapy and outcomes in patients with gastric cancer.** *Cancer Cell Int* 2020, **20**:92.
13. Bremnes RM, Al-Shibli K, Donnem T, Sirera R, Al-Saad S, Andersen S, Stenvold H, Camps C, Busund LT: **The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer.** *J Thorac Oncol* 2011, **6**(4):824–833.

14. Choi Y, Kim JW, Nam KH, Han SH, Kim JW, Ahn SH, Park DJ, Lee KW, Lee HS, Kim HH: **Systemic inflammation is associated with the density of immune cells in the tumor microenvironment of gastric cancer.** *Gastric Cancer* 2017, **20**(4):602–611.
15. Angell HK, Lee J, Kim KM, Kim K, Kim ST, Park SH, Kang WK, Sharpe A, Ogden J, Davenport A *et al*: **PD-L1 and immune infiltrates are differentially expressed in distinct subgroups of gastric cancer.** *Oncoimmunology* 2019, **8**(2):e1544442.
16. He Q, Li G, Ji X, Ma L, Wang X, Li Y, Fan C: **Impact of the immune cell population in peripheral blood on response and survival in patients receiving neoadjuvant chemotherapy for advanced gastric cancer.** *Tumour Biol* 2017, **39**(5):1010428317697571.
17. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA: **Profiling Tumor Infiltrating Immune Cells with CIBERSORT.** *Methods Mol Biol* 2018, **1711**:243–259.
18. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang CD *et al*: **The prognostic landscape of genes and infiltrating immune cells across human cancers.** *Nat Med* 2015, **21**(8):938–945.
19. Tomczak K, Czerwinska P, Wiznerowicz M: **The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge.** *Contemp Oncol (Pozn)* 2015, **19**(1A):A68-77.
20. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP: **Maftools: efficient and comprehensive analysis of somatic variants in cancer.** *Genome Res* 2018, **28**(11):1747–1756.
21. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters.** *OMICS* 2012, **16**(5):284–287.
22. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T *et al*: **Orchestrating high-throughput genomic analysis with Bioconductor.** *Nat Methods* 2015, **12**(2):115–121.
23. Qu F, Li R, He X, Li Q, Xie S, Gong L, Ji G, Lu J, Bao G: **Short telomere length in peripheral blood leukocyte predicts poor prognosis and indicates an immunosuppressive phenotype in gastric cancer patients.** *Mol Oncol* 2015, **9**(3):727–739.
24. Schrock AB, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, Miller VA, Lim D, Amanam I, Chao J *et al*: **Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer.** *Ann Oncol* 2019, **30**(7):1096–1103.
25. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtneess B *et al*: **Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial.** *Lancet Oncol* 2016, **17**(6):717–726.
26. Shitara K, Ozguroglu M, Bang YJ, Di Bartolomeo M, Mandala M, Ryu MH, Fornaro L, Olesinski T, Caglevic C, Chung HC *et al*: **Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial.** *Lancet* 2018, **392**(10142):123–133.
27. Boku N, Ryu MH, Kato K, Chung HC, Minashi K, Lee KW, Cho H, Kang WK, Komatsu Y, Tsuda M *et al*: **Safety and efficacy of nivolumab in combination with S-1/capecitabine plus oxaliplatin in patients**

- with previously untreated, unresectable, advanced, or recurrent gastric/gastroesophageal junction cancer: interim results of a randomized, phase II trial (ATTRACTION-4). *Ann Oncol* 2019, **30**(2):250–258.
28. Jiang W, Liu K, Guo Q, Cheng J, Shen L, Cao Y, Wu J, Shi J, Cao H, Liu B *et al*: **Tumor-infiltrating immune cells and prognosis in gastric cancer: a systematic review and meta-analysis.** *Oncotarget* 2017, **8**(37):62312–62329.
 29. Baxevas CN, Papamichail M, Perez SA: **Immune classification of colorectal cancer patients: impressive but how complete?** *Expert Opin Biol Ther* 2013, **13**(4):517–526.
 30. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS: **TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells.** *Cancer Res* 2017, **77**(21):e108-e110.
 31. Gambardella V, Castillo J, Tarazona N, Gimeno-Valiente F, Martinez-Ciarpaglini C, Cabeza-Segura M, Rosello S, Roda D, Huerta M, Cervantes A *et al*: **The role of tumor-associated macrophages in gastric cancer development and their potential as a therapeutic target.** *Cancer Treat Rev* 2020, **86**:102015.
 32. Zheng X, Song X, Shao Y, Xu B, Chen L, Zhou Q, Hu W, Zhang D, Wu C, Tao M *et al*: **Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: a meta-analysis.** *Oncotarget* 2017, **8**(34):57386–57398.
 33. Lee JS, Won HS, Sun S, Hong JH, Ko YH: **Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: A systematic review and meta-analysis.** *Medicine (Baltimore)* 2018, **97**(32):e11769.
 34. Becht E, de Reynies A, Giraldo NA, Pilati C, Buttard B, Lacroix L, Selves J, Sautes-Fridman C, Laurent-Puig P, Fridman WH: **Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy.** *Clin Cancer Res* 2016, **22**(16):4057–4066.
 35. Endris V, Buchhalter I, Allgauer M, Rempel E, Lier A, Volckmar AL, Kirchner M, von Winterfeld M, Leichsenring J, Neumann O *et al*: **Measurement of tumor mutational burden (TMB) in routine molecular diagnostics: in silico and real-life analysis of three larger gene panels.** *Int J Cancer* 2019, **144**(9):2303–2312.
 36. Li L, Ouyang Y, Wang W, Hou D, Zhu Y: **The landscape and prognostic value of tumor-infiltrating immune cells in gastric cancer.** *PeerJ* 2019, **7**:e7993.
 37. Chauveau C, Rowell J, Ferreiro A: **A rising titan: TTN review and mutation update.** *Hum Mutat* 2014, **35**(9):1046–1059.
 38. Li X, Pasche B, Zhang W, Chen K: **Association of MUC16 Mutation With Tumor Mutation Load and Outcomes in Patients With Gastric Cancer.** *JAMA Oncol* 2018, **4**(12):1691–1698.
 39. Aithal A, Rauth S, Kshirsagar P, Shah A, Lakshmanan I, Junker WM, Jain M, Ponnusamy MP, Batra SK: **MUC16 as a novel target for cancer therapy.** *Expert Opin Ther Targets* 2018, **22**(8):675–686.
 40. Bao Y, Wang L, Shi L, Yun F, Liu X, Chen Y, Chen C, Ren Y, Jia Y: **Transcriptome profiling revealed multiple genes and ECM-receptor interaction pathways that may be associated with breast cancer.** *Cell Mol Biol Lett* 2019, **24**:38.

41. Buttner P, Ueberham L, Shoemaker MB, Roden DM, Dinov B, Hindricks G, Bollmann A, Husser D: **Identification of Central Regulators of Calcium Signaling and ECM-Receptor Interaction Genetically Associated With the Progression and Recurrence of Atrial Fibrillation.** *Front Genet* 2018, **9**:162.
42. Zhai X, Xue Q, Liu Q, Guo Y, Chen Z: **Colon cancer recurrence-associated genes revealed by WGCNA coexpression network analysis.** *Mol Med Rep* 2017, **16**(5):6499–6505.
43. Duchartre Y, Kim YM, Kahn M: **The Wnt signaling pathway in cancer.** *Crit Rev Oncol Hematol* 2016, **99**:141–149.
44. Nusse R, Clevers H: **Wnt/beta-Catenin Signaling, Disease, and Emerging Therapeutic Modalities.** *Cell* 2017, **169**(6):985–999.
45. Kachrilas S, Dellis A, Papatsoris A, Avgeris S, Anastasiou D, Gavriil A, Horti M, Tseleni Balafouta S, Livadas K, Stravopodis DJ *et al*: **PI3K/AKT pathway genetic alterations and dysregulation of expression in bladder cancer.** *J BUON* 2019, **24**(1):329–337.
46. Cao Y, Luo Y, Zou J, Ouyang J, Cai Z, Zeng X, Ling H, Zeng T: **Autophagy and its role in gastric cancer.** *Clin Chim Acta* 2019, **489**:10–20.
47. Zhou H, Yuan M, Yu Q, Zhou X, Min W, Gao D: **Autophagy regulation and its role in gastric cancer and colorectal cancer.** *Cancer Biomark* 2016, **17**(1):1–10.
48. Gong LB, Wen T, Li Z, Xin X, Che XF, Wang J, Liu YP, Qu XJ: **DYNC111 Promotes the Proliferation and Migration of Gastric Cancer by Up-Regulating IL-6 Expression.** *Front Oncol* 2019, **9**:491.
49. Ren J, Liu J, Sui X: **Correlation of COX-2 and MMP-13 expressions with gastric cancer and their effects on prognosis.** *J BUON* 2019, **24**(1):187–193.
50. Dezutter-Dambuyant C, Durand I, Alberti L, Bendriss-Vermare N, Valladeau-Guilemond J, Duc A, Magron A, Morel AP, Sisirak V, Rodriguez C *et al*: **A novel regulation of PD-1 ligands on mesenchymal stromal cells through MMP-mediated proteolytic cleavage.** *Oncoimmunology* 2016, **5**(3):e1091146.
51. Xiao GQ, Sherrod AE, Hurth KM: **ZBTB16: A new biomarker for primitive neuroectodermal tumor element / Ewing sarcoma.** *Pathol Res Pract* 2019, **215**(10):152536.

Figures

Figure 1

The flow diagram of DEGs analysis.

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The flow diagram of DEGs analysis.

Figure 2

TCGA gastric adenocarcinoma mutation cohort. (A) Overview of TCGA gastric adenocarcinoma cohort mutations; (B) Waterfall of the top 30 mutated genes in the TCGA gastric adenocarcinoma cohort.

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Figure 3

Differential analysis of tumor-infiltrating immune cells (TIICs) between high-group (red) and low-TMB groups (blue).

Figure 3

Figure 4

The heatmap of DEGs between TMB-high group and TMB-low group.

Figure 5

GO analysis of the top ten enriched biological processes (BPs) and cell composition (CC) and molecular function (MF) of DEGs.

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GO analysis of the top ten enriched biological processes (BPs) and cell composition (CC) and molecular function (MF) of DEGs.

Figure 6

KEGG enrichment analysis of DEGs.

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KEGG enrichment analysis of DEGs.

Figure 7

Kaplan–Meier analysis of 3 survival-related genes and reconfirmed by Kaplan Meier-plotter.

Figure 7

Kaplan–Meier analysis of 3 survival-related genes and reconfirmed by Kaplan Meier-plotter.

Figure 8

PPI network analysis A: PPI network; B: Histogram of core genes.

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PPI network analysis A: PPI network; B: Histogram of core genes.