Biological Omics Analysis of Tumor Mutation Burden Combined with Immune Infiltrates in Uterine Corpus Endometrial Carcinoma

Yuan Zhuang  
Department of Radiation Oncology, The First Affiliated Hospital of China Medical University, 155 North Nanjing Street, Heping District, Shenyang, 110001, Liaoning, China

Chang Liu  
Department of Radiation Oncology, The First Affiliated Hospital of China Medical University, 155 North Nanjing Street, Heping District, Shenyang, 110001, Liaoning, China

Jin Huang  
Department of Radiation Oncology, The First Affiliated Hospital of China Medical University, 155 North Nanjing Street, Heping District, Shenyang, 110001, Liaoning, China

Guang Li (✉️ zyannis0701@163.com)  
China Medical University Hospital

Research

Keywords: Tumor mutation burden (TMB), uterine corpus endometrial carcinoma (UCEC), immune infiltration, survival

DOI: https://doi.org/10.21203/rs.3.rs-37252/v1

License: ☕️ 🛑 This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: In various malignancies, whether tumor mutation burden (TMB) is associated with improved survival outcomes or enhanced immunotherapy remains controversial. We are committed to exploring the prognosis of TMB and its potential association with immune infiltration of uterine corpus endometrial cancer (UCEC).

Methods: We downloaded transcriptome data and somatic mutation data from the Cancer Genome Atlas (TCGA) database, and visualized the mutation profiles using the "maftools" package. TMB was calculated and the samples were divided into two groups by the median. Kaplan-Meier analysis and Wilcoxon test were used to compare the differences in survival and clinicopathological characteristics. Furthermore, we performed functional enrichment analysis to screen for significant contributing pathways. The "CIBERSORT" package was used to estimate the abundance of immune components. Differential analysis was performed using the "limma" package to compare gene expression profiles. Multivariate analysis was used to identify risk genes related to TMB. Based on these genes, a risk model was established, and the receiver operating characteristic (ROC) curve was drawn to assess the predictive accuracy. Finally, we evaluated the relationship between risk genes and immune infiltration using the Timer database.

Results: The mutation data included 542 UCEC patients. The waterfall plot summarized the specific mutation information. Higher TMB levels indicated improved overall survival (OS) (p =0.048) and were associated with advanced grades. Pathway analysis showed that the differential signals were enriched in multiple immune-related crosstalks. We screened 108 differentially expressed genes in two TMB groups and identified four risk-related genes. The model based on these four risk genes had good predictive value, the area under the curve (AUC) = 0.670, and patients with higher risk scores showed worse OS (p <0.001). Additionally, CD8 T cells memory-activated, CD4 T cells, and M1 macrophages in the high TMB group showed higher infiltrates, while regulatory T cells (Tregs) and M2 macrophages showed lower infiltrates.

Conclusions: Higher TMB was associated with better survival outcomes and increased the immune infiltration in the tumor microenvironment of UCEC.

1. Introduction

Uterine corpus endometrial cancer (UCEC) is a group of epithelial malignancies occurring in the endometrium (the lining of the uterus or womb), most generally in perimenopausal and postmenopausal women(1). UCEC is one of the most common tumors of the female reproductive system, with nearly 200,000 new cases each year. In the past few decades, the incidence and mortality of endometrial cancer have been on the rise. This makes it the third most common cause of death in cancers which only affect women, behind ovarian and cervical cancer(2, 3). According to the pathogenesis and biological behavioral characteristics, UCEC can be divided into estrogen-dependent type (type I) and non-estrogen-dependent type (type II)(4). Approximately 40% of cases are related to obesity(5, 6). Besides, endometrial
cancer is also associated with excessive estrogen exposure, hypertension, and diabetes. The prognosis of UCEC is based largely on histologic grade and clinical stage(7, 8). Although patients with early-stage disease have a 5-year overall survival rate of about 80%, those with advanced-stage have a 5-year overall survival rate of less than 20%(3). As overall survival remains poor in advanced and recurrent cases, the emergence of new targeted therapeutics and immunotherapies for these sub-patients offers hope for improved outcomes(9).

In recent years, there has been a lot of good news about cancer immunotherapy(10). Currently, it has manifested strong anti-tumor capability in the treatment of solid tumors such as melanoma(11), non-small cell lung cancer(12), kidney cancer(13), and prostate cancer(14). Several immunotherapy drugs have been approved for clinical application by the Food and Drug Administration (FDA)(15). Due to its prominent efficacy and innovation, "cancer immunotherapy" was named the most important scientific breakthrough of the year by "science" in 2013. In 2018, American immunologist James P. Allison and Japanese immunologist Tasuku Honjo received the Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation(16). Immunotherapies can be categorized as active, passive, or hybrid (active and passive)(17). Active immunotherapies direct the immune system to attack tumor cells by targeting tumor antigens, while passive immunotherapies enhance existing anti-tumor responses(18). Immunotherapies mainly involve immune checkpoint inhibitors (ICIs), therapeutic antibody, cancer vaccines, immune system modulators, cell therapy, and small synthetic molecule inhibitors. Endometrial cancer cells and tumor microenvironment have been shown to modulate immune responses. The strategies utilizing ICIs monotherapy, ICIs combination regimens, and ICIs combined with other fundamental targeted therapies have become promising treatments to improve the anti-tumor immune response of advanced endometrial cancer(19).

Tumor mutational burden (TMB, the number of mutations within a targeted genetic region in the cancerous cell's DNA) is a promising biomarker for predicting the effect of immunotherapy(20, 21). Goodman AM et al. (2017) indicated that higher TMB is a promising biomarker that predicts favorable outcome to PD-1/PD-L1 blockade in melanoma and non-small cell lung cancer (NSCLC)(22). TMB may be an independent predictor of immunotherapy response. Thomas A et al. (2018) found that TMB swayed immune-related survival outcomes in breast cancer patients(23). In contrast, there was growing evidence that higher TMB in renal cell carcinoma (RCC) was correlated with immune cell exclusion and the immunologically cold phenotypes. Accordingly, higher TMB was supposed to be associated with worse survival in RCC(24, 25). These findings stated that TMB levels may have contradictory predictions in different tumor types.

With the continuous development of bioinformatics, we have the opportunity to obtain helpful resources about genomics and therapeutic responses from various public databases. Zhang C et al. performed a multi-omics analysis of TMB and immune infiltration in bladder urothelial carcinoma using the Cancer Genome Atlas (TCGA) database(26). Given the impressive results achieved by immunotherapies, especially ICIs in the treatment of UCEC, we performed this study to explore the prognostic role of TMB and its potential association with immune infiltration in UCEC.
2. Materials And Methods

2.1 Data Collection

Somatic mutation data were downloaded from the Genomic Data Commons (GDC) Data Portal of TCGA (https://portal.gdc.cancer.gov/). Next, the “Masked Somatic Mutation” data was selected and processed based on the VarScan2 software. We input the downloaded mutation annotation format (MAF) data and implemented the visualization process for genomic analysis by executing the “maftools” R package. We acquired the transcriptome RNA-sequencing data with HTSeq-FPKM type and corresponding clinical follow-up information of UCEC also from the TCGA database by the GDC tool.

2.2 Assessment of TMB and prognostic analysis

TMB was defined as the total number of mutations per coding area of a tumor genome(27). In our study, the TMB was calculated as (total number of variants) / (total length of exons), and the variants included base substitution, deletion, or cross-base insertion. We then operated the Perl script based on the JAVA8 platform to calculate the frequency of the variants of each sample. According to the median as the cutoff value, UCEC patients were divided into the low-TMB group and high-TMB group. The TMB levels were merged with the corresponding survival data of each sample according to the sample ID. The "survival" R package was applied to Kaplan-Meier analysis to compare OS between the two groups. Moreover, the correlation between the TMB levels and other clinicopathological factors was further judged, where Wilcoxon rank-sum test was utilized.

2.3 Differential gene analysis and functional enrichment analysis

First, the transcriptional data of UCEC samples were divided into low and high TMB groups. Differential gene analysis was performed using the “limma” package, with parameters of log fold change (FC)>1 or<-1 and false discovery rate (FDR)<0.05. And we applied the “heatmap” package to visualize the heatmap. Then, functional enrichment analysis was conducted through gene ontology (GO) and “Kyoto Encyclopedia of Genes and Genomes” (KEGG) analysis to explore the potential functions of TMB, using “ggplot2”, “enrichplot”, and “clusterProfiler” packages. Additionally, based on the TMB level as the phenotypes, gene set enrichment analysis (GSEA) was performed, in which “c2. Cp. Kegg. V6.2. Symbols. GMT gene set” was selected as the reference gene set. The way to download GSEA software is http://software.broadinstitute.org/gsea/index.jsp. The gene set was obtained from the MSigDB database (http://software.broadinstitute.org/gsea/msigdb/). Set the false discovery rate (FDR) less than 0.05 as the threshold.

2.4 Analysis of immune cell infiltration
Based on the CIBERSORT analysis tool (https://cibersort.stanford.edu/), we estimated the expression abundance of 22 immune cells. The box plot showed the infiltration proportion of 22 types of immune cells in each UCEC patient. Wilcoxon rank-sum test was used to accurately evaluate the differential density between high and low TMB groups, which was visually represented by the violin plot.

2.5 Identification of TMB-related immune signature

The "Gene Summary" file containing the information of immune-related genes was downloaded from the immunology database – Immport (https://www.immport.org/). Based on the immune-related gene data and the differential gene expression data between the TMB high and low groups, the "VennDiagram" package was used to screen out the differentially expressed immune genes between the two groups.

2.6 Construction of prognostic risk model based on differential TMB-related immune genes

Multivariate Cox regression analysis was applied to opt for the differential TMB-related immune genes (DTIGs) associated with prognosis risk, and resulting weighted scores were calculated as risk scores for each patient. The score of the prediction model was determined as $S = (\text{exprDTG}_1 \times \text{coef}_1) + (\text{exprDTG}_2 \times \text{coef}_2) + ... + (\text{exprDTG}_n \times \text{coef}_n)$ and the score was described TMB Prognostic index (TMBPI). According to the median score as a cut-off value, UCEC patients were divided into the low-risk group and high-risk group. Kaplan-Meier analysis was used for survival analysis. Using the "survivalROC" R package, we drew the ROC curve and calculated the area under the curve (AUC) to verify the predictive capability of this model. Besides, based on the "SCNA" module of the TIMER database (https://cistrome.shinyapps.io/timer/), we further assessed the association between DTGs in the UCEC prognosis model and the infiltration of 6 immune cells.

2.7 Statistical analysis

All analyses were conducted using R software (version 3.6.2). Wilcoxon rank-sum test was selected for the non-parametric statistical test used to compare the differences between the two groups. Survival analysis was performed using the log-rank test. Multivariate analyses were performed via Cox regression. P values<0.05 were considered significant.

3. Results

3.1 Landscape of mutation profiles of UCEC

We downloaded the somatic mutation information of 542 UCEC patients from TCGA, in which the "Masked Somatic Mutation" data type and "VarScan2" workflow type were selected. In the TCGA
database, there are four types of mutation data: Annotated Somatic Mutation, which is an annotation file of somatic mutations in the format of VCF; Raw Simple Somatic Mutation, which is the original file of somatic mutations in the format of VCF; Aggregated Somatic Mutation, which is protected Mutation annotation file in the format of MAF; Masked Somatic Mutation, which is open access annotation file in the format of MAF. Next, we input the prepared MAF files and visualizing the results of the patients' mutation data using the “maftools” package. The detailed mutation information of each UCEC patient was shown in the waterfall plot (Figure 1). The clinical baseline of all 545 UCEC patients was summarized in Table 1, of whom the mean age was 63.93±11.14. To discuss the patient’s variant details in more depth, we further categorized and summarized the mutations. To sum up, we found that missense mutation was the most frequently occurring variant classification (Figure 2a), single nucleotide polymorphism (SNP) was the most common variant type (Figure 2b), and C>T accounted for the largest proportion in single-nucleotide variation (SNV, Figure 2c). In addition, the number of variants for each patient sample was calculated and displayed (Figure 2d), and variant classification levels were presented again in a box plot (Figure 2e). Subsequently, we listed the top ten frequently mutated genes, which were PTEN (64%), PIK3CA (48%), ARID1A (44%), TTN (38%), TP53 (37%), PIK3R1 (30%), KMT2D (26%), CTCF (26%), MUC16 (25%), and CTNNB1 (24%, Figure 2f). What’s more, our study continued to examine the consistency and exclusivity correlations among these mutated genes, with green for co-occurrence and brown for mutual exclusion. It can be observed from the figure that there were coexistence relationships across numerous mutated genes, while the mutually exclusive relationships between PTEN and TP53 and between TP53 and ARID1A were obvious (Figure 2g). Finally, the genetic cloud map was drawn to distinctly recap the mutated genes again (Figure 2h).

3.2 The relationship between TMB level and survival prognosis and tumor grades in UCEC

Tumor mutation burden (TMB) is defined as the total number of somatic gene coding errors, base substitutions, gene insertions, or deletion errors detected per million bases. The TMB level of each UCEC sample was calculated, and patients were divided into high TMB group and low TMB group with the median as the cut-off value. Then, according to the sample ID, TMB levels were merged with the patient survival information and the clinicopathological characteristics information. Comparing the survival outcomes of the two groups, it was found that patients with high-level TMB kept better overall survival (OS), and the results were statistically significant (p = 0.048, Figure 3a). Surprisingly, higher TMB was correlated with advanced pathological grades of UCEC (p = 0.002, Figure 3b). It seemed that TMB was higher in patients aged 65 and younger than in older patients, but not statistically significant (p = 0.893, Figure 3c).

3.3 Differential expression gene identification and functional enrichment analysis
Transcriptome RNA-sequencing data of HTSeq-FPKM type were downloaded from TCGA, including 552 UCEC tissues and 23 adjacent non-tumor tissue samples. Comparing the transcriptome genes of the two TMB groups, 427 differential genes were obtained. Then, we selected the 40 genes with the most significant differences to draw a gene heatmap (Figure 4a). Moreover, we performed enrichment analyses on differential genes, including gene ontology (GO) analysis, Kyoto gene and genome encyclopedia (KEGG) analysis, and gene set enrichment analysis (GSEA). It is well known that GO can be divided into three parts: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). Our study found that in the BP group, humoral immune response, lymphocyte-mediated immunity, and complement activation were frequently enriched. In the CC group, differential genes were mainly involved in immunoglobulin complex, external side of the plasma membrane, and immunoglobulin complex, circulating. In the MF group, antigen binding, immunoglobulin receptor binding, and receptor-ligand activity were the three main terms (Figure 4b). According to the above results, we discovered that the major GO terms enriched by differential genes were mainly concerned with the immune response. The top ten KEGG pathways were Neuroactive ligand-receptor interaction, Alzheimer disease, Cytokine-cytokine receptor interaction, Breast cancer, Cell adhesion molecules (CAMs), Gastric cancer, Hippo signaling pathway, Proteoglycans in cancer, MAPK signaling pathway and Wnt signaling pathway (Figure 4c). Finally, we listed four excellent results of GSEA, in which the high TMB levels were significantly enriched in pyrimidine metabolism, nucleotide excision repair, P53 signaling pathway, and fructose and mannose metabolism (Figure 4d).

3.4 Comparison of immune cells infiltration between two groups of high and low TMB levels

In 2015, Newman et al. of Stanford university school of medicine proposed a new method for analyzing single-cell types, called CIBERSORT, which is a computer algorithm that reconstructs the type and number of original cells based on the RNA content of all cell mixtures(28). Based on the CIBERSORT algorithm, our study evaluated the immune profile of each patient and compared the infiltration differences of these 22 immune cells between the high and low TMB groups. The immune infiltration profile of each sample was shown in Figure 5a, where each bar represented a patient, and different colors represented different cell components. Additionally, the violin-plot revealed that the infiltration levels of CD8 T cells, memory activated CD4 T cells, follicular helper T cells, and M1 macrophages in the high TMB group were significantly higher than those in the low TMB group (Figure 5b). Based on the above results, it was not difficult to recognize that higher TMB generally heightened the level of immune infiltration of UCEC samples and advanced the patients’ anti-tumor immune response.

3.5 Construction of a TMB-related immune genes risk model for UCEC patients
Through the intersection of immune-related genes and differential genes, 108 differential TMB-related immune genes (DTIGs) were obtained (Figure 6a). Next, we performed multivariate Cox regression analysis on these DTIGs and acquired 4 risk-related DTIGs (EDN3, FGF19, IL13RA2, TRAV21) and their coefficients, which participated in the construction of the risk model (Table 2). The risk score in the model was calculated as \( \sum \text{coefficients} \times \text{expression values} \). Here, we defined this risk score as the “TMB Risk Index” (TMBRI). The median TMBRI was 1.1734604, which was regarded as a cut-off value, and patients were divided into the high-risk group (n=261) and low-risk group (n=262). Kaplan–Meier (KM) survival analysis showed that there was a significant difference in OS between the two groups, and OS in the high-risk group was even worse (Figure 6b). The 5-year survival rate of patients in the high-risk group was nearly 20% lower than that in the low-risk group (Table 3). Furthermore, ROC analysis was performed to verify the reliability of this risk model. The area under the curve (AUC) of the ROC curve was 0.670, indicating that the TMBRI risk model had certain applicability in predicting the prognosis of patients with UECE (Figure 6c). Besides, we further evaluated the potential relationship between the mutants of these DTIGs in the risk model and immune infiltration in the microenvironment. Using the SCNA module of the TIMER database, the relationship between END3 or FGF19 or IL13RA2 mutants, and the infiltration of 6 immune cells were exhibited by the box plot (Figure 6d-f).

4. Discussion

Endometrial cancer was decided for appropriate treatment based on the patient’s age, physical condition, lesion range, and histologic type. The majority of endometrial carcinoma is adenocarcinoma, which is not highly sensitive to radiotherapy, so the treatment is mainly surgery, and adjuvant therapy includes radiotherapy, chemotherapy, hormone therapy, and targeted therapy(7). New targeted and ICB therapies for advanced endometrial cancer held promise for improving prognosis, as overall survival for patients with advanced and recurrent endometrial cancer remains poor. Overexpression of PD-1 and PD-L1 was detected in EC tissues(29). Immunohistochemical results showed that more than 75% of endometrioid carcinomas were positive for PD-1 and PD-L1(30). Therefore, targeting PD-1 / PD-L1 may be a promising strategy to magnify the anti-tumor immune response. Le et al. (2015) demonstrated for the first time that Pembrolizumab, a humanized monoclonal antibody against PD-1, was clinically effective in tumors with mismatch repair defects, in a phase II study(31). In the Phase Ib study (KEYNOTE-028) of pembrolizumab in the treatment of PD-L1-positive advanced endometrial cancer, three of the 24 patients with recurrent and metastatic endometrial cancer achieved partial remission (PR), of which one had POLE mutation. Three other patients achieved stable disease (SD). The overall response rate (ORR) was 13%. As for toxicities, only mild side effects were observed in thirteen patients (54.2%), the most common being fatigue, itching, fever, and anorexia(32). Additionally, in the monotherapy for PD-L1-positive endometrial cancer, the efficacy of Atezolizumab (anti-PD-L1) and Nivolumab (anti-PD-1) were also examined, with ORR of 13% and 23%, respectively(3). According to existing investigations, the efficacy of ICB therapy was not only based on the expression of PD-1 / PD-L1 but also the release and presentation of tumor antigens and the infiltration of immune cells in the tumor microenvironment. Although the ICB has shown certain satisfactory therapeutic outcomes in anti-tumor treatment, only a small number of patients can benefit.
from it. Therefore, many studies are devoted to finding biomarkers that can effectively predict the efficacy of immunotherapy.

As the latest marker for evaluating the efficacy of PD-1 blockade, the predictive capability of TMB has been approved in the use of PD-1 antibody in the treatment of colorectal cancer with mismatch repair deficient (dMMR)(31, 33). Among 30 human cancers, endometrial cancer has the highest incidence of microsatellite instability (MSI) (34). Approximately 30% of primary endometrial cancer are MSI-H, and 13% to 30% of relapsed endometrial cancer are MSI- H or dMMR (35, 36). Based on the above conclusions, we speculated that TMB may play a crucial role in predicting the prognosis of endometrial cancer. Zhang et al. (2019) performed a multi-omics analysis of TMB and immune infiltration in bladder urothelial carcinoma on the TCGA public platform, demonstrating that TMB may be an independent biomarker for survival prediction of bladder cancer(26). In this study, a similar methodology was utilized to analyze the relationship between TMB level and survival status in endometrial cancer. Our results discovered that higher TMB resulted in better OS (p = 0.048, Figure 3a), which was consistent with similar results in most other malignancies, that higher TMB was more conducive to provoking local immune responses and improving prognosis. In the trial of PD-1 combined with CTLA-4 blockade therapy for NSCLC, Hellmann MD et al. (2018) observed that high TMB can predict improved objective response, long-term benefit, and progression-free survival (PFS). Moreover, TMB was independent of the expression of PD-L1, which was the strongest feature associated with a therapeutic effect in multivariate analysis(37). Lv et al. (2020) revealed that the prognosis of bladder cancer patients with higher TMB was better so that TMB was considered to be a powerful predictor of tumor behavior and response to immunotherapy (38). However, in some types of cancer, such as clear cell renal cell carcinoma, patients with high TMB levels showed worse survival outcomes (24).

So why did TMB predict the opposite across different tumor types? Chen (2013) (39) proposed the concept of the "cancer-immune cycle" and pointed out seven important steps for the anti-tumor immune response to function, including the release and presentation of tumor antigens and final T cell activation, etc. Therefore, the anti-tumor effect largely depended on the infiltration and activation of immune cells in the tumor microenvironment. In our study, patients in the high TMB group possessed higher levels of CD8 T cells, memory-activated CD4 T cells, follicular helper T (Tfh) cells, and M1 macrophage infiltration, while patients in the low TMB group held higher levels of regulatory T cells (Tregs) and M2 macrophages (Figure 5b). CD8 T cells are the leading anti-tumor effector cells. Memory CD4 T cells help the body obtain long-term protection against pathogen reinfection(40). Tfh cells secrete BCL-6, IL-21, CXCR5, and ICOS, which are mainly involved in the information transmission during B cell differentiation, helping to activate B cells and maintain humoral immune response for a long time(41, 42). Macrophages participate in the tumor immune response through different polarization methods: classic M1 macrophages produce IL-12 and promote tumor immune response: while M2 macrophages produce IL-10 to promote tumor progression(43). Tregs are a group of lymphocytes with negative regulation of the body's immune response, which are involved in the escape of tumor cells from the body's immune surveillance and chronic infection(44). From the above results, it can be seen that high levels of TMB in endometrial cancer were positively correlated with anti-tumor immune cells and negatively correlated with immune
cells that mediate tumor immune escape. Furthermore, we also observed that the level of TMB was correlated with the pathological grade of UCEC. The higher the level, the worse the differentiation, but patients with advanced-stage may be more prone to benefit from ICB (Figure 3b).

GO enrichment analysis of DTIGs indicated that these genes were mainly involved in humoral immune response, lymphocyte immunity, immunoglobulin complex, antigen binding, and other immune-related crosstalk. Subsequently, 4 risk-related DTIGs (positive correlation: FGF19 and IL13RA2, negative correlation: EDN3 and TRAV21) that were highly associated with survival were identified via multivariate cox analysis, and risk prediction models were developed based on these 4 genes. Patients with high TMBR1 showed poor survival results (p <0.001), and the AUC of the model’s ROC curve was 0.670, indicating excellent predictive reliability.

The binding of FGF19 to its specific receptor FGFR4 can inhibit apoptosis and NF-κB signaling, and up-regulate the expression of proliferation-related genes(45, 46). Both are linked with malignancy and risk prognosis and may be independent prognostic indicators for breast cancer and bladder cancer. IL13RA2 (interleukin-13 receptor Subunit Alpha 2) is a protein-coding gene related to diseases including glioblastoma and malignant glioma, which are mainly associated with poor prognosis and drug resistance(47, 48). Besides, Barderas et al. (2012) showed that interleukin 13 (IL-13) mediated the invasion, pro-metastatic effect, and poor prognosis in colorectal cancer through IL13Rα2 (49). At present, there are no researches on the association between IL13RA2 and the development, metastasis, and prognosis of endometrial cancer. END3 (endothelin-3) was down-regulated in a variety of tumor tissues, such as breast (50), cervical (51), and colon cancer (52, 53). In our study, EDN3 was also a negative risk factor, and the higher its expression, the lower the patient's risk value (coefficient = -0.016578188). However, some studies have suggested that EDN3 may be involved in the development of melanoma via Hypoxia-Inducible factor-1alpha(54). Therefore, the effect of EDN3 on endometrial cancer remains to be further explored.

5. Conclusions

In summary, our study demonstrates that in UCEC, higher TMB was associated with better survival outcomes, which may be due to the increased immune infiltration in the tumor microenvironment caused by high levels of TMB.

Abbreviations

AUC: area under the curve
BP: Biological Process
CAMs: Cell adhesion molecules
CC: Cellular Component
dMMR: mismatch repair-deficient
DTIGs: differential TMB-related immune genes
FC: fold change
FDA: Food and Drug Administration
FDR: false discovery rate
GDC: Genomic Data Commons
GO: gene ontology
GSEA: gene set enrichment analysis
ICIs: immune checkpoint inhibitors
KEGG: Kyoto Encyclopedia of Genes and Genomes
MAF: mutation annotation format
MF: Molecular Function
MSI: microsatellite instability
NSCLC: non-small cell lung cancer
ORR: overall response rate
OS: overall survival
PFS: progression-free survival
PR: partial remission
RCC: renal cell carcinoma
ROC: receiver operating characteristic
SD: stable disease
SNP: single nucleotide polymorphism
SNV: single-nucleotide variation
UCEC: uterine corpus endometrial cancer
TCGA: the Cancer Genome Atlas

Tfh: follicular helper T

Tregs: regulatory T cells

TMB: tumor mutation burden

TMBPI: TMB Prognostic index

Declarations

Ethics approval and consent to participate

No experiments have been performed including patients and/or animals.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this article and its additional files.

Conflict of interest

All authors declare that they have no conflict of interest.

Funding

This work was supported by the Natural Science Foundation of Liaoning Province under Grant [No. 20180530032].

Acknowledgment

Not applicable.

Author contributions

Zhuang Y and Li G designed the study and analyzed the data.
Zhuang Y and Liu C drafted the article.

Huang J. was responsible for language correction.

All authors finally approved the paper.

References

1. Kong A, Johnson N, Kitchener HC, Lawrie TA. Adjuvant radiotherapy for stage I endometrial cancer. The Cochrane database of systematic reviews. 2012;2012(4):Cd003916.

2. Moore K, Brewer MA. Endometrial Cancer: Is This a New Disease? American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting. 2017;37:435-42.

3. Green AK, Feinberg J, Makker V. A Review of Immune Checkpoint Blockade Therapy in Endometrial Cancer. American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting. 2020;40:1-7.

4. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet (London, England). 2005;366(9484):491-505.

5. Shaw E, Farris M, McNeil J, Friedenreich C. Obesity and Endometrial Cancer. Recent results in cancer research Fortschritte der Krebsforschung Progres dans les recherches sur le cancer. 2016;208:107-36.

6. Haggerty AF, Sarwer DB, Schmitz KH, Ko EM, Allison KC, Chu CS. Obesity and Endometrial Cancer: A Lack of Knowledge but Opportunity for Intervention. Nutrition and cancer. 2017;69(7):990-5.

7. Braun MM, Overbeek-Wager EA, Grumbo RJ. Diagnosis and Management of Endometrial Cancer. American family physician. 2016;93(6):468-74.

8. Yang X, Wang J. The Role of Metabolic Syndrome in Endometrial Cancer: A Review. Frontiers in oncology. 2019;9:744.

9. Lee YC, Lheureux S, Oza AM. Treatment strategies for endometrial cancer: current practice and perspective. Current opinion in obstetrics & gynecology. 2017;29(1):47-58.

10. Ito A, Kondo S, Tada K, Kitano S. Clinical Development of Immune Checkpoint Inhibitors. BioMed research international. 2015;2015:605478.

11. Franklin C, Livingstone E, Roesch A, Schilling B, Schadendorf D. Immunotherapy in melanoma: Recent advances and future directions. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2017;43(3):604-11.

12. Steven A, Fisher SA, Robinson BW. Immunotherapy for lung cancer. Respirology (Carlton, Vic). 2016;21(5):821-33.

13. Carlo MI, Voss MH, Motzer RJ. Checkpoint inhibitors and other novel immunotherapies for advanced renal cell carcinoma. Nature reviews Urology. 2016;13(7):420-31.

14. Bilusic M, Madan RA, Gulley JL. Immunotherapy of Prostate Cancer: Facts and Hopes. Clinical cancer research : an official journal of the American Association for Cancer Research.
15. Kitano S. [Development of immune checkpoint inhibitors]. [Rinsho ketsueki] The Japanese journal of clinical hematology. 2017;58(8):966-76.
16. Goldberg MS. Improving cancer immunotherapy through nanotechnology. Nature reviews Cancer. 2019;19(10):587-602.
17. Everson RG, Antonios JP, Liau LM. Cell-Based Immunotherapy of Gliomas. Progress in neurological surgery. 2018;32:90-100.
18. Johnston MP, Khakoo SI. Immunotherapy for hepatocellular carcinoma: Current and future. World journal of gastroenterology. 2019;25(24):2977-89.
19. De Felice F, Marchetti C, Tombolini V, Panici PB. Immune check-point in endometrial cancer. International journal of clinical oncology. 2019;24(8):910-6.
20. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science (New York, NY). 2015;346(6230):69-74.
21. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Annals of oncology: official journal of the European Society for Medical Oncology. 2019;30(1):44-56.
22. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Molecular cancer therapeutics. 2017;16(11):2598-608.
23. Thomas A, Routh ED, Pullikuth A, Jin G, Su J, Chou JW, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. Oncoimmunology. 2018;7(10):e1490854.
24. Zhang C, Li Z, Qi F, Hu X, Luo J. Exploration of the relationships between tumor mutation burden with immune infiltrates in clear cell renal cell carcinoma. Annals of translational medicine. 2019;7(22):648.
25. Yakirevich E, Patel NR. Tumor mutational burden and immune signatures interplay in renal cell carcinoma. Annals of translational medicine. 2020;8(6):269.
26. Zhang C, Shen L, Qi F, Wang J, Luo J. Multi-omics analysis of tumor mutation burden combined with immune infiltrates in bladder urothelial carcinoma. Journal of cellular physiology. 2020;235(4):3849-63.
27. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. The New England journal of medicine. 2017;377(25):2500-1.
28. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nature methods. 2015;12(5):453-7.
29. Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, et al. Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. JAMA oncology. 2015;1(9):1319-23.
30. Vanderstraeten A, Luyten C, Verbist G, Tuyaerts S, Amant F. Mapping the immunosuppressive environment in uterine tumors: implications for immunotherapy. Cancer immunology, immunotherapy : CII. 2014;63(6):545-57.

31. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England journal of medicine. 2015;372(26):2509-20.

32. Ott PA, Bang YJ, Berton-Rigaud D, Elez E, Pishvaian MJ, Rugo HS, et al. Safety and Antitumor Activity of Pembrolizumab in Advanced Programmed Death Ligand 1-Positive Endometrial Cancer: Results From the KEYNOTE-028 Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35(22):2535-41.

33. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science (New York, NY). 2017;357(6349):409-13.

34. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO precision oncology. 2017;2017.

35. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73.

36. Soumerai TE, Donoghue MTA, Bandlamudi C, Srinivason P, Chang MT, Zamarin D, et al. Clinical Utility of Prospective Molecular Characterization in Advanced Endometrial Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2018;24(23):5939-47.

37. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer. Cancer cell. 2018;33(5):843-52.e4.

38. Lv J, Zhu Y, Ji A, Zhang Q, Liao G. Mining TCGA database for tumor mutation burden and their clinical significance in bladder cancer. Bioscience reports. 2020;40(4).

39. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1-10.

40. Soon MS, Engel JA, Lee HJ, Haque A. Development of circulating CD4(+) T-cell memory. Immunology and cell biology. 2019;97(7):617-24.

41. Pepper M, Jenkins MK. Origins of CD4(+) effector and central memory T cells. Nature immunology. 2011;12(6):467-71.

42. Jogdand GM, Mohanty S, Devadas S. Regulators of Tfh Cell Differentiation. Frontiers in immunology. 2016;7:520.

43. Genin M, Clement F, Fattaccioli A, Raes M, Michiels C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. BMC cancer. 2015;15:577.

44. Wang H, Franco F, Ho PC. Metabolic Regulation of Tregs in Cancer: Opportunities for Immunotherapy. Trends in cancer. 2017;3(8):583-92.

45. Drafahl KA, McAndrew CW, Meyer AN, Haas M, Donoghue DJ. The receptor tyrosine kinase FGFR4 negatively regulates NF-kappaB signaling. PloS one. 2010;5(12):e14412.
46. Prieto-Dominguez N, Shull AY, Teng Y. Making way for suppressing the FGF19/FGFR4 axis in cancer. Future medicinal chemistry. 2018;10(20):2457-70.

47. Han J, Puri RK. Analysis of the cancer genome atlas (TCGA) database identifies an inverse relationship between interleukin-13 receptor α1 and α2 gene expression and poor prognosis and drug resistance in subjects with glioblastoma multiforme. Journal of neuro-oncology. 2018;136(3):463-74.

48. Sharma P, Debinski W. Receptor-Targeted Glial Brain Tumor Therapies. International journal of molecular sciences. 2018;19(11).

49. Barderas R, Bartolomé RA, Fernandez-Aceñero MJ, Torres S, Casal JI. High expression of IL-13 receptor α2 in colorectal cancer is associated with invasion, liver metastasis, and poor prognosis. Cancer research. 2012;72(11):2780-90.

50. Wiesmann F, Veeck J, Galm O, Hartmann A, Esteller M, Knüchel R, et al. Frequent loss of endothelin-3 (EDN3) expression due to epigenetic inactivation in human breast cancer. Breast cancer research : BCR. 2009;11(3):R34.

51. Sun DJ, Liu Y, Lu DC, Kim W, Lee JH, Maynard J, et al. Endothelin-3 growth factor levels decreased in cervical cancer compared with normal cervical epithelial cells. Human pathology. 2007;38(7):1047-56.

52. Olender J, Nowakowska-Zajdel E, Kruszniewska-Rajs C, Orchel J, Mazurek U, Wierzgoń A, et al. Epigenetic silencing of endothelin-3 in colorectal cancer. International journal of immunopathology and pharmacology. 2016;29(2):333-40.

53. Wang R, Löhr CV, Fischer K, Dashwood WM, Greenwood JA, Ho E, et al. Epigenetic inactivation of endothelin-2 and endothelin-3 in colon cancer. International journal of cancer. 2013;132(5):1004-12.

54. Spinella F, Rosanò L, Di Castro V, Decandia S, Nicotra MR, Natali PG, et al. Endothelin-1 and endothelin-3 promote invasive behavior via hypoxia-inducible factor-1alpha in human melanoma cells. Cancer research. 2007;67(4):1725-34.

Tables
Table 1. The clinical baseline of 545 USCS patients included in the study from the TCGA cohort

| Variables       | Number (%) |
|-----------------|------------|
| Vital status    |            |
| Alive           | 458 (84.04)|
| Dead            | 87 (15.96) |
| Age, y          | 63.93±11.14|
| Tumor grade     |            |
| G1/G2           | 221 (40.55)|
| G3              | 313 (57.43)|
| Undetected      | 11 (2.02)  |
| TMB level       |            |
| Low level       | 265 (48.62)|
| High level      | 269 (49.36)|
| Undetected      | 11 (2.02)  |
| TMBRI           |            |
| Low level       | 262 (48.07)|
| High level      | 261 (47.89)|
| Undetected      | 22 (4.04)  |

UCEC, uterine corpus endometrial carcinoma; TMB, tumor mutation burden.

Table 2. Risk related genes names and coefficients in the risk model.

| ID    | coefficient |
|-------|-------------|
| EDN3  | -0.01658    |
| FGF19 | 0.008823    |
| IL13RA2 | 0.019659    |
| TRAV21| -0.24836    |
Table 3. Five-year survival of high and low-risk groups based on the risk model

| Risk Group | 5-year-survival | lower 95% CI | upper 95% CI |
|------------|-----------------|--------------|--------------|
| High risk  | 67.70%          | 60.20%       | 76.20%       |
| Low risk   | 83.40%          | 76.90%       | 90.50%       |

Figures
Figure 1
Construction and assessment of TMBRI for UCEC. (A) Identification of TMB-related immune genes; (B) Kaplan–Meier analysis with log-rank test demonstrated that patients with higher TMBPI showed worse OS with $p < 0.001$. (C) AUC of ROC plot was 0.670 indicating the decent predictive accuracy of TMBRI. (D-F) The relationship between the three TMB-related mutants in the risk model and immune cell infiltration. TMB, tumor mutation burden; TMBRI, tumor mutation burden Risk Index; UCEC, uterine corpus endometrial carcinoma.
Figure 2

Comparisons of 22 important immune components between low and high TMB groups. (A) The immune infiltration profile of each sample was shown in a barplot, where each bar represented a patient, and different colors represented different cell components. (B) The violin plot showed higher infiltration levels of CD8 T cells, memory-activated CD4 T cells, follicle-assisted T cells, and M1 macrophages in the high TMB group. TMB, tumor mutation burden.
Figure 3

Differential analysis of gene expression profiles and enrichment pathways in high and low TMB groups. (A) Top 40 differentially expressed genes were shown in the heat map with $|\log(FC) > 1|$ and FDR < 0.05.
(B, C) GO and KEGG enrichment results revealed that these genes were involved in immune-related crosstalks; (D) Besides, GSEA showed the top TMB-related signaling pathways, including pyrimidine metabolism, nucleotide excision repair, P53 signaling pathway, and fructose and mannose metabolism. TMB, tumor mutation burden.
Figure 4

Prognosis of TMB and associations with risk clinicopathological characteristics. (A) Higher TMB levels correlated with better overall survival (OS) outcomes with P=0.048; (B) Higher TMB level correlated with advanced tumor grades with P=0.002, (C) while no significant difference was observed in patients’ age (P > 0.05). TMB, tumor mutation burden.
Figure 5
Summary of mutation profiling in UCEC samples. (A-C) Classification of mutation types, among which missense mutations account for the majority, SNP occurs more frequently than insertions or deletions, and C>T is the most common SNV. (D, E) Tumor mutation burden in specific samples; (F) The top 10 mutant genes in UCEC; (G) Consistency and exclusivity correlations among these mutated genes; (H) Gene cloud map of mutation information. SNP, single nucleotide polymorphism; SNV, single nucleotide variant; UCEC, uterine corpus endometrial carcinoma.

![Mutation Landscape](image)

**Figure 6**

The landscape of mutation profiles in UCEC samples. The detailed mutation information of each patient was shown in the waterfall plot, with different colors with specific annotations at the bottom indicating various types of mutations. The barplot above the legend showed the number of mutation burden. UCEC, uterine corpus endometrial carcinoma.