The prognostic value of a tumor microenvironment-based immune cell infiltration score model in colon cancer

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Abstract

Background

Tumor microenvironment consists of tumor cells, immune cells and other matric components. Tumor infiltration immune cells are associated with prognosis. But all the current prognosis evaluation system dose not take tumor immune cells other matrix component into consideration. In the current study, we aimed to construct a prognosis predictive model based on tumor microenvironment.

Method

CIBERSORT and ESTIMATE algorithms were used to reveal the immune cell infiltration landscape of colon cancer. Patients were classified into three clusters by ConsensusClusterPlus algorithm. Immune cell infiltration (ICI) scores of each patient were determine by principal-component analysis. Patients were divided to high and low ICI score groups. Survival, gene expression and somatic mutation of the two groups were compared.

Results

Patients with no lymph node invasion, no metastasis, T1-2 disease and stage I-II had higher ICI scores. Calcium signaling pathway, leukocyte transendothelial migration pathway, MAPK signaling pathway, TGF β pathway, and WNT signaling pathway were enriched in high ICI score group. Immune-checkpoint genes and immune-activity associated genes were significantly decreased in high ICI score. Patients in high ICI score group had better survival than low ICI score group. Prognostic value of ICI score was independent of TMB.

Conclusion

ICI score might serve as an independent prognostic biomarker in colon cancer.

Background

Colorectal cancer is the third most common cancer and it is the third leading cause of death in all cancers(1). The American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) TNM stage system is the most widely used prognosis predictive system(2). However, it was indicated that patients with the same TNM stage showed different clinical outcomes, revealing the defects of the TNM stage system(3). Other tumor cell characteristics were also used to classify colon cancer, including tumor morphology, tumor cell of origin, tumor gene expression and tumor mutation status, et al(4). But all the current cancer classifications are based on tumor cells and do not take immune status and microenvironment of tumor into consideration. The deficiency of immune system affects the ability of the body to eliminate tumor cells and promotes tumorigenesis(5). The components of tumor microenvironment, including fibroblasts, endothelia cells, cytokines, chemokines and metabolism

products, also play important roles in tumorigenesis(6, 7). A prognosis predictive model that takes the immune status and tumor microenvironment into consideration may work better in predicting prognosis.

In the current study, we constructed an immune cell infiltration (ICI) score model in colon cancer, described the somatic mutation characteristic and validated the prognostic value of the ICI score model.

Methods

Data resource

Transcriptome and clinical data of colon cancer patients were downloaded from TCGA (TCGA: TCGA-COAD, https://portal.gdc.cancer.gov/repository) and GEO (GEO: GSE17536, GSE17537, GSE28722, GSE29621, GSE38832, GSE39582, https://www.ncbi.nlm.nih.gov/geo/) database. Data was analysis by R software (R x64 v4.0.5, https://www.r-project.org/) and Perl software (strawberry-perl, v5.32.0, https://www.perl.org/). The expression data from TCGA was transformed into transcripts per kilobase million (TPMs). The ComBat algorithm was used to decreased the batch effects between different datasets(8).

Clustering of immune cell infiltration

TCGA and GEO data were merged and immune cell filtrations were calculated by CIBERSORT algorithm(9). Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE)(10) package was used to calculated immune score and stromal score. Total immune microenvironment score was the sum of the stromal score and immune score. Corrplot package was used to analyze the correlation of different contents in immune microenvironment. The clustering of samples was executed using ConsensusClusterPlus algorithm(11) and was repeated 1,000 times.

Analysis of gene expression difference

Limma package of R software was used to compare the gene expression difference of different ICIclusters. Adjusted $p<0.05$ and absolute fold-change > 1 was considered as significant.

Calculation of immune cell infiltration (ICI) score

Base on gene expression difference, patients were classified into different clusters using unsupervised clustering. Genes that were positively and negatively related to cluster signature was assigned as ICI gene signatures A and B. Boruta package was used to performed the dimension reduction(12). Then PCA analysis was used to calculate the immune cell infiltration score (ICI score) of each sample.

Somatic mutation analysis

Nucleotide variation data was down downloaded from TCGA database (https://www.cancer.gov/tcga/). The total number of non-synonymous mutation was counted. The somatic mutations of colon driver genes were evaluated using the Maftool package of R sofiware(13).

Statistical analysis

The correlation between different contents were analyzed by Pearson test. Survival difference between different groups were analyzed by log-rank test. Immune cell infiltration difference and TMB difference were analyzed by Wilcoxon test. Mutation difference in difference group was analyzed by chi-square test. Difference was considered to be statistically significant if $p<0.05$.

Results

The analysis of immune cell infiltration in tumor microenvironment of colon cancer

Totally 1576 cases with transcriptome information and clinical data were downloaded from TCGA and GEO database in March 2021. Patient characteristics were shown in table S1. Immune cell infiltration was quantified by CIBERSORT and ESTIMATE algorithms and clustering was performed to classify the patients into different clusters(9, 10).

According to the immune cell infiltration, patients were dived into three ICI clusters: ICI cluster A, ICI cluster B and ICI cluster C. Survival between the three ICI clusters was significantly different. ICI cluster A showed the best survival while ICI cluster B showed the worse survival ($p=0.006$) (figure 1A). We made a further comparison of the immune cell contents in tumor microenvironment of the three different ICI clusters. It was shown that high infiltration of CD8 T cell, follicular T helper cells, activated memory CD4 T cells, activated NK cells and M1 macrophages in ICI cluster A. ICI cluster B showed a lower infiltration of CD8 T cell, follicular T helper cells, activated memory CD4 T cells, activated NK cells, M1 macrophages, but had a higher level of resting NK cells, M0 macrophages and activated mast cells (figure 1B and 1C). We also made a correlation coefficient heatmap to visualized the immune cell interaction in tumor microenvironment (figure1D). Expression of two important immune checkpoint molecules, *PD-L1* and *CTLA4*, were examined. Both *PD-L1* and *CTLA4* expression were higher in ICI cluster A than ICI cluster C. But expression of *PD-L1* did not show difference between ICI cluster A and ICI cluster B. Expression of *CTLA4* did not show difference between ICI cluster B and ICI cluster C (figure 1E and 1F).

Immune gene cluster analysis

To reveal the gene expression characteristics in different ICI clusters, we used the limma packages of R software to analyze the transcriptome variations (table S2). Then an unsupervised clustering of the differentially expressed genes was performed. Patients were classified into three gene clusters: gene cluster A, gene cluster B and gene cluster C. Genes that were positively related to the gene cluster were assigned as ICI signature A, and the rest differentially expressed genes were assigned as ICI signature B(12) (table S3). A heatmap was used to visualized the transcriptomic profile difference between the three gene cluster(14) (figure 2A). Gene ontology analysis was performed to clarify the enriched biological processes in ICI signature A and signature B (figure 2B and 2C). Expression of *PD-L1* and *CTLA4* in the three gene clusters were examined. Both *PD-L1* and *CTLA4* expression were lower in gene cluster B than ICI cluster C. But no significant difference was observed between gene cluster B and cluster

A (figure 2D and 2E). To explore the prognostic value of gene clusters, we performed the survival analysis. It was shown that patients in gene cluster B had better survival than patients in the other two gene clusters (p=0.023) (figure 2F). Gene cluster C had higher infiltration of CD8 T cells, M1 macrophages, dendritic cells, NK cells than gene cluster B. And immune score and stromal score in cluster C were also higher than cluster B. However, gene cluster B showed a higher level of B cells, plasma cells and lower level of M1 macrophages (figure 2G).

Construction of immune cell infiltration score model

ICI scores of each patient were calculated by principle-component analysis (PCA). The optimal cutoff value was found out using the cut-off package of R software. Patients from TCGA database were assigned to high ICI score group or low ICI score group according to their ICI scores (figure 3A). We compared the ICI scores of different subgroup patients. It was indicated that ICI score was higher in patients who had no lymph node invasion and no metastasis. Patients with T1-2 and stage I-II disease also showed higher ICI score (figure 3B).

We evaluated the expression level of immune-checkpoint and immune-activity associated genes. *PDCD1*, *HAVCR2*, *CTLA4*, *CD274*, *LAG3* were selected as immune-checkpoint associated genes and *CXCL9*, *CXCL10*, *GZMB*, *GZMA*, *TNF*, *CD8A*, *TBX2*, *PRF1*, *IFNG* were selected as immune-activity associated genes(15-17). We found that most of the immune-checkpoint genes and immune-activity associated genes, except *PDCD1*, were significantly decreased in high ICI score group (figure 3C and 3D.). Gene set enrichment analysis (GSEA) was performed and it was revealed that calcium signaling pathway, leukocyte transendothelial migration pathway, MAPK signaling pathway, TGF β pathway, and WNT signaling pathway were enriched in high ICI score group while base excision repair pathway, cell cycle pathway, homologous recombination pathway, mismatch repair pathway and nucleotide excision repair pathway were enriched in low ICI score group (figure 3E and 3F). To evaluate the prognostic value of the ICI scores, Kaplan-Meier analysis was performed in the TCGA cohort. Patients in high ICI score group showed better survival than patients in the low ICI group (p=0.017) (figure 3G). We did a further validation of the prognostic value of the ICI score in the alll patients form both TCGA and GEO cohort. It was revealed that high ICI group also showed a better OS than low ICI group in total patient cohort (p=0.002) (figure 3H).

The relationship between ICI score and TMB

It has been indicated that tumor mutation burden (TMB) is associated with patients' response to immunotherapy(18, 19). Patients with high TMB showed an improved response to PD-1 inhibitors and other antitumor therapy. High TMB also predicted better prognosis in colorectal cancer patients (20, 21). Thus, an exploration was performed to analyze the correlation between ICI score and TMB. We compared the TMB level of patients in high and low ICI score groups. It was shown that there was no significant difference on TMB between the two group patients (figure 4A). Correlation analysis revealed that ICI score was not correlated with TMB (figure 4B). Survival analysis demonstrated that patients with high TMB had better OS than patients with low TMB (p=0.040) (figure 4C). To found out whether the combination of

TMB and ICI score can better predict the prognosis of colon cancer patients, a stratified survival analysis was performed. Survival differences was observed between high ICI score and low ICI score patients both in high TMB and low TMB subgroups (figure 4D). The above results suggested that ICI score is independent of TMB and might serve as a potential prognostic biomarker in colon cancer patients.

Distribution of somatic mutation in high and low ICI score group

The distribution of somatic mutation of driver genes in colon cancer was evaluated in both high and low ICI score groups. The driver genes were evaluated using the maftools package of R software(13). We further analyzed the top twenty genes with the highest mutation frequency and result was shown in figure 4E and 4F. There were totally 133 genes with significantly different mutation frequencies between high and low ICI score groups (Table S4). The results might provide information for further studying the mechanism of immune cell infiltration and gene mutation in immune therapy.

Discussion

Tumor infiltrating immune cells are important components of tumor microenvironment and immune cell infiltration (ICI) is associated with tumor prognosis(22). However, most of the current prognostic assessment system in colon cancer do not take tumor microenvironment or ICI into account.

It has been pointed out that tumor-infiltrating immune cells offered important prognostic information in cancer patients. An immune risk score (dIRS) which was constructed based on tumor infiltration immune cells was proved to be an independent prognostic biomarker for relapse-free survival in patients with resectable colon cancer(23). Basing on CD3 + and cytotoxic CD8 + T cells in tumor, another study constructed an immunescore system and revealed that the immunescore system provides reliable information on the risk of recurrence in patients with colon cancer(24). Tumor infiltration immune cells also proved to be a prognostic biomarker in other solid cancer, including lung cancer(25), head and neck squamous cell carcinoma(26), breast cancer(27), etc.

In the current research, we constructed an ICI score model based on tumor immune environment using bioinformatics analysis. The ICI score model developed in this study can evaluate the prognosis of colon patients in some extent. The ICI score was independent on TMB. The combination of ICI score and TMB worked better than TMB alone in assessing prognosis.

Our analysis demonstrated that ICI cluster with increased infiltration of CD8 + T cell, CD4 + T cells, activated NK cells, M1 macrophages and ICI score had a better prognosis. The result was consistent with the previous study(28, 29). We analyzed the gene characteristics of different ICI clusters and classified the patients into three gene clusters. Though gene cluster C had higher immune score and stromal score than cluster B, patients in gene cluster B showed the better OS. There may be several reasons. Firstly, although gene cluster C had higher infiltration of T cells, gene cluster B had higher infiltration of B cells and plasma cells. Secondly, other components in tumor microenvironment, such as inflammatory factors and metabolism products, also contributes to the anti-tumor immune response of the host. The abnormal

alteration of these molecules during tumor development may affect the interaction between the immune cells and break the balance between immunity tolerance and immunity activity(30).

Due to individual differences of immune cell infiltration, it is very important to make an individual immune score for each patient. In this study, we constructed an ICI score model using the Boruta algorithm. GSEA revealed that calcium signaling pathway, leukocyte transendothelial migration pathway, MAPK signaling pathway, TGF β pathway, and Wnt signaling pathway were enriched in high ICI score group. Previous study also indicated that hyperactivated of calcium signaling pathway and MAPK signaling pathway was associated high infiltration of immune cell(28). Although it was indicated that TGF β was an suppressor factor to anti-tumor immunity, TGF β play an important role in suppressing tumor development by inhibiting cell cycle progression, promoting apoptosis and decreasing expression of growth factor, cytokine and chemokine(31). Wnt signaling pathway also have dual effects on anti-tumor immunity. On one hand, Wnt signaling executes an adverse effect on anti-tumor immunity by suppressing effect T cell differentiation, inhibiting expansion of CD8 + T cells, promoting M2-like polarization of TAM, etc. On the other hand, Wnt signaling promotes DC maturation and activation, and promoting the trafficking of DC and T cells to tumor tissue(32).

TMB was regarded as a predictive biomarker of immunotherapy. We also evaluated the TMB in the current study and found that patients with high TMB showed better survival. There was no significant difference on TMB between high and low ICI score group patients. Besides, there was no correlation between TMB and ICI score. ICI score and TMB represented different aspects of tumor immunity and ICI score was a prognostic biomarker independent of TMB in colon cancer.

Conclusions

In conclusion, we analyzed the immune cell infiltration landscape and provided a sight on the anti-tumor response regulation in colon cancer. And we constructed an ICI score model that might serve as a potential prognostic biomarker independent of TMB. However, it should be noted that the ICI score model was constructed and validated in colon cancer patients lacking information about their treatment. For patients who received specific treatment, such as surgery or immunotherapy, the predictive value of this ICI score system needs further validation.

Declarations

Availability of data and materials

The datasets analysed during the current study are available in the TCGA database (https://portal.gdc.cancer.gov/).

Competing interests

The authors declare that they have no competing interests

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Author Contributions

XT, ML, XL, MZ, SH and XP had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. XT, ML, XL, MZ, SH and XP designed the study. Acquisition of data: XT, ML and XL. Analysis and interpretation of data: XT, SH and XP. Statistical analysis: XT, SH and XP. Drafting of the manuscript: XT, SH and XP.

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Figures

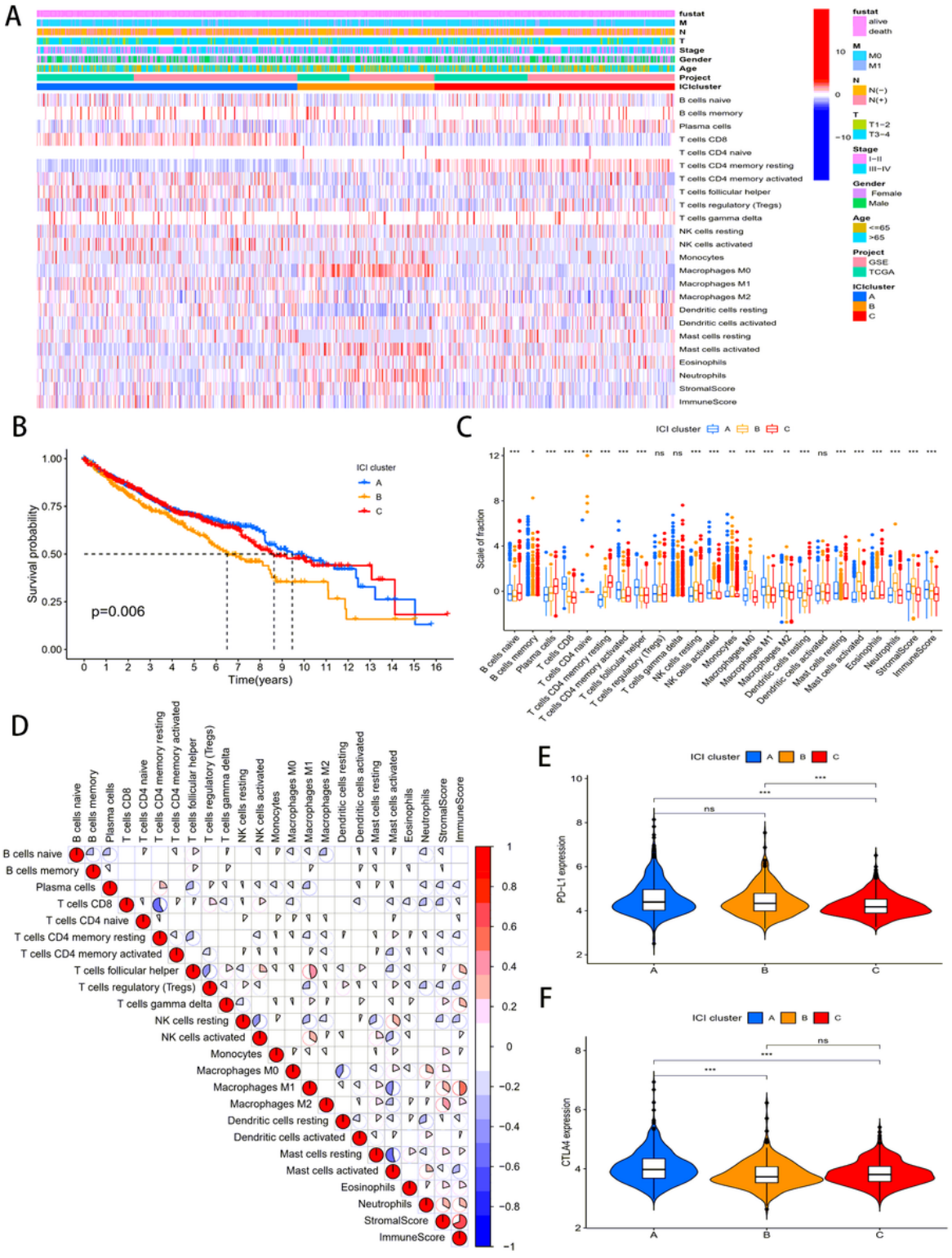

Figure 1

Immune cell infiltration in tumor environment of colon cancer patients. (A) Colon cancer patients were classified into three clusters according to immune cell infiltration in tumor micro environment. Rows represent immune cells infiltrated in tumor microenvironment, and columns represent samples. (B) Overall survival of colon patients in the three ICI clusters. (Log rank test, p = 0.006). (C) Cellular interaction of the tumor-infiltrating immune cell types. (D) The proportion of immune cells in three ICI

clusters. The immune score and stromal score of three ICI clusters were also plotted. (Kruskal-Wallis test. *p < 0.05; **p < 0.01; ***p < 0.001). (E) PD-L1 expression and (F) CTLA4 expression in the three ICI clusters (Kruskal-Wallis test, ***p < 0.001).

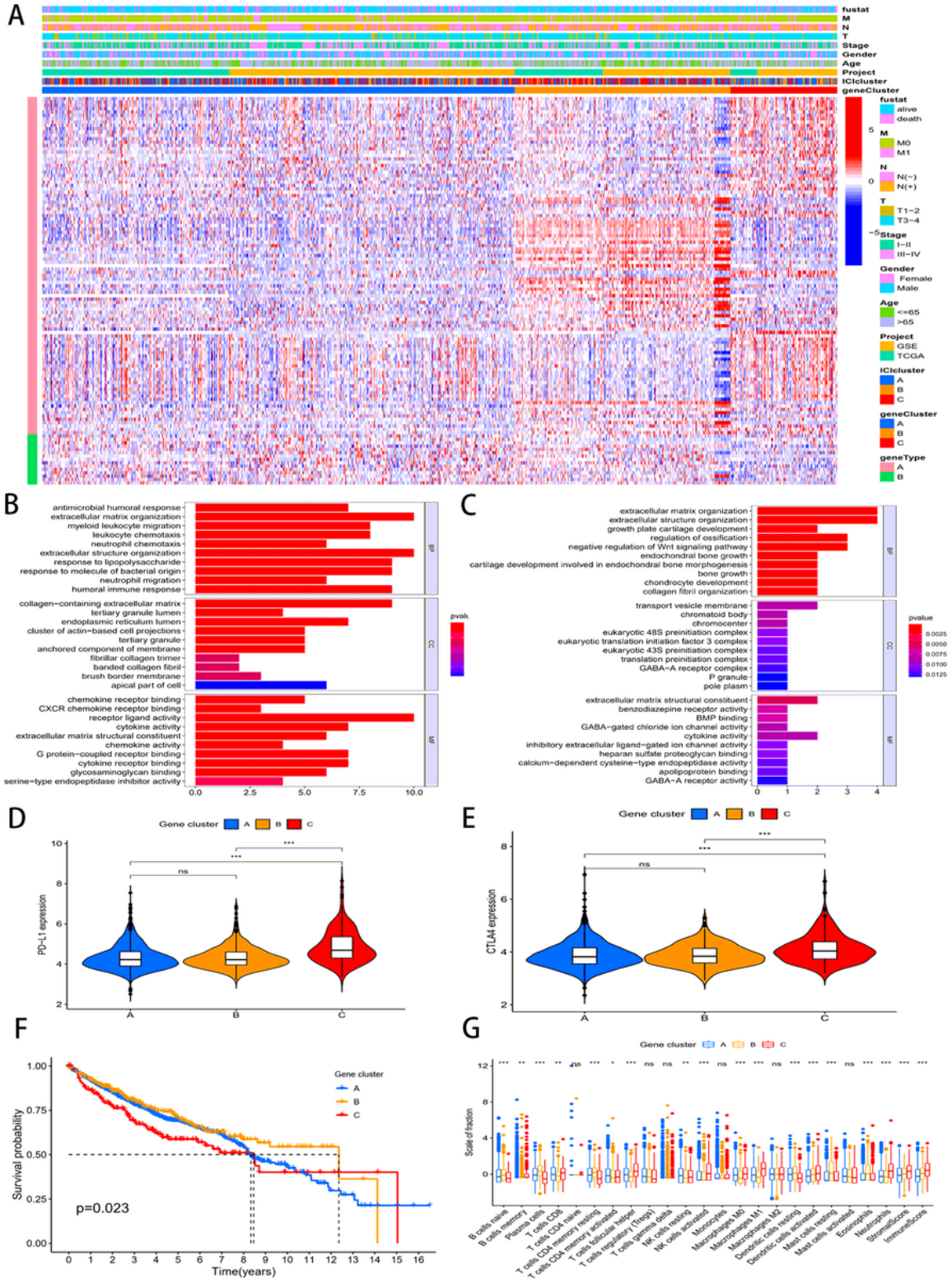

Figure 2

Clustering of immunogenic gene (A) An unsupervised clustering of the differentially expressed genes was used to classified colon patients into three gene clusters: gene cluster A, gene cluster B and gene cluster

C. (B and C) Enriched biological processes in ICI signature A (B) and signature B (C). (D) PD-L1 and (E) CTLA4 expression in the three gene clusters (Kruskal-Wallis test, ***p < 0.001). (F) Overall survival for patients in the three gene clusters (The log rank test, p = 0.023). (G) The proportion of immune cells in three gene clusters. The immune score and stromal score of three ICI clusters were also plotted. (Kruskal-Wallis test. *p < 0.05; **p < 0.01; ***p < 0.001).

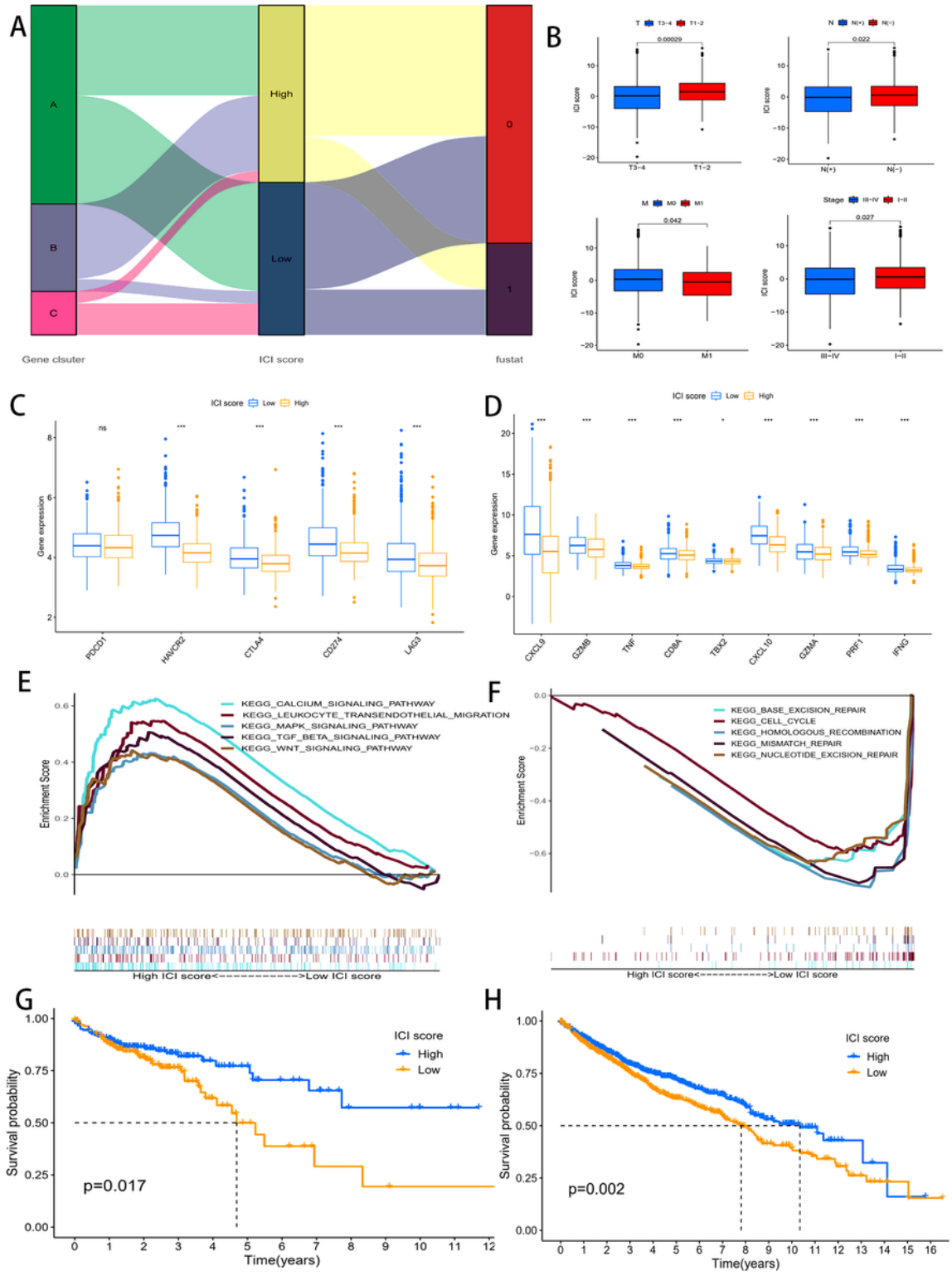

Figure 3

Construction of immune cell infiltration score model (A) Alluvial diagram of patient distribution. (B) ICI scores of patients in different subgroups (Kruskal-Wallis test, *p < 0.05; **p < 0.01; ***p < 0.001). (C) Immune-checkpoint associated genes (PDCD1, HAVCR2, CTLA4, CD274 and LAG3) and (D) Immune-activation associated genes (CXCL9, GZMB, TNF, CD8A, TBX2, CXCL10, GZMA, PRF1 and IFNG) expressed in ICI high and low groups (Kruskal-Wallis test, *p < 0.05; **p < 0.01; ***p < 0.001). (C) GSEA showed that calcium signaling pathway, leukocyte transendothelial migration pathway, MAPK signaling pathway, TGF β pathway, and WNT signaling pathway were enriched in high ICI score group. (D) Base excision repair pathway, cell cycle pathway, homologous recombination pathway, mismatch repair pathway and nucleotide excision repair pathway were enriched in low ICI score group. (E) Overall survival of colon patients in ICI high and low groups in the TCGA cohort (Log rank test, p = 0.017) (F) Overall survival of colon patients in ICI high and low groups in the total patient cohort (Log rank test, p = 0.002).

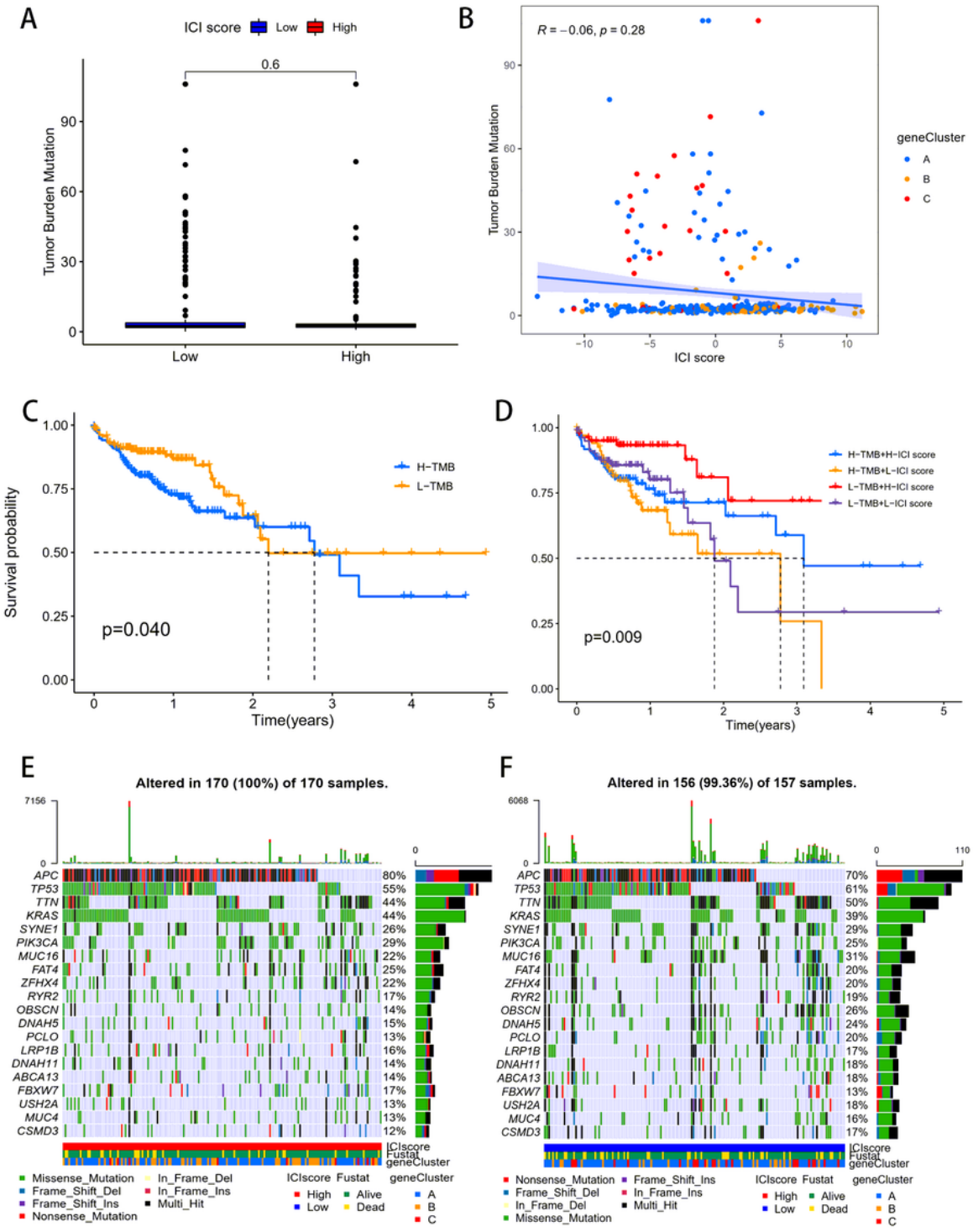

Figure 4

The relationship between ICI score and somatic mutation (A) There was no significant difference on TMB between the two group patients. (B) Correlation analysis revealed that ICI score was not correlated with TMB. (C) Overall Survival of colon patients with high and low TMB (Log rank test, p = 0.040). (D) Overall Survival for colon patients stratified by ICI score and TMB (Log rank test, p = 0.009). (E and F) The top

twenty genes with the highest mutation frequency in ICI high (E) and low (F) group. Rows represent genes and columns represent samples.

Supplementary Files

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