An immune-associated gene prognostic index risk model for stomach adenocarcinoma

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Research Article

Keywords: stomach adenocarcinoma, immune-associated gene, prognosis, tumor immune microenvironment, immune therapy

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-841317/v1>

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Abstract

Background

Stomach adenocarcinoma (STAD) is one of the most common malignant tumors worldwide. In this study, we attempt to construct a valid immune-associated gene prognostic index risk model which could predict the survival of HCC patients and the efficacy of immune check point inhibitors (ICIs) treatment.

Methods

The transcriptome, clinical and gene mutational data were obtained from the TCGA database. And immune-related genes were downloaded from the ImmPort and InnateDB databases. Functional and enrichment analysis was performed to identify the potential molecular function and mechanism of these differentially expressed immune-associated genes. And then candidates genes related to overall survival (OS) of STAD was obtained by weighted gene co-expression network analysis (WGCNA). Next, the immune prognostic risk model was constructed via multivariate Cox regression analysis and verified with GEO STAD cohort. Afterwards, the association between the risk model and the immune characteristics and was estimated. Finally, the correlation between the risk model and efficacy of ICIs therapy.

Results

A total of 493 immune-related genes were identified to enriched in function associated to immune response as well as in immune and tumor-related pathways. Based on the cox regression analysis, we constructed an immune-associated gene prognostic index (IAGPI) risk model based on 8 genes (RNASE2, CGB5, INHBE, PTGER3, CTLA4, DUSP1, APOA1 and CD36). Patients were divided into two subsets according to risk score. Patients in low risk set had a better OS than those in high. In the low risk set, there were more CD8 T cells, activated memory CD4 T cells, follicular helper T cells and M1 macrophages, while monocytes, M2 macrophages, eosinophils and neutrophils were more plentiful in the high. And patients in the low risk set were more sensitive to ICIs therapy.

Conclusion

The IAGPI risk model can precisely predict prognosis, reflect tumor immune microenvironment and predict the efficacy of ICIs therapy in STAD patients.

Introduction

Stomach adenocarcinoma (STAD) is the sixth highest incidence of cancer and the second leading cancer-related mortality[1]. Since most of the patients with STAD are diagnosed in the middle and advanced stage, even if they receive surgery-based comprehensive treatment, the recurrence rate is still very high

and the prognosis is poor, the five-year survival rate of the patients is less than 30%[2]. Although great progress has been made in recent years in the treatment of surgery, chemotherapy and targeted therapy, the five-year survival rate of patients with advanced STAD remains low[3]. Therefore, there is an urgent need to predict the survival of patients with STAD and improve their clinical prognosis.

With the continuous progress and deepening of the research on programmed death receptor-1 (PD-1), programmed death ligand (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA4), immune checkpoint inhibitors (ICIs) therapy has become a cutting-edge topic in comprehensive tumor therapy[4–6]. Previous studies have confirmed that anti-PD-1, PD-L1 and CTLA4 can improve the prognosis and quality of life of patients with STAD[7–9]. However, although ICIs is a promising treatment for patients with STAD, the patient response rate to ICIs therapy is still limited and new strategies need to be developed to maximize the efficacy of ICIs therapy[10, 11]. Studies have confirmed that the response to ICIs therapy is related to the immune cells in tumor immune microenvironment (TIME)[12]. Some studies have found that TIME can be used as a major prognostic indicator and can also improve the potential of precision treatment[13, 14]. Identifying potential prognostic markers associated with TIME could improve immunotherapy outcomes in patients with STAD. Regrettably, there is little known about the TIME of STAD, therefore, suitable and effective model with prognosis and therapy are imminently needed.

In our study, we attempted to develop a prognostic marker for STAD that could predict the outcome of conventional and immunological therapy. We used all immune-related genes in STAD transcriptome information of TCGA database, and selected immune-related hub genes associated with patient prognosis through weighted gene co-expression network analysis (WGCNA), and constructed immune-associated gene prognosis index (IAGPI) risk model. We then analyzed the molecular and immune characteristics of IAGPI risk model, identified its prognostic value in patients with immunotherapy, and compared it with tumor immune dysfunction and exclusion (TIDE) model and tumor inflammatory characteristics (TIS) model. The results suggested that IAGPI risk model is a prospective prognostic signature for patients receiving routine and immunological therapy.

Method

Data collection and collation

The RNA transcriptome profiling of 407 STAD samples, including 375 tumor tissues and 32 none-tumor tissues, the clinicopathological and gene mutation annotation information and immune subtype data were obtained from the TCGA database (<https://gdc.cancer.gov/>). The transcriptome and survival information of 433 STAD samples in GSE84437 were downloaded from the GEO database. The battery of immune-related genes were downloaded from the ImmPort (<https://www.immport.org/shared/genelists>) and InnateDB(<https://www.innatedb.ca/annotatedGenes.do?type=innatedb>) databases.

Extraction and identification of immune-associated hub gene

According to the RNA transcriptome profiling of STAD samples (375 tumor and 32 none-tumor samples) from TCGA and immune-associated genes from the ImmPort and InnateDB databases, series of differential expression genes were screened through the limma and pheatmap packages in R by the criteria of A fold change of (FC) >1 and *P* value <0.05. And then the immune-associated genes were analyzed and identified by means of clusterProfiler, org.Hs.eg.db, enrichplot, ggplot2 and GOplot packages in R to get Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

To identify immune-associated hub genes WGCNA was performed by limma and WGCNA packages in R. Firstly, a gene co-expression network was constructed for two genes using as Pearson's correlation matrix to get the similarity matrix based on the immune-associated differential expression genes. And subsequently, we chose the soft-threshold $\beta=3$ to convert similarity matrix into adjacency matrix, and then into a topological overlap matrix (TOM). The TOM is a method to describe the degree of correlation between nodes. Next, the dynamic tree was constructed to identify the module eigengene (ME), and the genes in ME with the lowest *P* value was performed to construct co-expression network by igraph package in R, and the genes in the network were the immune-associated candidate genes. Finally, survival and survminer package was used to bring immune-associated candidate genes into the STAD patient cohort of TCGA to verify the survival rate, and immune-associated hub gene was above-mentioned genes with survival significance.

To identify the gene mutation status of immune-associated hub gene with survival significance, we used the maftools package in R to visualize the gene mutation in the STAD patient cohort of TCGA database.

Modeling and verification of the immune-associated gene prognostic index (IAGPI)

The IAGPI risk model was constructed according to the immune-associated genes with survival significance via multivariate Cox regression with the following formula: risk score = $\text{coef}_1 * \text{Exp}_{\text{mRNA}1} + \text{coef}_2 * \text{Exp}_{\text{mRNA}2} + \text{coef}_3 * \text{Exp}_{\text{mRNA}3} + \dots + \text{coef}_n * \text{Exp}_{\text{mRNA}n}$. "coef" is the regression coefficient of mRNA, and "Exp" is the expression of the mRNA. And then, the robustness of the model was verified by log-rank test using patients with STAD in the TCGA and GEO cohorts. Finally, univariate and multivariate Cox regression analysis of independent prognostic was performed to verify the value of the risk model.

Analysis of molecular characteristics of different IAGPI risk subsets

In the previous analysis, patients with STAD in TCGA cohort were divided into high-risk and low-risk sets based on IAGPI scores and gene set enrichment analysis (GSEA) was performed by limma, org.Hs.eg.db, clusterProfiler and enrichplot packages in R to identify the signaling pathways that differentially expressed genes involved in.

Analysis of immune characteristics of different IAGPI risk subsets

In order to further understand the immunological characteristics of the IAGPI risk subsets, the relationship between gene mutations and subsets was analyzed using Maftools package in R. Next, limma, ggplot, ggpubr and ggExtra packages was applied to explore the relationship between different IAGPI risk subsets and PD1, PD-L1 expression and tumor mutation burden (TMB).

In order to identify the immunological characteristics of 407 tissues with STAD, limma, e1071, parallel and preprocessCore packages in R were used to import express information into CIBERSORT and repeated 1000 times and evaluate the relative proportions of 22 immune cells. Subsequently, in order to analyze the composition of immune cells in different IAGPI risk subsets, limma, reshape2 and ggpubr packages in R were used to compare the distribution of immune cells in different IAGPI risk subsets with Wilcoxon test. And clinicopathological factors in different IAGPI risk subsets was visualized by ComplexHeatmap package in R. To reveal the relationship between immune cell content and survival rate of STAD patients in TCGA cohort, Kaplan-Meier curves were drawn by limma, survival and survminer packages in R.

Single sample GSEA (ssGSEA) analysis was executed by limma, GSVA, GSEABase, ggpubr and reshape2 packages in R to explore differential immune-related functions in two different IAGPI risk subsets. According to the immune-related functions obtained in the previous step, they were brought into the STAD patients cohort of TCGA for survival analysis and verification of immune-related function by limma, survival and survminer packages in R.

RColorBrewer package in R was used to analyze the correlation between clinical information and two different IAGPI risk subsets, and the correlation between immune subtype and two different IAGPI risk subsets.

Analysis of immune escape and ICIs therapy in different IAGPI risk subsets

To assess the potential clinical efficacy of immunotherapy on patients in the two IAGPI risk subsets by analysis of immunoescape and immunotherapy, we put the gene expression profiling of STAD patients in TCGA into online tool TIDE ([HTTP://tide.dfci.harvard.edu/](http://tide.dfci.harvard.edu/)), and downloaded the data including TIDE score, MSI expression, Dysfunction and exclusion. Following, Limma and ggpubr packages were used to analyze the potential ICIs therapy. In addition, to further verify the prognostic power of our risk model, we compared the IAGPI risk model with the TIDE and TIS model through limma, survival, survminer and timeROC packages in R.

Statistical analysis

The t-test was used for the comparison of two sets of numerical continuous variables. The chi-square test was suitable for categorical variable. Cox regression analysis was used for multivariate survival analysis. Wilcoxon test was taken to compare the TID score and IAGPI risk sets. The log-rank test was used to analyze OS.

Results

Identification of immune-associated hub gene

Through analysis of 375 tumor tissues and 32 normal tissues in TCGA database, a total of 8833 differentially expressed genes were obtained, of which 1335 genes were down-regulated and 7498 genes were up-regulated (Figure1A). The intersection of the above-mentioned differentially expressed genes with immune-associated genes obtained from ImmPort and InnateDB was used to obtain a total of 493 differentially expressed immune-related genes, of which 184 genes were down-regulated and 309 were up-regulated (Figure1B). Through GO analysis, the function of the immune-associated differentially expressed genes was divided into three parts: cellular component (CC), molecular function (MF) and biological process (BP). As shown in Figure1C, in BP, genes were mainly enriched in leukocyte migration, regulation of inflammatory response and regulation of immune effector process etc. , in CC, genes were mostly enriched in immunoglobulin complex and external side of plasma membrane etc. , and in MF, genes were largely enriched in receptor ligand activity, cytokine activity and cytokine receptor binding etc. As shown in Figure 1D, was the top 8 GO terms and the name of the gene that corresponds to each term. KEGG pathway analysis certified that these genes were conspicuously enriched in cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction and chemokine signaling pathway (Figure 1E). And in Figure 1F, it was shown that the top 8 KEGG terms and the name of the gene that corresponds to each term.

In order to obtain immune-related hub genes, 493 candidate genes were analyzed by WGCNA, and a total of 5 modules were identified (Figure 2A). The turquoise module was closely associated with STAD tumors, and the top 77 immune-associated hub genes were obtained according to the threshold value (Figure 2B). In the end, as shown in Figure 3 and 4, 36 immune-related genes were closely associated with overall survival (OS) in STAD patients ($p < 0.05$).

We then identified the mutational characteristics of 36 immune-associated hub genes, as demonstrated in Figure 5A, we found that mutations in 28 of the 36 immune-associated hub genes.

Survival outcomes in two different IAGPI risk sets

To identify independent prognostic genes, a multivariate Cox regression analysis was performed for 36 immune-associated hub genes. After calculation, we found that only 8 genes were the independent prognostic factors associated with the OS of STAD. Then the following formula was constructed: Risk score = $0.28 \times \text{Exp}_{\text{RNASE2}} + 0.26 \times \text{Exp}_{\text{CGB5}} + 0.50 \times \text{Exp}_{\text{INHBE}} + (-0.35) \times \text{Exp}_{\text{PTGER3}} + (-0.34) \times \text{Exp}_{\text{CTLA4}} + 0.26 \times \text{Exp}_{\text{DUSP1}} + 0.07 \times \text{Exp}_{\text{APOA1}} + 0.23 \times \text{Exp}_{\text{CD36}}$. Univariate Cox regression analysis showed that age, stage and IAGPI risk score were significantly correlated with the prognosis of STAD (Figure 5B). Multivariate Cox regression analysis showed that IAGPI risk model was an independent prognostic factor (Figure 5C).

In the cohort of STAD patients in TCGA database, patients with high IRGPI risk had better OS than those with low, using median IRGPI risk score as a cutoff value (Figure 5D, $p < 0.05$). And then, the robustness of the model was verified by using STAD patients cohort in GSE84437 of GEO database. As shown in Figure 5E, the patients in the high risk set had a distinctly better prognosis than those in the low risk set ($p < 0.05$).

Molecular and immune characteristics of different IAGPI risk subsets

GSEA was performed to identify genes enriched in different IAGPI risk subsets. The genes of samples with high IAGPI risk were enriched in immune response pathway (Figure 6A), while the genes of samples with low IAGPI risk were enriched in spliceosome, cell cycle and DNA replication etc. which were related to pathway of tumor (Figure 6B).

Gene mutations were then analyzed to further understand the immunological characteristic of the two IAGPI risk subsets. The number of mutations in the high IAGPI risk subset was significantly higher than that in the low IAGPI risk subset, and the top 20 genes with the highest mutation rates in the IAGPI risk subsets were identified (Figure 6C).

Next, the relationship between IAGPI risk score and TMB level, PDL1 expression, and PD1 expression was respectively investigated. As demonstrated in Figure 7A, TMB level of samples in low IAGPI risk set was significantly higher than that in high ($p < 0.05$), and the IAGPI risk score had a weak negative correlation with TMB level ($R = -0.29$, $p < 0.05$). The CD274 expression level of samples in low IAGPI risk set was evidently higher than that in high ($p < 0.05$), and the IAGPI risk score had a weak negative correlation with CD274 expression level ($R = -0.17$, $p < 0.05$). And the PDCD1 expression level of samples in low IAGPI risk set was obviously higher than that in high ($p < 0.05$), and the IAGPI risk score had a weak negative correlation with PDCD1 expression level ($R = -0.21$, $p < 0.05$).

The result of the composition of immune cells in different IAGPI risk subsets was shown in Figure 8A, it was obvious that CD8 T cells, activated memory CD4 T cells, follicular helper T cells and M1 macrophages were more plentiful in the low-IAGPI risk subset, while monocytes, M2 macrophages, eosinophils and neutrophils were more plentiful in the high-IAGPI risk subset. The immune and clinicopathological factors in different IAGPI risk subsets were demonstrated in Figure 8B.

According to the immune cell content of each STAD sample in the TCGA database, we found that CD8 T cells, resting NK cells, M0 macrophages, M2 macrophages, resting dendritic cells and resting mast cells were significantly related to the OS of STAD (Figure 9A-F, $p < 0.05$).

The result of differential immune-related functions in two different IAGPI risk subsets was shown in Figure 9G, CCR, DCs, iDCs, macrophages, mast cells, neutrophils, T helper cells and type II IFN response were more abundant in the high IAGPI risk subset, while cytolytic activity, inflammation promoting, MHC class I, T cell co-inhibition and th1 cells were more abundant in the low IAGPI risk subset.

We further explore if the prognostic value of the IAGPI risk model was affected by differential immune-related functions. As shown in Figure 10A-F, it was revealed that patients in high risk group of APC co-inhibition, check point, cytolytic activity, inflammation promoting, T cell co-inhibition and Th2 cells had a better outcome, while patients with more neutrophils, more parainflammation, more iDCs, more Th1 cells, more mast cells and more type II IFN response had a worse prognosis (Figure 10G-L). Therefore, the IAGPI risk model was affected by better immunoregulation.

The correlation between clinical information and two different IAGPI risk subsets was shown in Figure 11A, the patients number of each stage in the two IAGPI risk subsets was roughly similar, and the chi-square test showed no statistical significance. And the correlation between immune subtype and two different IAGPI risk subsets (low=181, high=167) was seen in Figure 11B, the patients number of each immune subtype in the two IAGPI risk subsets (low=173,high=170) was shown to be statistically significant by chi-square test ($p < 0.05$).

ICIs therapy in different IAGPI risk subsets

TIDE was used to evaluate the potential clinical efficacy of immunotherapy in different IAGPI risk subsets. A higher TIDE predictive score indicated a higher likelihood of immune escape, indicating that patients did not respond well to ICIs therapy. Our results was shown in the Figure 12A, TIDE scores were higher in the high IAGPI risk subset than that in the low IAGPI risk subset, which means that patients with low IAGPI risk are better candidates for ICIs therapy than patients with high IAGPI risk. In addition, a higher TIDE prediction score was related to a poorer prognosis. Therefore, the high IAGPI risk set with low TIDE score may have a better prognosis than the low IAGPI risk set with high TIDE score. Moreover, we found that the high IAGPI risk subset had higher microsatellite instability (MSI) scores, higher T cell exclusion scores, and higher T cell dysfunction scores. Besides, we used ROC curve to identify the robustness of the IAGPI risk model, as shown in Figure 12B, the AUC at 1, 2 and 3 year indicated that a commendable value of the IAGPI risk model to predict prognosis of patients with ICIs therapy. In addition, Compared to TIDE and TIS models, the IAGPI risk model had a better predictive value (Figure 12C).

Discussion

Stomach adenocarcinoma is the most common histological subtype of gastric cancer, and has an relatively unsatisfactory survival rate[15]. ICIs therapy has been confirmed to achieve success for advanced patients with STAD[16]. However, the overall positive response rate to ICIs treatment still remains low, so it is critical to determine which patients are better able to receive immunotherapy[17–19]. In the last few years, genomics and bioinformatics have made possible prognostic signature for STAD based on lncRNA, miRNA and mRNA, but we still have not found a validated biomarker to predict the overall survival and efficacy of immunotherapy of STAD patients. Therefore, it is essential to establish a model to predict the survival of patients with STAD and to benefit the effective the STAD patients of immunotherapy.

In the present study, a total of 493 immune-associated genes of STAD were identified, and their biological processes, molecular functions and signaling pathway results including “leukocyte migration”, “receptor ligand activity” and “cytokine-cytokine receptor interaction” etc. are closely related to immune, which was corresponded with previous reports[20–22]. Subsequently, 36 immune-associated hub genes related to OS were identified using WGCNA and then according to Cox regression analysis, IAGPI risk model was constructed based on 8 genes which were independent prognostic factors of OS. The 8 genes that make up the IAGPI risk model are RNASE2, CGB5, INHBE, PTGER3, CTLA4, DUSP1, APOA1 and CD36. Subsequently, IAGPI risk model has been verified to be a potent prognostic immune-associated signature for STAD. As validated in TCGA and GEO STAD patients cohorts, patients in low IAGPI risk subset had better survival than those in high.

RNASE2 is a member of ribonuclease A family which was previously reported played an important role in dendritic cell activation, immune response regulation and TLR2 activation[23]. In addition, it was reported that RNASE2 might effectively predict the prognosis of clear cell renal cell carcinoma[24]. Chorionic gonadotropin subunit beta 5 (CGB5) was proved to be significantly overexpressed in gastric cancer and has a good independent prognostic value in gastric cancer patients[25]. INHBE is inhibin subunit beta E which encodes a member of the transforming growth factor- β (TGF- β) superfamily of proteins. INHBB is the homologous gene of INHBE which has been reported to be down-regulated in patients with lung cancer and endometrial adenocarcinoma[26, 27]. In addition, inhibin has been shown to be associated with immunomodulation[28]. PTGER3 is one of four receptors identified for prostaglandin E2 (PGE2) that is abundant in the tumor microenvironment and plays an important role in immune regulation, epithelial cell proliferation and invasion[29–31]. PGE2-PTGER3 signaling has been reported to be associated with tumor-associated angiogenesis and tumor growth[32]. As a member of the immunoglobulin superfamily, CTLA4 is one of the most widely accepted immune checkpoints, and its main role is to competitively bind CD28, blocking the B7-CD28 signaling pathway necessary for T cell activation, as well as binding to B7 molecules to generate negative regulatory signals, thereby inhibiting T cell function and ultimately leading to a downregulation of the immune response[33–35]. CTLA4 has been extensively reported to be associated with cancer and could be used as a biomarker to guide immunotherapy and prognosis[36–38]. DUSP1 has been reported to be involved in a variety of functions, including cell proliferation and apoptosis, and is associated with prognosis in cancer patients[39–41]. Moreover, DUSP1 was found to play an significant role in immune escape[42, 43]. A research of Guo et al. demonstrated that APOA1 is associated with the density of immune cells in the tumor microenvironment[44]. In addition, ApoA1 has also been reported as a marker of survival in a variety of cancers[45, 46]. CD36 was reportedly related to prognosis in various cancer[47, 48]. A series of recent studies have shown that CD63 plays an important role in migration of immune cell[49, 50]. In conclusion, IAGPI risk model was a signature related to the prognosis of patients and active immunity.

Based on the gene mutations in the two different IAGPI risk subsets, we further investigated the effect of patients receiving immunotherapy. We found that the differences in mutations between the two sets was in TTN, TP53 and MUC16 etc. mutations, which were more frequent in the samples of high-IAGPI risk set those that in the low. TTN was identified as the most frequently mutated gene in the pan-cancer cohort,

and its mutation number was most associated with TMB[51]. In addition, there is a study confirmed that TTN and MUC16 gene mutations were associated with gastric cancer prognosis and TMB, and could be used as biomarkers for the treatment of ICIs[52]. A study have demonstrated that TP53 mutations and tumor immunity was closely related, suggesting that TP53 mutation status could be a effective biomarker for predicting response to cancer immunotherapy in different types of cancer[53]. TMB reflects the number of cancer gene mutations, and higher TMB is clinically associated with better ICIs outcomes[54]. PD-1/PD-L1 is one of the most common immune checkpoint molecules, targeting PD-1 and PD-L1, immune checkpoint inhibitors reactivate cytotoxic T cells to anti cancer cells[55]. Patients with higher PD-1/PD-L1 expression were more likely to respond positively to the anti- PD-1/PD-L1 agents[56, 57]. In our study, TMB, CD274 (PD-L1) and PDCD1 (PD1) in low-IAGPI risk set was significantly higher than that in high. Moreover, we considered the IAGPI risk score had a slight correlation with TMB, CD274 (PD-L1) and PDCD1 (PD1). Consequently, we thought that the IAGPI model could identify which patients were more likely to respond to immunotherapy.

Immune infiltrating cells in TIME have been shown to play an important role in tumor development and would influence clinical outcomes in cancer patients[58]. We used CIBERSORT to evaluate the relative proportions of 22 immune cells in each STAD specimen. Studies have shown that the complexity and diversity of TIME is a key link in causing immune escape and promoting the development of gastric cancer[59–61]. In general, high plentitude of activated memory CD4 T cells and CD8 T cells facilitated the development of an immune response, and contributed to a better prognosis[62]. Follicular helper T cell was reported to be involved in the information transmission in the process of B cell differentiation, assists in the activation of B cells, promotes the formation of germinal centers and the class conversion of immunoglobulin, and maintains a long-term humoral immune response[63]. Macrophages could be mainly divided into M1 macrophages and M2 macrophages. M1 macrophages, by secreting proinflammatory cytokines and chemokines and presenting antigens exclusively, participated in the positive immune response and played the function of immune surveillance, while M2 macrophages only have weak antigen presenting ability, and through the secretion of inhibitory cytokines IL-10 or TGF- β to down-regulate the immune response[64, 65]. Monocytes had the ability to differentiate into macrophages and dendritic cells. Immature dendritic cells (iDCs) interacted with T and B cells and participated in the immune response only when they were activated into mature dendritic cells[66, 67]. Studies of eosinophils were controversial, while they might synthesized and secreted granule proteins that kill cancer cells, they also produced mediators that promoted angiogenesis and matrix remodeling to promote cancer growth[68]. It has been verified that neutrophils could release some substances (reactive oxygen species and reactive nitrogen species etc.) and weaken the immune system that promote tumor formation, and neutrophils count is an unfavorable prognostic factor[69–71]. Our findings are consistent with this, there were more CD8 T cells, activated memory CD4 T cells, follicular helper T cells and M1 macrophages in the low-IAGPI risk subset, while monocytes, M2 macrophages, eosinophils and neutrophils were more plentiful in the high-IAGPI risk subset.

In terms of a STAD immune subtype classification, in low-IAGPI risk subset there were more patients with C2 (IFN- γ Dominant) and fewer patients with C4 (Lymphocyte Depleted). Studies have shown that IFN- γ

contributed to the creation of a tumor microenvironment conducive to T cell infiltration and cancer cell target recognition[72]. Besides, a study of B Soldevilla et al. figured out C2 had higher densities of CD8, CD4 activated, follicular helper T cells, which contributed to better immune response. It was also found that C4 showed moderate cell proliferation and tumor heterogeneity, and macrophages showed Th1 inhibition and high M2 response[73]. Therefore, our research conformed to the above characteristics.

Recent studies have revealed two distinct mechanisms of tumor immune evasion: in some tumors, although cytotoxic T-cell infiltration is high, these T cells were often in a dysfunctional state. In other tumors, immunosuppressive factors could exclude T cells infiltrating the tumor tissue. Given to these characteristics, TIDE was generally considered a model to effectively predict the effect of immune checkpoint suppression therapy. In our study, exclusion, dysfunction and MSI were lower in low-IAGPI risk group, which means a weaker immune escape. Previous studies have demonstrated that MSI is prevalent in gastric cancer and that MSI could lead to a high mutational load that is sensitive to ICIs therapy[74]. Hence, the IAGPI risk model can effectively distinguish which patients are more suitable for ICIs treatment.

In conclusion, we constructed an IAGPI risk model that is associated with TIME to distinguish efficacy of ICIs and predict prognosis of STAD patients, but further research of the model are necessary.

Declarations

Funding

This work was supported by the Taishan Scholars Program of Shandong Province (2019010668); Shandong Higher Education Young Science and Technology Support Program (2020KJL005)

Conflicts of Interest:

The authors have no conflict of interest to disclose.

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Figures

Figure 1

Differential expression genes in STAD. A: heatmap of differential expression genes (DEGs) between 375 tumor tissues and 32 normal tissues in TCGA ($p < 0.05$). B: heatmap of immune-associated DEGs

Figure 3

Prognostic curves of 36 immune-associated hub genes. Kaplan-Meier survival analysis of 36 immune-associated hub genes which related to overall survival of STAD in TCGA database ($p < 0.05$).

Figure 4

Prognostic curves of 36 immune-associated hub genes. Kaplan-Meier survival analysis of 36 immune-associated hub genes which related to overall survival of STAD in TCGA database ($p < 0.05$).

Figure 5

Establishment of IAGPI risk model. A: waterfall plot of 36 immune-associated hub genes mutations in TCGA database. B: Univariate Cox regression analysis of clinical factors and the IAGPI risk score ($p < 0.05$). C: Multivariate Cox regression analysis of IAGPI risk score ($p < 0.05$). D: Kaplan-Meier survival analysis of the IAGPI subsets in the TCGA cohort ($p < 0.05$). E: Kaplan-Meier survival analysis of the IAGPI subsets in the GEO cohort ($p < 0.05$).

Figure 6

Analysis of molecular characteristics in different IAGPI subsets. A: gene set enrichment analysis (GSEA) in high-IAGPI risk set ($p < 0.05$). B: GSEA in low-IAGPI risk set ($p < 0.05$). C: characteristics of Top 20 genes mutations in different IAGPI subsets.

Figure 7

The relationship between IAGPI risk score and TMB, expression of PD-1 and PD-L1. A: box plot of TMB in two different IAGPI risk subsets ($p < 0.05$). B: correlation analysis of IAGPI risk score and TMB ($p < 0.05$). C: box plot of PD-L1 expression in two different IAGPI risk subsets ($p < 0.05$). D: correlation analysis of IAGPI risk score and PD-L1 expression ($p < 0.05$). E: box plot of PD-1 expression in two different IAGPI risk subsets ($p < 0.05$). F: correlation analysis of IAGPI risk score and PD-1 expression ($p < 0.05$).

Figure 8

Immune characteristics in two different IAGPI risk subsets. A: the proportion of 22 immune-related cells in different IAGPI risk subsets (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). B: the relationship between IAGPI subsets and immune-associated cells proportion in 443 patients in the TCGA cohort.

Figure 9

Analysis of immune cells and function in different IAGPI risk subsets. A: survival analysis of resting dendritic cells in the TCGA cohort ($p < 0.05$). B: survival analysis of M0 macrophages in the TCGA cohort ($p < 0.05$). C: survival analysis of M2 macrophages in the TCGA cohort ($p < 0.05$). D: survival analysis of resting mast cells in the TCGA cohort ($p < 0.05$). E: survival analysis of resting NK cells in the TCGA

cohort ($p < 0.05$). F: survival analysis of CD8 T cells in the TCGA cohort ($p < 0.05$). G: immune function in two different IAGPI risk subsets (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 10

Kaplan-Meier survival analysis of immune-related function in different risk subsets. A: survival analysis of APC co-inhibition in the TCGA cohort ($p < 0.05$). B: survival analysis of check point in the TCGA cohort ($p < 0.05$). C: survival analysis of cytolytic activity in the TCGA cohort ($p < 0.05$). D: survival analysis of inflammation promoting in the TCGA cohort ($p < 0.05$). E: survival analysis of T cell co-inhibition in the TCGA cohort ($p < 0.05$). F: survival analysis of Th2 cells in the TCGA cohort ($p < 0.05$). G: survival analysis of neutrophils in the TCGA cohort ($p < 0.05$). H: survival analysis of parainflammation in the TCGA cohort ($p < 0.05$). I: survival analysis of iDCs in the TCGA cohort ($p < 0.05$). J: survival analysis of Th1 cells in the TCGA cohort ($p < 0.05$). L: survival analysis of type II IFN response in the TCGA cohort ($p < 0.05$).

Figure 11

The correlation between clinical information and two different IAGPI risk subsets. A: the number and proportion of different patients clinical stages in different IAGPI risk subsets. B: the number and proportion of patients with different immune subtypes in different IAGPI risk subsets ($p < 0.05$).

Figure 12

The prognostic value of IAGPI risk model in patients with ICIs therapy. A: TIDE, MSI, and T cell exclusion and dysfunction score in different IAGPI risk subsets (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). B: ROC curves that predict the prognosis of patients treated with ICIs at 1, 2, and 3 years. C: The ROC curve of IAGPI risk model compared with TIDE model and TIS model.