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Integrated analysis of tumor mutation burden and immune infiltrates in endometrial cancer

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ABSTRACT

To explore the prognostic value of tumor mutation burden (TMB) and its correlation with immune infiltrates in endometrial cancer. Transcriptome and somatic mutation profiles of Uterine Corpus Endometrial Carcinoma (UCEC) were downloaded from TCGA database. Somatic mutations were analyzed by "maftools" and visualized in waterfall plot. We calculated TMB of each patients and divided all patients into the high-TMB group and the low-TMB group by the median threshold. Survival analysis and Wilcoxon test were used to investigate the prognostic value of TMB and its association with clinical variables. Differentially expressed genes (DEGs) were identified in 2 TMN groups and functional analysis was performed to find out significant biological pathways. A TMB-related signature was conducted by multivariate analysis, receiver operating characteristic (ROC) curve was performed to predict accuracy of the model, meanwhile, a validation cohort from Fudan University Shanghai Cancer Center (FUSCC) was obtained to verify the signature. Then we estimated association between TMB and immune infiltrates by CIBERSORT algorithm and figured out prognostic immune cells of UCEC in TIMER database. Total 575 samples including 25 normal tissues and 552 tumor samples were enrolled from TCGA database. PTEN mutations accounted for the most and single nucleotide polymorphism and C>T transitions were most frequent forms of somatic mutations in UCEC. The low-TMB group possessed worse survival than the high-TMB group ($P = 0.004$). DEGs in 2 TMB groups were mostly enriched in adaptive immune response and immunoglobulin/immune receptor component. A TMB-related signature consisting of GFAP, EDN3, CXCR3, PLXNA4, SST presented good predictability

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with area under the curve (AUC) = 0.686. In FUSCC validation cohort, the high-risk group possessed worse survival outcome than the low-risk group ($P = 0.015$). Immune infiltrates was correlated to survival in UCEC and low TMB were associated with less immune infiltrates, which suggested poor immune response. TMB was not only related to overall survival but also immune infiltrates in UCEC. The TMB-related signature (GFAP, EDN3, CXCR3, PLXNA4, SST) had good predictability for overall survival in endometrial cancer. Our study might have some merits in elucidating potential mechanism of TMB and immune infiltrates in UCEC and providing guidance of immunotherapy for endometrial cancer.

ARTICLE INFO

Keywords: TCGA; Tumor mutation burden; Immune infiltrate; Endometrial cancer; Survival

Introduction

Endometrial cancer was the leading gynecological malignancy in the United States and ranked fourth in women cancers worldwide.[1,2] Cancer statistics from the American Cancer Society revealed that there were estimated 61,880 new cases and 12,160 deaths of uterine corpus carcinoma in 2019.[1] The age-standardized annual incidence rate and mortality rate of endometrial cancer was 8.4% and 1.8%, respectively.[3] Majority of patients were diagnosed in early stage and possessed a favorable outcome with 5-year survival rate of 95%, however, patients in advanced stage had a decreased 5-year survival rate of 68% and 17% for stage III and stage IV, respectively.[4] Endometrial cancer was generally classified into 2 histological types: Type I as estrogen-dependent endometrioid adenocarcinomas (EAC) and type II as estrogen-independent serous adenocarcinomas (SAC).[4] Radical surgery was initial treatment for early-stage patients, but patients with advanced-stage/distant diseases could not be cured by current treatment strategies.[5] Novel treatment strategies for recurrent/metastatic patients were needed exploration.

Immunotherapy, including checkpoint inhibitors, adoptive cellular transfer, vaccines, was already an available option in various human cancers, most notably in melanoma, nonsmall cell lung cancer and renal malignancies.[6-9] Antiprogrammed death-1 (PD-1) or antiprogrammed death-ligand-1 (PD-L1) had greatly implemented therapeutic advancements in recurrent/metastatic human cancers.[10,11] As for endometrial cancer, the cancer genome atlas (TCGA) group had divided it into 4 subgroups by its molecular features as “POLE-ultramutated”, “microsatellite instability (MSI)-hypermutated”, “copy-number low” and “copy-number high”.[12] The first 2 groups were characterized by high neoantigen loads and number of tumor infiltrating lymphocytes, which was counterbalanced by overexpression of PD-1 and PD-L1.[13] Pembrolizumab (anti–PD-1 monoclonal antibody), approval by Food and Drug Administration (FDA), was usually recommended to MSI tumor types, unfortunately, the majority of endometrioid (72%) and serous (98%) endometrial cancers belonged to the copy-number low or copy-number high groups, which lacked evidence of MSI.[2]

Tumor mutation burden (TMB), a novel biomarker for predicting immunotherapy effect, was calculated as (total count of variants)/(the whole length of exons), where gene variants were defined as base substitutions, insertions, or deletions across bases.[14] High TMB might predict favorable outcome to PD-1/PD-L1 blockade in diverse tumors.[11] Thomas et al.[15] discovered that TMB was a determinant of immune-mediated survival of breast cancer patients. Goodman et al.[16] revealed that high-TMB tumors with microsatellite stable (MSS) were correlated to better response to pembrolizumab with longer progression-free survival than low/intermediate TMB tumors. Based on previous findings, we guessed whether TMB played an essential role in estimating the response to immunotherapy for endometrial cancers despite of microsatellite stability.

In previous researches, TMB was largely calculated by whole-exome sequencing, which was expensive to widely implemented in single institutions. Given this dilemma, in this study, we would like to acquire large-scale sequencing data from available public database and explore the prognostic value of TMB and its correlation with tumor immune infiltrate in endometrial cancer.

Material and methods

Transcriptome data and somatic mutation profiles acquisition

First, we downloaded gene expression information and transcriptome data of 575 samples including 25 normal samples and 552 uterine corpus endometrial carcinoma (UCEC) samples from TCGA database UCEC project (https://portal.gdc.cancer.gov/). Second, we obtained somatic mutation profiles of all tumor samples from "Masked Somatic Mutation" category in TCGA database, which included four types of mutation data based on diverse processing software and we selected "MuTect2 Variant" process with 530 samples for further mutation analysis. Then R software "maftools" package was used to perform various mutation analysis and provide visualization process of mutation analysis results. Third, we acquired clinical information on age at diagnosis, race, ethnicity, menopause status, histological type, tumor grade, percent of tumor invasion, peritoneal cytology, clinical stage, survival time and survival status from 545 patients in TCGA dataset via GDC portal (https://portal.gdc.cancer.gov/).

Estimation of TMB for each samples and survival analysis

TMB was defined as the total number of somatic gene coding errors, base substitutions, and gene insertions or deletions detected across per million bases. In this study, we calculated TMB of each patients by the formula: TMB = (total count of variants)/(the whole length of exons) via Perl script (version:5.30.2) (https://www.perl.org/), then we divided all patients into 2 groups: The low-TMB group and the high-TMB group according to the median TMB count. Kaplan-Meier survival analysis was performed between the 2 groups. We analyzed the association between TMB and clinical variables by Wilcoxon ranked-sum test for 2 groups or Kruskal-Wallis test for more than 2 groups.

Differentially expressed genes (DEGs) in two TMB groups and functional analysis

We screened for DEGs between the low-TMB group and the high-TMB group by R software "limma" package with P value <0.05 and $|\text{Log FC}| \geq 1$. Top 20 significant DEGs were presented in heatmap by R "pheatmap" package. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis were implemented to investigate significant functional biological pathways for these DEGs with false discovery rate (FDR) <0.05. Gene Set Enrichment Analysis (GSEA) enrichment analysis was performed to find out top 50 significant pathways with FDR <0.05 in these 2 groups.

Construction of TMB-related signature in TCGA dataset

We obtained 2498 immune genes from IMMPORT website (https://www.immport.org/) and found 1811 immune genes were prognostic for overall survival with $P < 0.05$ by Kaplan-Meier survival analysis. Then we selected out 123 intersect genes between TMB-related DEGs with $|\text{Log FC}| \geq 1$ and 1811 significant immune genes. Univariate and multivariate analysis were performed to screen for 123 possible prognostic TMB-related immune genes and find out independent ones. Moreover, we constructed a prognostic signature according to a linear combination

of gene expression values multiplied by a regression coefficient (β) accessed from the multivariate Cox proportional hazards regression model of each gene. The formula was as follows: risk score = expression of $gene_1 \times \beta_1 gene_1$ + expression of $gene_2 \times \beta_2 gene_2 + \ldots$ expression of $gene_n \times \beta_n gene_n$. All patients were classified into 2 groups: the high-risk group and the low-risk group by the median risk score. Receiver operating characteristic (ROC) curve and survival analysis were performed to assess the predictive accuracy and prognostic value of the signature.

External validation of the signature in FUSCC set

For validation, we obtained 30 patients with endometrial cancer who underwent radical resection from Fudan University Shanghai Cancer Center (FUSCC) between January 2015 and May 2015. Total 30 patients were fully informed consent and clinical characteristics of them were summarized in supplementary table S1. RNAs were extracted from 30 samples using TRIzol Reagent (cat. no. 15596026; Thermo Fisher Scientific, Inc.). RNAs were converted to cDNAs by reverse transcription using PrimeScript RT Master Mix kit (cat. no. RR036A, Takara Biotechnology Co., Ltd.) and then quantitative polymerase chain reaction (PCR) was performed by TB Green Premix Ex Taq II (Tli RNaseH Plus kit, cat. no. RR820A, Takara Biotechnology Co., Ltd.). We measured the concentration of PCR production to calculate relative gene mRNA expression level. GAPDH mRNA level was used as internal reference. Primers for 5 genes were listed in supplementary table S2. We calculated risk scores of each patients according to the signature, then we divided total 30 patients into the low-risk group and the high-risk group according to the median risk-scores. Kaplan-Meier survival analysis and Log-rank test were used in these 2 groups to compare survival.

Immune infiltrates in UCEC

We calculated relative percent of 22 immune cells in each UCEC samples from TCGA database by CIBERSORT algorithm which included gene expression of 22 leukocyte subtypes. Then we compared 22 immune cells infiltrates level between the low-TMB group and the high-TMB group by Wilcoxon ranked-sum test. We estimated the prognostic value of immune cells for overall survival in UCEC via TIMER database (https://cistrome.shinyapps.io/timer/). Kaplan-Meier survival analysis by Log-rank test and multivariate cox regression model were constructed to identify independent immune cells for overall survival. Moreover, we investigated association between copy number alterations of 5 genes in the signature and immune infiltrates level via TIMER database.

Statistical analysis

Descriptive analysis was used to summarize the demographics and clinicopathological characteristics of patients with UCEC in TCGA cohort and in FUSCC validation set. All statistical analyses were performed by R software (version 4.0.0). Kaplan-Meier and Log-rank test were used for survival analysis. The cut-off value of continuous variables such as age at diagnosis, TMB and risk scores were determined by their median values. Statistical significance was set by $P < 0.05$.

Results

Landscape of genome-wide mutation files in UCEC

We obtained somatic mutation profiles of 530 patients with UCEC from “MuTect2” process in TCGA database. Around 525 (99.06%) samples possessed somatic mutations, and mutation

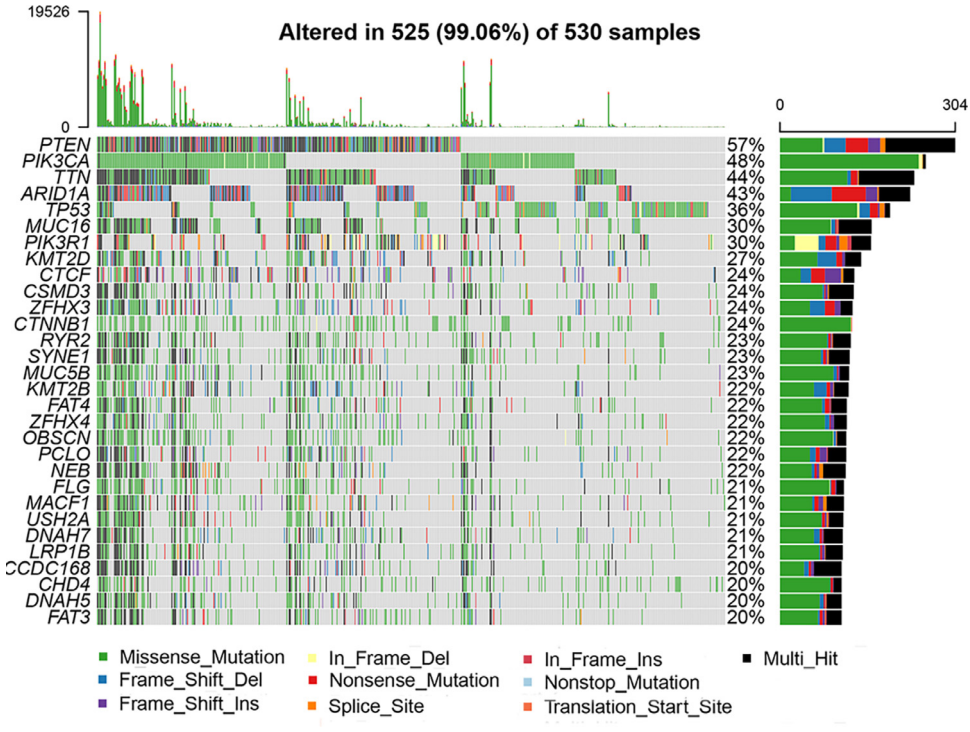

Fig. 1. Landscape profile of somatic gene mutations in 530 UCECs from TCGA database. Mutations of each genes in each samples were shown in waterfall plot. Each column presented each samples. Name of mutated genes was listed in the left, different forms of somatic mutation types were in different colors shown in the bottom and percent of gene mutation was shown in the right. (Color version of figure is available online.)

information of each gene in all samples were shown in waterfall plot (Fig. 1). As for top 10 mutated genes shown in Fig. 1 and Fig. 2F, we discovered that gene PTEN mutated most frequently approximately accounting for 57%, followed by PIK3CA (48%), TTN (44%), ARID1A (43%), TP53 (36%), MUC16 (30%), PIK3R1 (30%), KMT2D (27%), CTCF (24%) and CSMD3 (24%). Gene variants were usually classified into 9 types shown in Fig. 2A, among these alterations, missense mutation was the most frequent form. Single nucleotide polymorphism (SNP) was the more frequent variant type than insertion or deletion (Fig. 2B) and C>T alterations accounted mostly than other types of SNP (Fig. 2C). Then we calculated number of variant of each samples (median number: 41) and exhibited variant types with different colors in boxplot (Fig. 2D and 2E). Co-occurrence or mutually-exclusive expression of mutated genes was shown in Fig. 2G.

Demographics and clinicopathological characteristics of 545 patients from TCGA database were listed in Table 1. The median age at diagnosis was 64 years ranging from 31 years to 90 years. Around 319 (71.74%) patients were diagnosed in stage I-II and 154 (28.26%) patients were in stage III-IV.

TMB associated with survival and clinical variables

We calculated TMB of each patients and divided total patients into the low-TMB group and the high-TMB group by the median TMB threshold, and we discovered that the low-TMB group possessed worse survival than the high-TMB group ($P = 0.004$). Then we investigated the association between TMB and clinical variables, and we found that patients with low TMB were prone

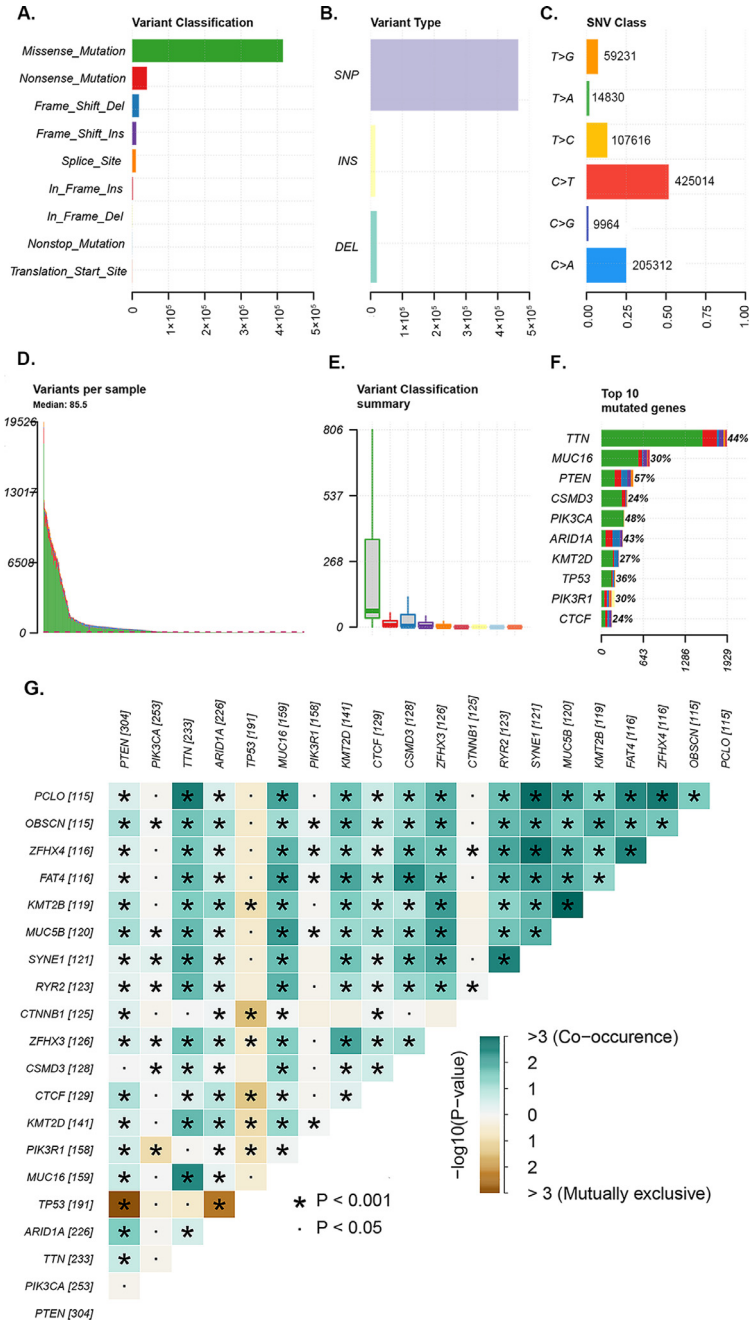

Fig. 2. Summary of mutation profiles in UCEC. (A) Nine common variant mutation forms in all samples. Missense mutation accounted for the most compared with other types of variants. (B) Three variant types in all samples. SNP was more frequent than deletion or insertion. (C) Six subclasses of single nucleotide polymorphism (SNP) in all samples. C>T transition was the most frequent. (D-E) Summary of variant classification in all samples. (F) Top 10 mutated genes in all samples. (G) Co-occurrence or mutually exclusive interaction of mutated genes. (Color version of figure is available online.)

Table 1
Demographics and clinicopathological characteristics of 545 patients with UCEC from TCGA database

Clinical variables	Number	%
Age at diagnosis	median: 64y (31y-90y)	
Race		
African	109	20.00%
Caucasian	373	68.44%
Other	63	11.56%
Ethnicity		
Hispanic/Latino	15	2.75%
Not Hispanic /Latino	376	68.99%
Unknown	154	28.26%
Menopause status		
Pre	35	6.42%
Post	462	84.77%
Unknown	48	8.81%
Tumor grade		
Grade 1-2	221	40.55%
Grade 3-4	324	59.45%
Histological type		
EAC	402	73.76%
SAC	135	24.77%
Other	8	1.47%
Percent of tumor invasion		
<50%	263	48.26%
>=50%	210	38.53%
Unknown	72	13.21%
Peritoneal cytology		
Negative	352	64.59%
Positive	58	10.64%
Unknown	135	24.77%
Clinical stage		
Stage I-II	391	71.74%
Stage III-IV	154	28.26%

EAC, endometrioid adenocarcinomas; SAC, serous adenocarcinoma.

to possessing positive peritoneal cytology ($P = 0.034$) and endometrioid adenocarcinoma ($P < 0.001$). Moreover, we discovered that the low-TMB group tended to have worse tumor differentiation with higher grade ($P = 0.074$) and more advanced stage ($P = 0.062$) but without statistical significance (Fig. 3).

DEGs in two TMB groups and functional analysis

We selected DEGs between the low-TMB group and the high-TMB group with |Log FC|≥1 and FDR < 0.05. Finally, 516 DEGs were identified and top 20 genes of them were plotted in heatmap (Fig. 4A). As for functional analysis in Fig. 4B, we found that most DEGs were enriched in immune response of biological process (BP), immunoglobulin component/immune receptor of cellular component (CC), and antigen/immunoglobulin receptor binding of molecular function (MF) by GO analysis. By KEGG pathway analysis, DEGs were mostly enriched in cell adhesion molecules and cytokine-cytokine receptor interaction. GSEA analysis revealed that DEGs could participate in killer cell mediated cytotoxicity, T cell receptor signaling pathway and P53 signaling pathway both with $P < 0.05$ (Fig. 4C).

Construction and external validation of TMB-related signature

We selected 123 intersect genes between 1811 prognostic immune genes and DEGs of 2 TMB groups with |Log FC|≥ 1 shown in Supplementary Fig. 1. Fourteen genes were verified to be

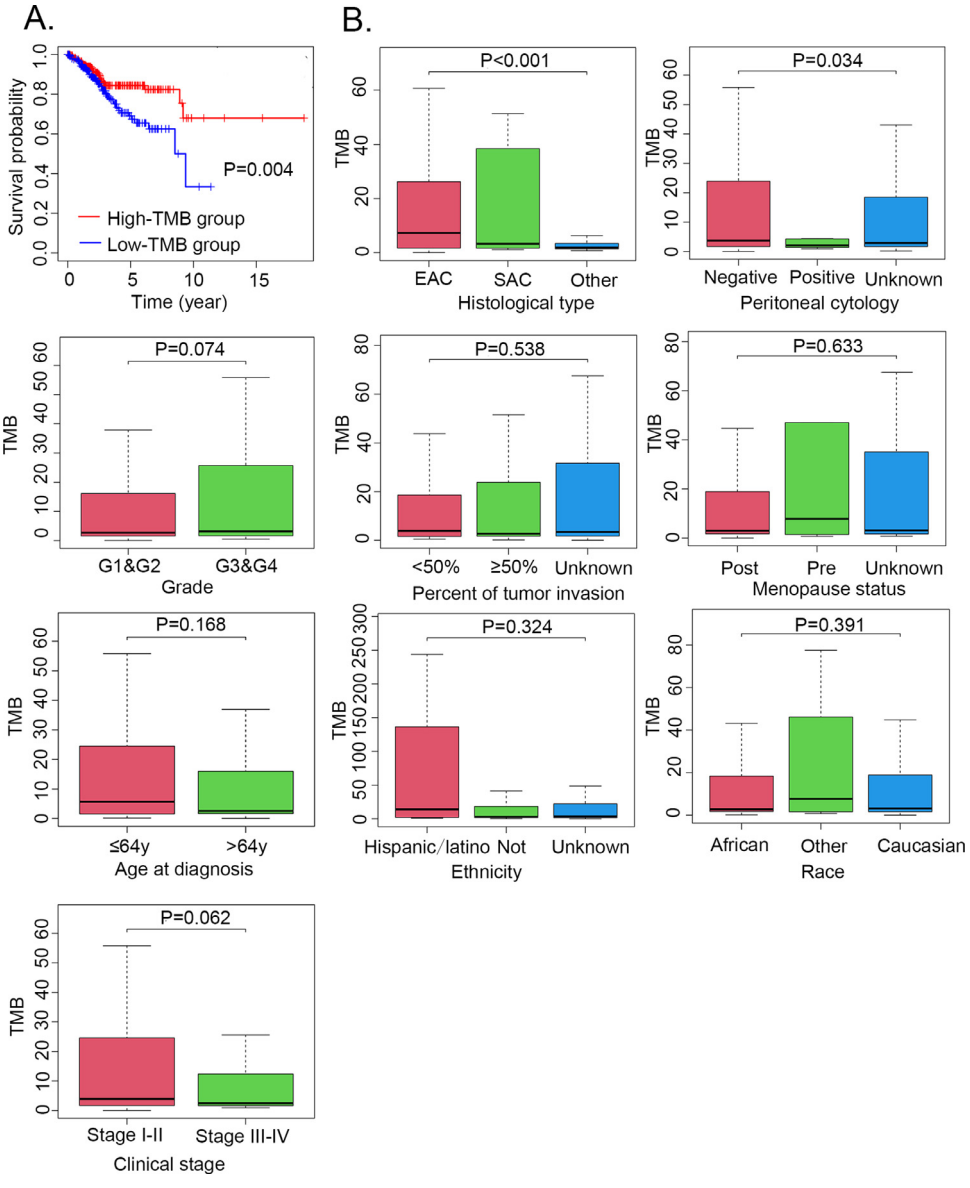

Fig. 3. Association between TMB and survival outcome (A) and clinical variables (B). Clinical variables included histological type, peritoneal cytology, grade, percent of tumor invasion, menopause status, age at diagnosis, ethnicity, race, and clinical stage. (Color version of figure is available online.)

prognostic for overall survival in UCEC by univariate analysis (in Supplementary table S2) and a signature including 5 genes (GFAP, EDN3, CXCR3, PLXNA4, SST) of these 14 genes was conducted by multivariate analysis. Shown in Table 2, each gene had their coefficients to survival and risk scores could be calculated by the following criteria: Risk scores = GFAP * (0.22) + EDN3 * (-0.14) + CXCR3 * (-0.30) + PLXNA4 * (0.25) + SST * (0.07). Each patients had their own risk scores according to the signature (Fig. 5A and Fig. 5B), and all patients could be divided into the high-risk group and the low-risk group by the median risk scores, and we discovered that

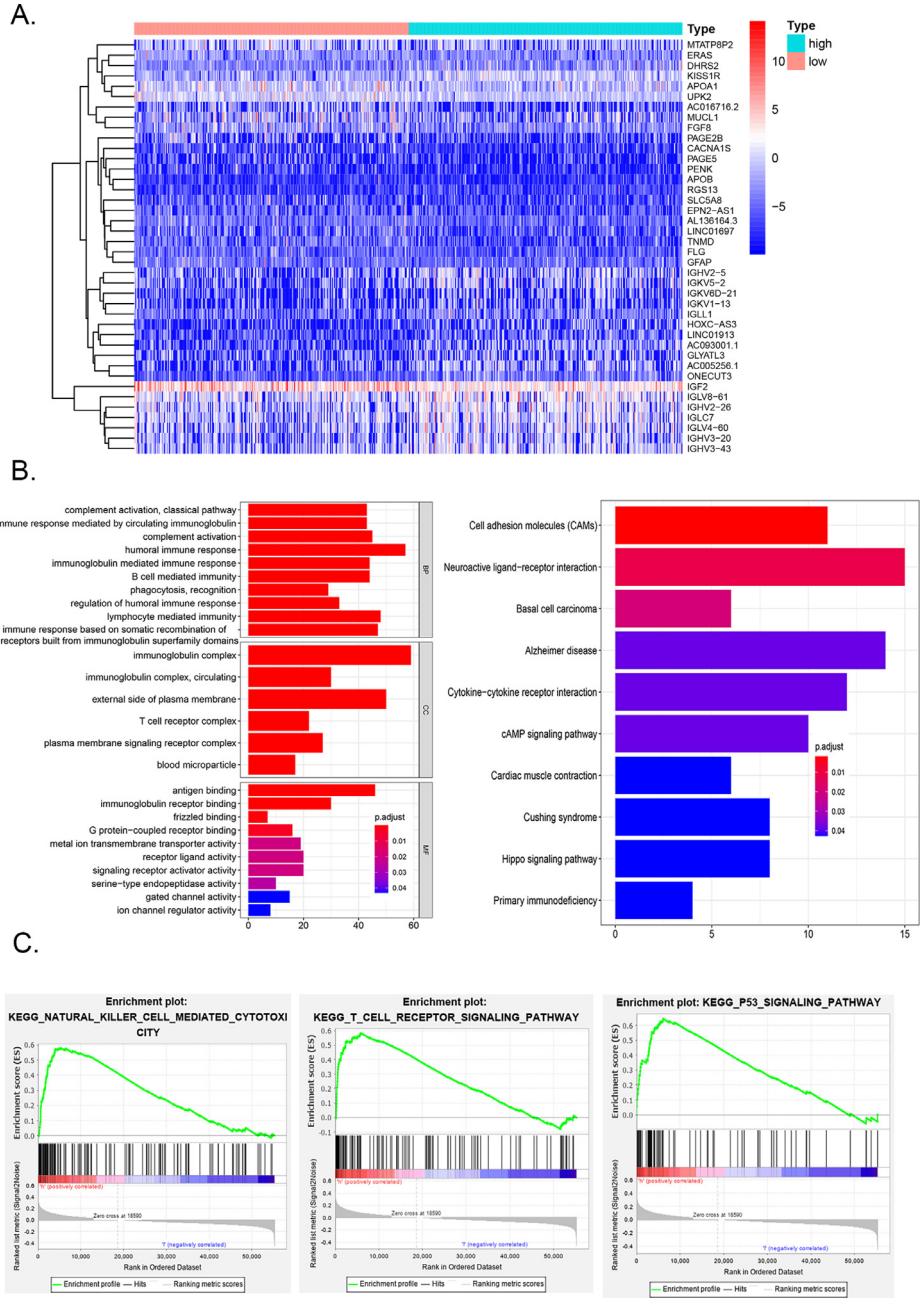

Fig. 4. Functional analysis of DEGs in 2 TMB groups. (A) Heatmap of top 20 DEGs in 2 TMB groups. Each column represented each sample. The colors in the heatmaps from blue to red represent expression level from low to high. The red dots in the volcano plots represent up-regulation, the blue dots represent down-regulation and white dots represent genes without differential expression. (B) GO (left) and KEGG (right) functional analysis for DEGs. BP, biological process; CC, cellular component; MF, molecular function. (C) GSEA enrichment analysis for DEGs. (Color version of figure is available online.)

Table 2
Construction of a TMB-related signature in UCEC

Gene name	Coefficients	HR (95%CI)	*P* value
GFAP	0.22	1.25 (1.01-1.54)	0.040
EDN3	-0.14	0.87 (0.76-0.99)	0.042
CXCR3	-0.30	0.74 (0.58-0.94)	0.014
PLXNA4	0.25	1.29 (1.05-1.58)	0.015
SST	0.07	1.08 (1.01-1.15)	0.035

HR, hazard ratio; CI, confidence interval.

the high-risk group possessed worse survival than the low-risk group ($P < 0.001$) (Fig. 5B). ROC analysis manifested good predictivity of the signature with 0.686 of area under the curve (AUC) (Fig. 5C). For validation, we obtained 30 patients with endometrial cancer from FUSCC and detected mRNA expression of 5 genes. As the same, we calculated risk scores of 30 patients and discovered that patients in high risk associated with worse prognosis than those in low risk ($P = 0.015$) (Fig. 5D).

Immune infiltrates in UCEC

To investigate the association between TMB and immune infiltrates in endometrial cancer, we calculated percent of 22 leukocyte cells of all samples in UCEC by CIBERSORT algorithm (Fig. 6A) and then compared immune cell fractions in 2 TMB groups (Fig. 6B). We discovered that low-TMB group had more rested memory CD4 T cells ($P = 0.016$), less activated CD4 T cells ($P < 0.001$), less helper T cell ($P < 0.001$), less macrophages 1 ($P < 0.001$), and less activated dendritic cells ($P = 0.001$). It manifested that patients with low-TMB possessed less immune cell infiltrates than the high-TMB group, which might predict poor adaptive immune response and worse survival.

Furthermore, we investigated prognostic immune cells in UCEC in TIMER database and we discovered that low percent of B cells and CD8 T cells was significantly related to poor survival in UCEC with $P < 0.05$ (Fig. 6C). Meanwhile, we discovered that copy number alternations of 5 genes in the signature were associated with immune infiltrates level in UCEC (Supplementary Figure 2).

Discussions

In recent years, promising clinical trials of immunotherapy (anti-PD-1/anti-PD-L1) had been initiated in recurrent/metastatic endometrial cancer. A study conducted by Howitt et al.,[13] revealed that POLE-mutated or MSI endometrial cancers presented large number of tumor infiltrate immune cells and tumor neoantigen, which suggested good response to immunotherapy. A phase 2 clinical trial of pembrolizumab administration in fifteen mismatch repair (MMR) protein-deficient endometrial cancers presented good outcomes with 3 cases complete response and 5 cases partial response.[17-19] However, in a KEYNOTE-28 study (pembrolizumab) of endometrial cancer, one patient with microsatellite stability (MSS) responded and one patient with MSI had progressive diseases.[20] Therefore, based on above findings, we guessed that POLE mutation/MMR-deficiency and MSI could not completely reflect or estimate the effect of immunotherapy in endometrial cancer.

TMB presented a number of gene mutations that existed in tumors. High TMB manifested large number of gene mutations which could promote to produce numerous altered peptides, resulting in neoantigens to which the immune system could generate an antitumor response.[10,14] TMB could be a candidate biomarker for estimating potential response to immunotherapy.[10,11]

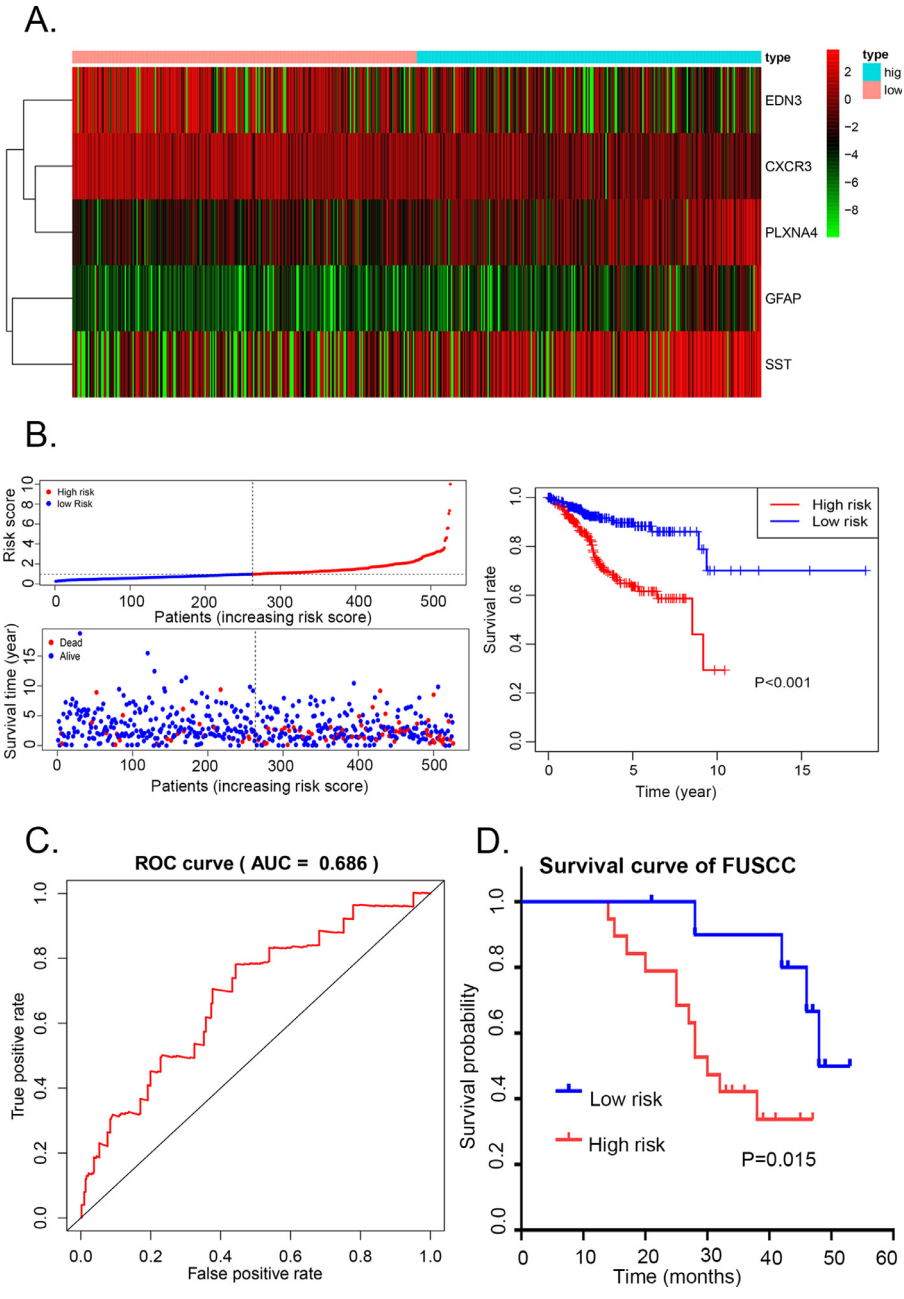

Fig. 5. Construction and validation of a TMB-related signature in UCEC. (A) Heatmap of 5 genes in the signature for each sample. Each column represented each samples. The colors in the heatmaps from green to red represent expression level from low to high. The red dots in the volcano plots represent up-regulation, the green dots represent down-regulation and black dots represent genes without differential expression. (B) Risk-score plot (left) and survival curve for the signature (right). All patients were divided into the high-risk group and the low-risk group by the median risk scores. Survival analysis was performed to compare overall survival of 2 groups. (C) ROC curve for the signature. (D) Survival curve of FUSCC validation cohort. (Color version of figure is available online.)

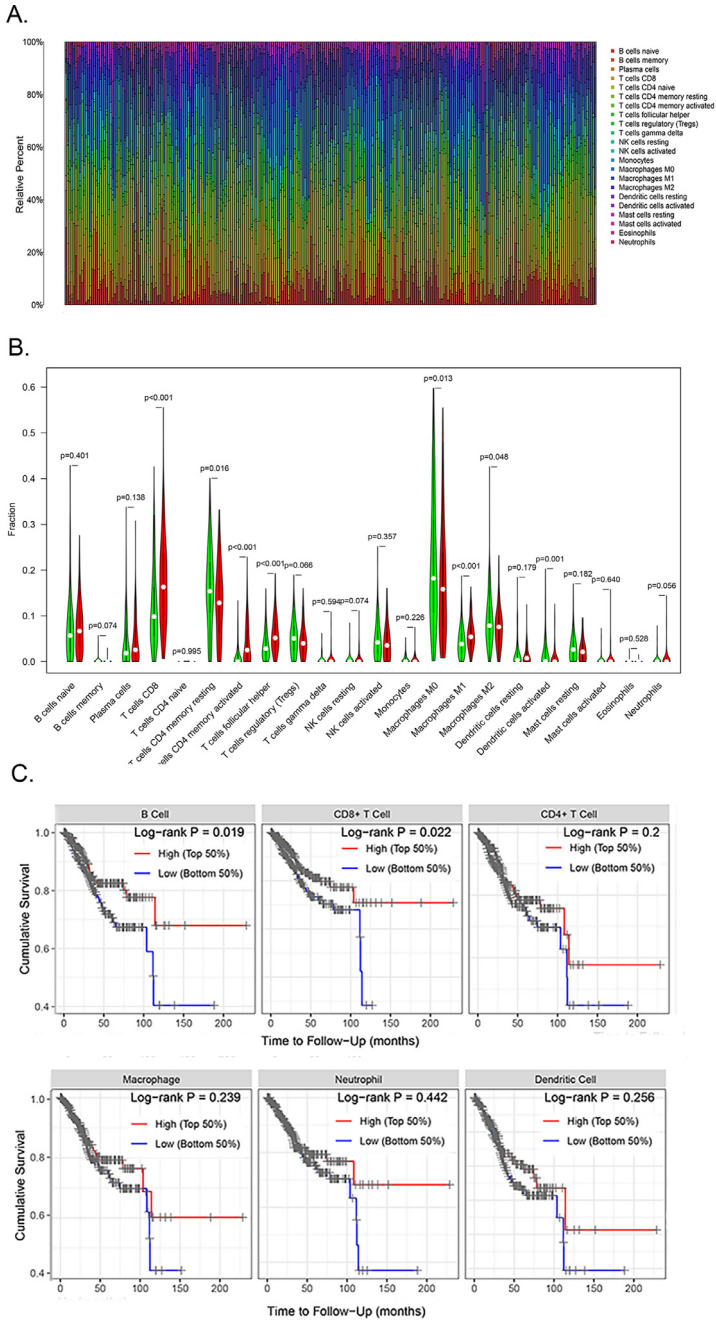

Fig. 6. Immune cells in UCEC. (A) Estimated 22 immune leucocytes fractions in UCEC by CIBERSORT algorithm. Each chart exhibited the cell proportions of each patients and 22 immune leucocytes were in different colors in the right. (B) Immune cell fractions in the low-TMB group (green) and the high-TMB group (red). (C) Survival curve of 6 common immune cells in UCEC from TIMER database. (Color version of figure is available online.)

Budczies et al.[21] found that high TMB was strongly positive correlated with MSI-high or MMR deficiency in UCEC. In the present study, we found patients with low TMB were significantly correlated to worse survival in UCEC ($P = 0.004$), which might be associated with poor immunotherapy response. Consistent to a study conducted by Samstein et al.,[22] they enrolled large number of cancers including 350 nonsmall cell lung cancers, 321 melanomas, 151 renal cell carcinomas (RCC), 214 bladder cancers, and 138 head and neck squamous cell cancers, and they revealed that higher TMB was associated with longer overall survival time after immunotherapy across multiple cancer types. The similar association between higher TMB and improved survival outcomes was also observed in diverse cancers.[11] Moreover, in this study, we found patients with low TMB were prone to possessing higher grade and more advanced stage but it did not reach statistical significance.

Tumor infiltrating lymphocytes (TILs) were verified increasingly important implications of immunotherapy.[23] However, few researches focused on the prognostic value of tumor immune infiltrates in endometrial cancer despite many studies investigated correlation of TILs and microsatellite status.[24,25] In the present study, we revealed that low percent of B cells and CD8 T cells was associated with poor survival in UCEC. We were the first to elucidate the association between TMB and TILs in endometrial cancer. Interestingly, we figured out that low TMB was strongly correlated to poor immune infiltrates with less activated CD4 T cells, CD8 T cells, macrophages, and dendritic cells, that might explain why patients with low-TMB possessed poor immune response and worse survival compared with high-TMB group.

Besides, we explored potential functional pathways of DEGs in 2 TMB groups by KEGG and GO analysis. We discovered that DEGs were mostly enriched in adaptive immune response process and immunoglobulin/immune receptors components. Meanwhile, we firstly established and validated the prognostic value of a TMB-related signature consisting of 5-hub genes (GFAP, EDN3, CXCR3, PLXNA4, SST) in UCEC. We verified that patients with high risk scores possessed worse survival both in TCGA cohort and FUSCC validation set.

Five genes in the signature were rarely reported in endometrial cancer. GFAP was initially discovered in multiple sclerosis and mostly studied in brain tumors.[26] Higher GFAP expression reflected an increase of tumor grade in astrocytoma.[27] EDN3 was a frequent target of epigenetic inactivation in human breast cancer and attenuated EDN3 expression was associated with adverse outcome in breast cancer.[28] Anti-CXCR3 could inhibit tumor growth of colon cancer and tumor metastasis of lung cancer and breast cancer.[29-31] Silenced PLXN4 could inhibit tumor proliferation and affect cytoskeletal organization in glioblastoma cells.[32] SST could inhibit vascular endothelial growth factor and metalloprotease-2 mRNA expression to attenuate endometrial stromal cells proliferation.[33] These 5 biomarkers in the signature might be novel targets for endometrial cancer.

Conclusions

Our group was the first to investigate the association between TMB and TILs in endometrial cancer based on large-scale mutation data in public database. In the present findings, we innovatively elaborated that TMB was not only associated with survival but also with TILs in UCEC. Furthermore, we constructed and validated a TMB-related prognostic signature, which presented good predictability for overall survival in UCEC. Our research might have some merits in elucidating potential mechanism of TMB and TILs in endometrial cancer and providing evidence on high TMB as an indicator for immunotherapy in endometrial cancer.

Author contributions

Xi Cheng, Haoran Li, and Hongyu Zhou contributed to the conception of the study. Hongyu Zhou and Lihua Chen performed main experiment and wrote the main part of the manuscript.

Yajie Lei and Tianjiao Li helped collect data and performed statistics analysis. All authors approved the final manuscript.

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Declaration of competing interest

All authors declared that there was no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2020.100660.

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