Submitted: 03 April, 2023 Accepted: 06 July, 2023 Published: 15 August, 2023 DOI:10.22514/ejgo.2023.065

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Identification of immune subtypes of uterine corpus endometrial adenocarcinoma based on tumour microenvironment

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Abstract

Uterine corpus endometrial adenocarcinoma is the prevalent gynaecological malignancy. The related morbidity and mortality are high despite the progress made in treatments. Therefore, efficient prognostic indicators and reliable predictive factors for the treatments are vital. In this study, the transcriptome and clinical data of endometrial adenocarcinoma samples were screened and downloaded from The Cancer Genome Atlas Program (TCGA) database. The relation between immune cell types and clinicopathological grade of endometrial adenocarcinoma was explored. The endometrial adenocarcinoma samples were divided into six immune subtypes based on immune microenvironment scores. The differential genes in immune subtypes were classified according to the score, and correlation enrichment analysis was made to explore the immune pathways related to prognosis and survival. They were divided into high and low risk groups according to the median risk score in order to explore the survival outcomes of the various immune scores. Finally, the relationship between tumour mutation burden, immune subtypes, and prognosis was discussed. Herein, the endometrial adenocarcinoma is classified based on immune microenvironment which demonstrates good predictive potential of immune-based classification strategy. The predicted outcomes are described for the patients at high risk of endometrial adenocarcinoma to improve the treatment strategies. Immune risk score can be used as an independent risk factor for overall survival of endometrial adenocarcinoma patients. This immune-based classification system can prognose endometrial adenocarcinoma patients at high risk.

Keywords

Uterine corpus endometrial adenocarcinoma; Tumour microenvironment; Immune cell infiltration

1. Introduction

Endometrial cancer is one of the three most prevalent gynaecological malignancies with its worldwide incidence on the rise [1, 2]. It was estimated that 65,620 new cases and 12,590 subsequent deaths occurred in 2020 in the United States [3], where ~80% were the estrogen-dependent type I endometrial cancer, also known as endometrial endometrioid adenocarcinoma [4]. The relapse and metastasis occur in 13–25% of patients despite the endometrial endometrioid adenocarcinoma has good prognosis. Metastasis and tumour spread are often the main causes of poor prognosis [5]. The endometrial adenocarcinoma survival rates in developing countries are lower than those in developed ones and vary between geographical regions. The effective prognostic indicators and guided treatments are thus crucial [6]. Classification systems for uterine corpus endometrial cancer have been developed over time to predict patient outcomes and define adequate treatment strategies.

The molecular classification of endometrial adenocarcinoma mainly based on gene expression profiling and selected IHC can accurately reflect the prognosis of patients. Several studies have explored the molecular aspects of endometrial adenocarcinoma based on gene expression and mutational burden; however, limited studies are available on the immune types of endometrial adenocarcinoma. The tumour microenvironment (TME) is an extracellular matrix secreted by tumour, stromal, endothelial, immune, and tumour-associated cells. The components in TME interact with tumour cells to regulate their growth and development [7]. It has been demonstrated in recent years that development and metastasis of malignant cells are associated with TME [8, 9]. Moreover, TME has role in uterine corpus endometrial adenocarcinoma development and its response to immunotherapy as a number of immune cells and cytokines are involved in endometrial adenocarcinoma [10].

In this study, the data from TCGA database was screened

and selected in relation to endometrial adenocarcinoma, excluding the data linked to mucous, serous, clear cell carcinoma, and carcinosarcoma. Immune-related subtypes of uterine corpus endometrial adenocarcinoma were explored based on the abundance and microenvironment scores of immune cells infiltrating the tumours. Their clinical features and prognostic significance were also evaluated. Uterine adenocarcinoma was further assessed according to the differential analysis of identified immune subtypes based on stable genotypes, and the relationships between signalling pathways profiles and clinical characteristics, prognostic significance, and specific heterogeneity. Finally, the relationships between immunotype groups, tumour mutational burden, and prognostic significance were investigated. The study outcomes can provide theory for future immunotherapy to treat endometrial adenocarcinoma.

2. Materials and methods

2.1 Data processing

Uterine corpus endometrial adenocarcinoma datasets were obtained from The Cancer Genome Atlas (TCGA). TCGA Knowledge Base (https://portal.gdc.cancer.gov/repository), UCSC Xena (https://xenabrowser.net/datapages/), and cBioPortal for Cancer Genomics (http://www.cbioportal.org/) databases were used to collect transcriptome data, mutation data, and clinical information. Sample data of 405 endometrial adenocarcinoma patients were downloaded.

2.2 Identification and genotyping of uterine corpus endometrial adenocarcinoma subtypes based on immune cells

Clustering may have bias because of the small number of samples per pathological grade in endometrial adenocarcinoma. All endometrial adenocarcinomas were used for the immunoassay to explore relation between immune cell types and clinicopathologic grades through TME scores. The 28 immune gene sets were used to determine tumour immunity [11]. The gene set variation analysis package was employed for single-sample Gene Set Enrichment Analysis (ssGSEA) of 28 immune cells types for each uterine corpus endometrial adenocarcinoma dataset. The identified subsets were further genotyped using limma R package (v3.50.0). The consensus clustering, molecular subtype screening of ssGSEA scores (k = 2–9), and genotype screening (k = 1–12) of immune subtypes were conducted by The Consensus Cluster Plus package. Briefly, clustering was performed with 50 iterations (each using 80% samples). The optimal cluster number was determined from the clustering score of cumulative distribution function curve, and relative changes in area under the curve were evaluated. Genes meeting the screening criteria ($p < 0.05$, cut-off criteria, and |log fold-change| > 1.0) were divided into high and low expression groups according to the relationship between gene expression and genotyping. The two groups were compared.

2.3 Immune cell correlation assessment

The ESTIMATE algorithm [12] was implemented using estimation package to identify the specific features associated with stromal and immune cells infiltration in the tumour, based on transcriptome data. The corrplot package was employed to find correlations between immune cells.

2.4 Heatmap

The equation xi' = (xi-xmin)/(xmax-xmin) converted ssGSEA score (xi') of uterine corpus endometrial adenocarcinoma sample i to xi', where xmax and xmin are the maximum and minimum ssGSEA scores for samples in uterine corpus endometrial adenocarcinoma dataset, respectively. The heatmap was visualized by heatmap package.

2.5 Overall survival curve

Mapping the Kaplan-Meier curve revealed relation between patient overall survival and genomic expression profiles. The relationship was tested using log-rank test. $p < 0.05$ in relation to the cut-off value was employed for selecting the survival-dependent immune cell infiltration (ICI) scores.

2.6 Functional annotation of gene clustering

The ClusterProfiler package was utilized to perform Gene ontology (GO) [13, 14], and Kyoto Encyclopaedia of Genes and Genomes (KEGG) [15] pathway enrichment analyses.

2.7 Gene set enrichment analysis (GSEA)

The samples expression matrix classified as immune scores in TCGA dataset was used for GSEA with "c2.cp.kegg.v7.0.symbols.gmt" as the reference gene set. Using GSEA v4.0, the number of permutations was set to 1000, and false discovery rate <0.05 as the filtering threshold.

2.8 Correlation estimates between immunisation scores and TMB

Tumour mutation burden (TMB) is the sum of mutations and genetic variations in tumour cells. All base substitutions and insertions-deletions in the target gene coding region were thus counted. Silent mutations failing to contribute toward amino acid changes were not considered. The sample TMB score was calculated by counting the total number of mutations in relation to the exome size 45 Mb as exome size estimate [16]. Two maf files having somatic cell variants of uterine corpus endometrial adenocarcinoma samples were detected by VarScan and visualised *via* the maftools package [17]. The immune scores were correlated to TMB using the ggpubr R package (v0.4.0).

2.9 Assessment of immune score and clinical relevance

Immune scores were combined with the clinical data from TCGA database to assess the clinical survival outcomes of high and low immune scores patients and explore the differences

between these groups under various clinical conditions. Logistic regression using Kaplan-Meier methods assessed the correlations between clinical stages, immune scores, and overall survival.

2.10 Statistical analysis

Statistical analysis was performed using the free statistical computing software R (v3.6.0, R Project, Vienna, Austria). Kaplan-Meier survival analysis determined the survival curve reflecting association between genetic mutations and prognosis *via* the log-rank test. Two-tailed p value < 0.05 was considered statistically significant for all comparisons.

3. Results

3.1 Identification and immunologic characteristics of uterine corpus endometrial adenocarcinoma subtypes based on immune profile

Data of 405 endometrial adenocarcinoma samples were downloaded from TCGA database. All samples had been employed for immunoassay. First, the relationship between immune cell infiltration and pathological grade was studied. The results exhibited that Memory B cells, T-helper type 2 cells, effector memory cluster of differentiation 4 (CD4) T cells, eosinophils, type 17 helper T cells, activated CD4 T cells, plasmacytoid dendritic cells, monocytes, central memory CD8 T cells, macrophages, regulatory T cells, CD56 dim natural killer cells, and $\gamma\delta$T cells were correlated with pathological grades ($p < 0.05$). Memory B cells, type 2 helper T cells, effector CD4 T cells, eosinophils, type 17 helper T cells, and activated CD4 T cells were significantly correlated ($p < 0.0001$), indicating strong correlation between immune cell types and clinicopathological grades (**Supplementary Fig. 1**). The ssGSEA scores were then implemented on 28 samples associated with immune cells. Results depicted that the samples could be divided into 6 subtypes (Fig. 1A). Further analysis of various subtypes according to clinical outcomes revealed that the immune subtypes impacted the clinical prognosis ($p = 0.039$; Fig. 1B). The R software package ESTIMATE evaluated the Stromal Score, Immune Score, and ESTIMATE Score between A, B, C, D, E and F subtypes. The ssGSEA investigated 28 immune cell types. ESTIMATE results showed that subtype C had the highest tumour immune infiltration, while subtype E had the lowest. The ssGSEA results indicated that activated B cells, activated CD 4 T cells, activated CD 8 T cells, immature myeloid-derived suppressor cells (MDSC), and immune cell abundance in C subtype were higher than those of the other five subtypes (Fig. 1C). The correlation analysis confirmed that the clustering was associated with immune cells presence (Fig. 1D). The heat maps of tumour-infiltrating immune cell (TIIC) distribution across six subtypes are shown in Fig. 1E. It is speculated that the subtype C may respond better to immunotherapy.

3.2 Genotyping and characterisation of uterine corpus endometrial adenocarcinoma based on immune subtypes

The genotyping of uterine corpus endometrial adenocarcinoma dataset revealed that the samples could be divided into seven subtypes (1–7) according to differentially expressed gene patterns (Fig. 2A–C). However, this genotypic classification did not affect the clinical prognosis of patients with $p > 0.05$ (Fig. 2D). ESTIMATE evaluated the matrix and immune scores among seven subtypes of differentially expressed genes. The ssGSEA evaluated the 28 immune cell types. ESTIMATE results exhibited genotype G with the highest tumour immune infiltration, while genotype A with the lowest. The ssGSEA results depicted that the abundance of immature cells, MDSC cells, Monocyte cells, T follicular helper cells, and Type 1 T helper cells in G subtype were higher than those in the other six subtypes. The abundance of Effector memory CD4 T cells, Memory B cells, and Plasmacytoid dendritic cells in subtype C were higher than those in other six subtypes. The abundance of most immune cells in subtype A was lower than that in other six subtypes. The abundances of CD56 bright natural, natural killer T cells, Eosinophil, Neutrophil, and Type 17 T helper cells were not different among the seven subtypes (Fig. 2E). A heat map of TIIC distribution for the seven genetic subtypes is shown in Fig. 1E. The genotype A was positively associated with uterine corpus endometrial adenocarcinoma genotyping having more genotypes and increased gene expression, while the other subtypes were negatively associated with genotyping having more genotypes but decreased gene expression (Fig. 2F).

3.3 Immune scoring and GO enrichment analysis

The clinical prognosis of uterine corpus endometrial adenocarcinoma was evaluated based on the genotypically defined cancer subtypes. No significant difference in the clinical prognosis of uterine corpus endometrial adenocarcinoma was found according to the genotypic cancer subtypes ($p > 0.05$; Fig. 3A). However, GO and KEGG enrichment analysis revealed that B cell activation and immunoglobulin receptors were enriched in the high ICI group, while RNA splicing, and ribosomes were enriched in the low ICI group. The high ICI group may thus respond better to immunotherapy (Fig. 3B–E). Based on uterine corpus endometrial adenocarcinoma genotyping, most genotype A patients and all subtypes D and F patients had high ICI scores, whereas most genotypes B and C patients and all genotype E patients had low ICI scores. Patients of high ICI scores had higher survival rates than those with low ICI scores (Fig. 3F). *Proprotein Convertase Subtilisin/Kexin Type 4* (*PCSK4*), *Cyclin dependent kinase inhibitor 2A* (*CDKN2A*), *Indian hedgehog homolog* (*IHH*), *Leucine Rich Repeat Containing 8 VRAC Subunit D* (*LRRC8D*), *Cathepsin W* (*CTSW*) and *TNF Receptor Superfamily Member 18* (*TNFRSF18*) were analysed in high and low score groups as per the literature [13, 14]. *IHH*, *PCSK4*, and *LRRC8D* expressions were significantly different. *IHH* and *PCSK4* were upregulated in the low ICI group, and *LRRC8D* in high ICI group (Fig. 3G).

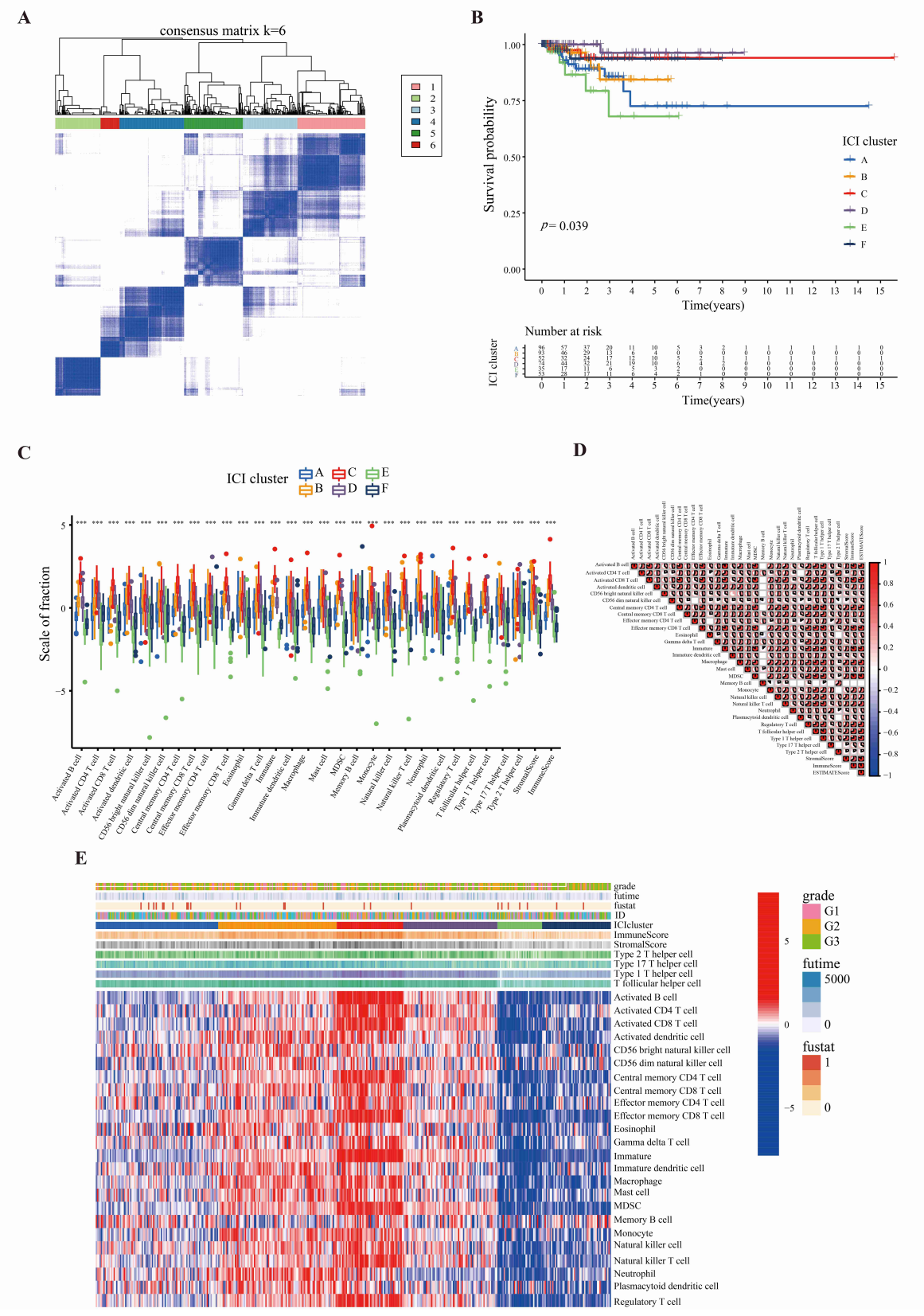

FIGURE 1. Identification and immunologic characteristics of endometrial carcinoma subtypes defined based on the immune profile. (A) Consensus clustering of uterine corpus endometrial carcinoma (UCEC) TCGA cohorts. NMF cluster consensus map. (B) Overall survival curve of the different UCEC immune subtypes. $p = 0.039$. (C) Comparison of UCEC subtypes based on 24 immune cell types. (D) Correlation analysis of immune cells. (E) Comparison of the six immune ssGSEA scores among the UCEC subtypes. ICI: Immune cell infiltration; CD: cluster of differentiation; MDSC: myeloid-derived suppressor cells.

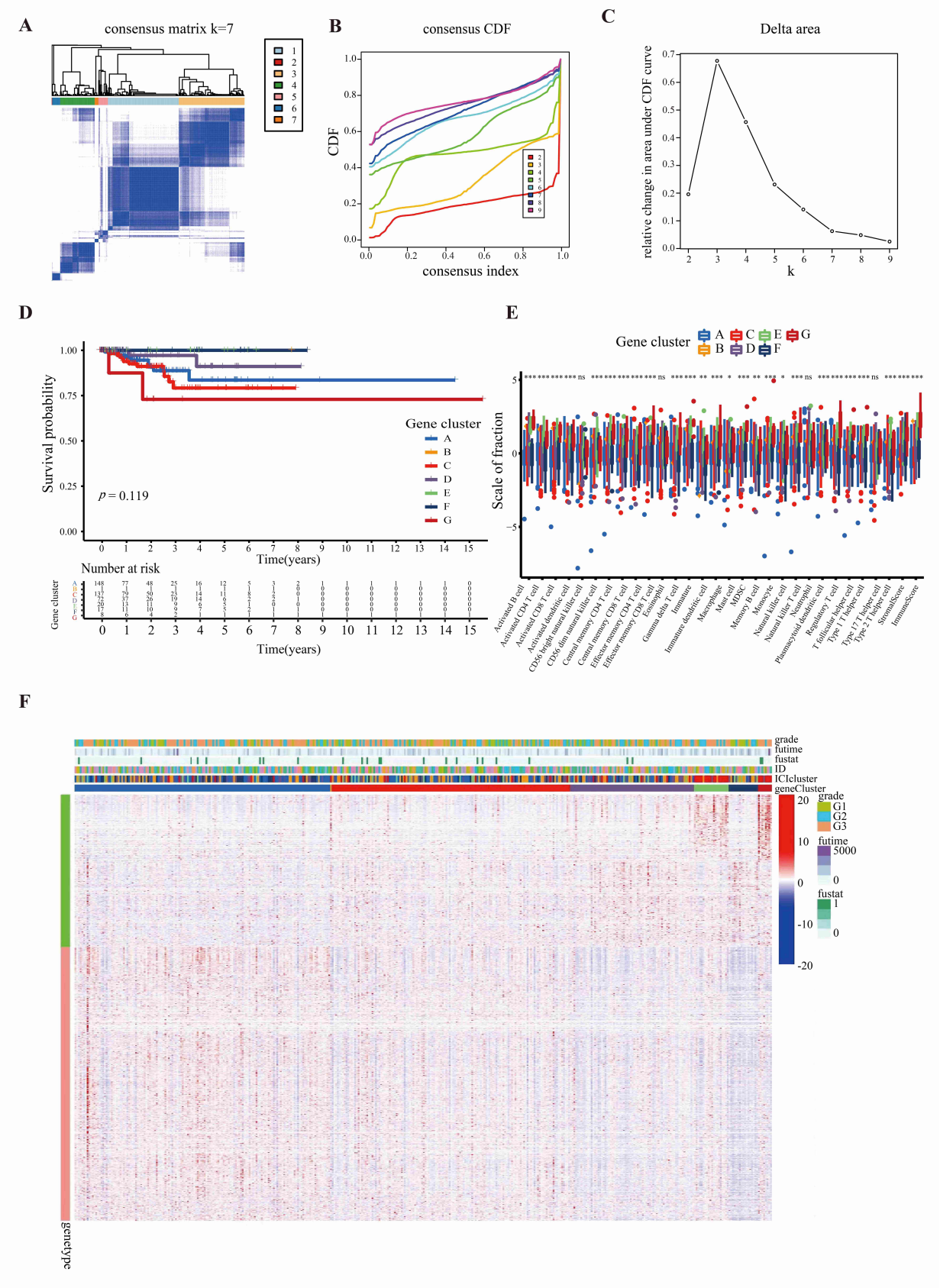

FIGURE 2. Genotyping and characterization of UCEC based on immune subtypes. (A) NMF cluster consensus map. (B) Cumulative distribution function curve of the consistence score for different subtype numbers (k = 1–12). (C) Delta area plot of the relative increase in cluster stability when k = 7. (D) OS curve of UCEC molecular gene subtypes. $p > 0.05$. (E) Expression of 28 immune cells in different UCEC subtypes. (F) Heatmap of the seven immune subtypes based on ssGSEA scores for 24 immune gene sets. CDF: Cumulative Distribution Function; CD: cluster of differentiation; MDSC: myeloid-derived suppressor cells.

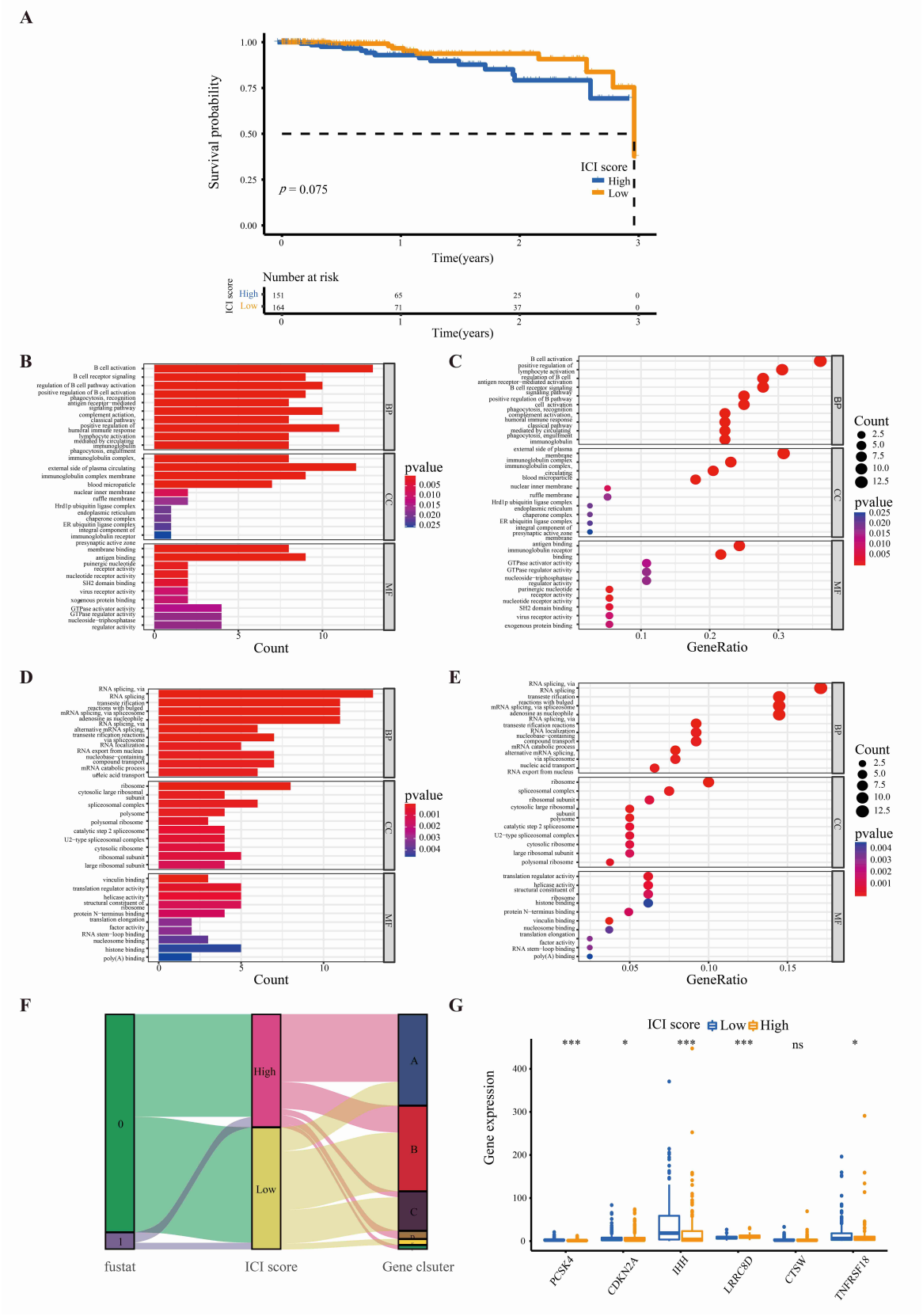

FIGURE 3. Analysis of immune scoring and gene ontology enrichment. (A) Kaplan-Meier curves of high and low immune cell infiltration (ICI) scores in patients with genotyping-defined UCEC subtypes. (B–E) Gene ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis of high and low ICI groups. (F) Alluvial plot of genotyping clusters in groups with different genotyping based UCEC subtypes, genotyping scores, and survival outcomes. (G) Expression patterns of immune checkpoint-related genes in different UCEC subtypes. ***$p < 0.001$. PCSK4: Proprotein convertase subtilisin/kexin type 4; CDKN2A: cyclin-dependent kinase inhibitor 2A; IHH: Indian hedgehog homolog; LRRC8D: leucine rich repeat containing 8; CTSW: Cathepsin W; TNFRSF18: Tumour necrosis factor receptor superfamily member 18.

3.4 Tumour mutational burden score and GSEA analysis

KEGG analysis of biological changes associated with each uterine corpus endometrial adenocarcinoma subtype showed that E2F-targets, G2M-checkpoint, mitotic spindle, and MYC proto-oncogene (MYC)-targets-V1 pathways were enriched in the high and low ICI score groups (Fig. 4A). The genes *DEP Domain Containing 1* (*DEPDC1*) and *Diaphanous Related Formin 3 (DIAPH3)* depicted abnormal expression in E2F-targets (Fig. 4B). There were abnormalities in *microtubule associated protein-2* (*MAP2L1*), *Exonuclease 1* (*EXO1*), *Structural Maintenance of Chromosomes 4* (*SMC4*), and *Kinesin Family Member 15* (*KIF15*) metabolism of G2M-checkpoint, mitotic spindle, and xenobiotic spindle (Fig. 4C–E). ICI score subtype affected TMB ($p = 1.1 \times 10^{-5}$; Fig. 4F) having correlation between immune genotyping and TMB ($p = 3.2 \times 10^{-8}$; Fig. 4G).

3.5 Relationship between high/low TMB and clinical correlation analysis of immune scores

The overall survival was not improved in patients with high TMB than those with low TMB ($p = 0.054$; Fig. 5A). However, uterine corpus endometrial adenocarcinoma patients with high TMB and low ICI scores had the highest overall survival, while those with low TMB and high ICI scores had the worst ($p = 0.007$; Fig. 5B). PTEN mutation frequency in high ICI scores group was higher than that in low ICI scores patients (Fig. 5C,D). Overall, the high and low ICI scores patients had similar survival rates (Fig. 5E). There were no differences in immune scores of various clinical traits or in the patients who survived and died ($p = 0.63$, Fig. 5F). The immune scores were thus unsuitable for analysing the patients of different clinical stages ($p > 0.05$; Fig. 5G–J).

4. Discussion

The identification of uterine corpus endometrial adenocarcinoma immunotyping was focussed based on the TME. The immune subtypes were thus identified regarding their potential diagnostic, prognostic, and therapeutic implications. First, the strong associations between various immune cell types and clinicopathological grades were demonstrated, and then six immune subtypes were identified based on the abundance of immune cells and their interrelationships. The subtype D had the highest ICI score and the lowest tumour purity, while subtype E had the lowest score and the highest tumour purity. Next, seven uterine corpus endometrial adenocarcinoma subtypes were identified based on differential gene expression profiles of immune-derived subtypes. Several ICI modulations into the TME were associated with genotyping outcomes, however no significant differences were observed in the survival of patients having different immune types. GSEA enrichment analysis revealed that the high and low groups were enriched in E2F-targets, G2M-checkpoint, mitotic spindle, and MFY-targets-V1 pathways, where *MAD2L1*, *EXO1*, *SMC4* and *KIF15* genes showed aberrant expression.

There are limitations of this study. The conclusions are based on sequencing data and bioinformatics analyses. Large-scale clinical studies are warranted to validate these findings. Previous studies have analysed high-risk uterine corpus endometrial cancer by constructing models such as molecular typing and genetic prognosis. Endometrial adenocarcinoma is focused in this study. Most endometrial cancers are adenocarcinoma. This research is thus targeted, accurate and reliable. The uterine corpus endometrial adenocarcinoma is classified according to the immune microenvironment which demonstrate good predictive performance of immune-based classification strategy. This can predict outcomes in high risk patients of uterine corpus endometrial adenocarcinoma for improved treatment strategies.

PCSK4, *CDKN2A*, *IHH*, *LRRC8D*, *CTSW* and *TNFRSF18* genes were selected for this study as per reported literature [18–21]. Zheng *et al*. [22] found that *MAD2L1* was related to the prognosis of endometrial cancer patients through characterising trunk characteristics of endometrial cancer based on machine learning, and identifying prognostic sub pathways. *MAD2L1* was thus highly expressed in endometrial cancer tissues [22]. *EXO1* gene encoded an evolutionarily conserved exonuclease [23], involved in genomic DNA metabolic processes and had complex pathophysiological roles [24]. Its mechanism in endometrial adenocarcinoma had not been reported and remained unclear. Yang *et al*. [25] showed that *SMC4* silencing inhibited the endometrial adenocarcinoma cell proliferation and promoted apoptosis by regulating Forkhead box O1 (FoxO1) activity. In a study on Non-SMC Condensin I Complex Subunit H (*NCAPH*) role in endometrial adenocarcinoma [26], the gene ensemble enrichment analysis method identified mitotic spindle, G2M-checkpoint, MYC-targets-V1, E2F_targets, MYC-targets-V2, and Mechanistic target of rapamycin complex 1 (mTORC1)-signalling as the *NCAPH* upregulation pathways in endometrial cancer which were consistent with our enrichment results of high and low ICI score groups. There were differences in the *IHH*, *LRRC8D* and *PCSK4* expressions of high and low ICI score groups.

Several studies aimed at finding prognostic biomarkers of endometrial adenocarcinoma followed by efficient immunotherapy. A study developed a genetic signature of six genes (*CTSW*, *PCSK4*, *LRRC8D*, *TNFRSF18*, *IHH* and *CDKN2A*) to prognose endometrial cancer using a robust survival model [19]. Establishing a predictive risk scoring system might provide new biomarkers for prognosing endometrial adenocarcinoma. Moreover, understanding the therapeutic targets of endometrial adenocarcinoma is vital. Liu *et al*. [19] selected eight genes *Zinc finger, SWIM-type containing 1* (*ZSWIM1*), *Nitrogen permease regulator-like 3* (*NPRL3*), *Golgi autoantigen, golgin subfamily a, 7* (*GOLGA7*), *ST6 N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 4* (*ST6GALNAC4*), *cell division cycle 16 homolog* (*CDC16*), *Inositol-tetrakisphosphate 1-kinase* (*ITPK1*), *Proprotein Convertase Subtilisin/Kexin Type 4* (*PCSK4*) and *Coronin 1B* (*CORO1B*) by screening endometrial cancer modules related to regulatory T (Treg) cells. A Tregs-associated risk profile was developed and validated to assess the endometrial cancer prognosis and reflect immune status [27]. Further characterisation of the somatic mutation pattern of endometrial adenocarcinoma immune subtypes showed

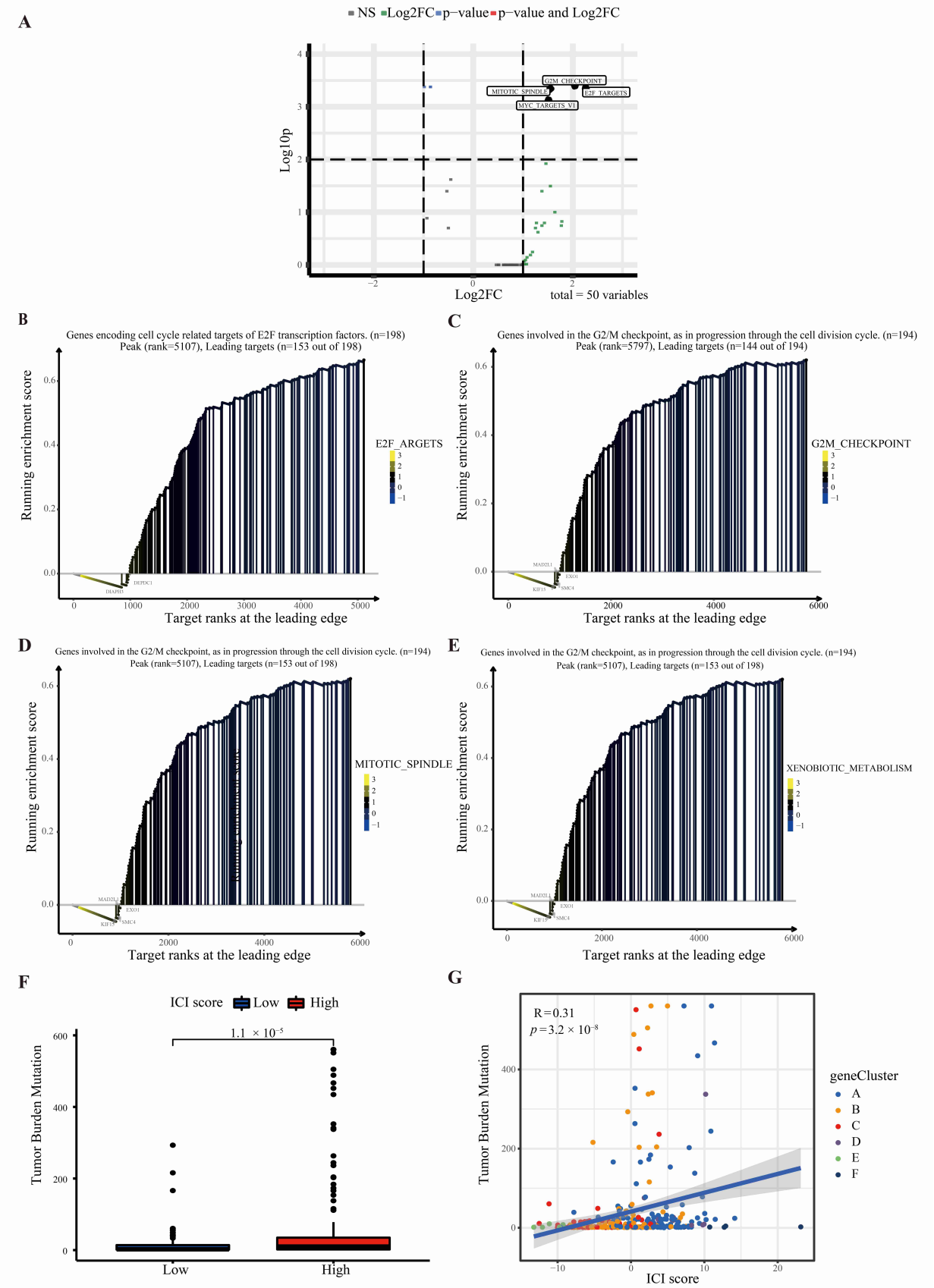

FIGURE 4. Tumour mutation burden score and GSEA analysis. (A–E) Enriched gene sets annotated by KEGG analysis between the high and low genotyping-based UCEC subtypes. (F) Effect of immunological scoring on TMB. ($p = 1.1 \times 10^{-5}$). (G) Effect of genotyping clustering on TMB. ($p = 3.2 \times 10^{-8}$). DEPDC1: The genes DEP domain containing 1; DIAPH3: Diaphanous- related formin-3; MAD2L1: MAD2 mitotic arrest deficient-like 1; EXO1: exocytic Inhibitor 1; SMC4: structural maintenance of chromosome protein-4; KIF15: kinesin family member 15.

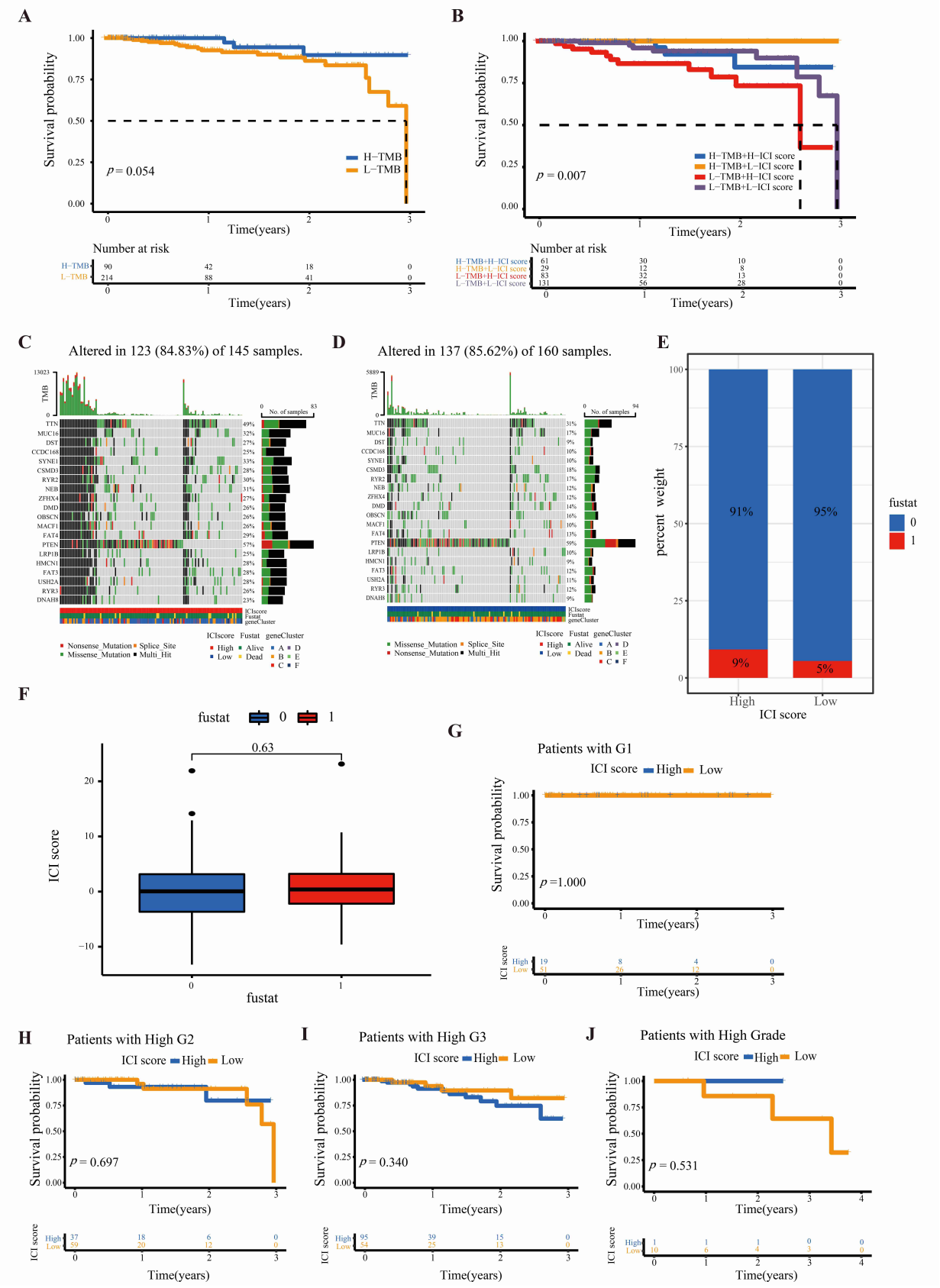

FIGURE 5. Clinical correlation analysis of UCEC immune scoring. (A) Kaplan-Meier curves of TMB in UCEC patients ($p = 0.054$). (B) Kaplan-Maier curve of TMB and ICI scores in UCEC patients ($p = 0.007$). (C,D) OncoPrint was constructed according to high and low ICI scores. Individual patients are represented in each column. (E) Rate of in high and low ICI score subgroups in UCEC. (F) Distribution of ICI scores according to different survival status (0 means alive and 1 means dead) ($p = 0.63$). (G–J) Kaplan-Maier curves of patients with different clinical UCEC staging according to high and low ICI scoring. *$p <$ 0.05, **$p < 0.01$, n.s.: not significant.

that patients of high ICI scores had higher *PTEN* mutation rates than patients of low ICI scores. Long-term molecular observations revealed that *PTEN* inactivation was driving the endometrial adenocarcinoma [28]. The somatic mutations in *PTEN* were associated with PI3 kinase- Phosphatase and Tensin Homolog-the serine/threonine kinase AKT-mammalian target of rapamycin (PI3K-PTEN-Akt-mTOR) axis activation and occurred in 69–80% endometrial tumours. This was the most common genomic abnormality in endometrial cancer. Therefore, *PTEN* mutations were an early event in endometrial cancer pathogenesis as found by several *in vivo* studies. A relatively large proportion of endometrial hyperplasia and complex dysplasia cases harboured *PTEN* mutations [29, 30]. The molecular aberrations in PI3K-PTEN-Akt-mTOR signalling pathway [31] were known for PTEN loss, and high expressions of PI3K and mTOR proteins were associated with poor outcome in TNBC patients. PTEN deletions were the major cause of reduced or absent PTEN expression in Triple negative breast cancer (TNBC) [32]. Somatic mutations in catalytic (p110α) and regulatory (p85α) subunits encoding *PI3K* were also common (40–56% and 20–43%, respectively), in addition to *PTEN* aberrations [33]. Thus, the PI3K-PTEN-Akt-microsphere pathway was a conducive therapeutic target for endometrial cancer. The endometrial cancer classification was complicated. The immune aspects of endometrial cancer were analysed by immuno-subtype and differential genotyping. It could be speculated that patients with certain immune subtypes had better response to immunotherapy. The high immune subtype group had better prognosis to guide regarding the treatment with certain clinical significance.

In this study, immune subtypes of uterine corpus endometrial adenocarcinoma were explored based on the abundance and microenvironment scores of immune cells infiltrating the tumours. Their clinical features and prognostic significance were evaluated. According to the differential analysis of identified immune subtypes, uterine corpus endometrial adenocarcinoma was further assessed based on stable genotypes and relationships between signalling pathway profiles, clinical characteristics, prognostic significance, and specific heterogeneity. Finally, the relationships between immunotype groups, tumour mutational burden, and prognostic significance were investigated. The study outcomes provide an experimental framework for future immunotherapies of uterine corpus endometrial adenocarcinoma.

The prognosis of high-risk uterine corpus endometrial adenocarcinoma remains poor because of inefficient treatment strategies including conventional surgery, radiotherapy, and chemotherapy. Previous studies have shown many immune cells and cytokines infiltrating the uterine corpus endometrial adenocarcinoma microenvironment [26]. Therefore, the immunological classification and prognostic markers of uterine corpus endometrial adenocarcinoma are important for improving prognosis, clinical diagnosis, and treatment. Based on this study, the relationship between immunophenotyping and endometrial cancer immunotherapy can be explored for helping patients in their treatments.

5. Conclusions

Memory B cell, Type 2 T helper cell, Effector memory CD4 T cell, Eosinophil, Type 17 T helper cell, and Activated CD4 T cell were correlated with the pathological grade of endometrial adenocarcinoma ($p < 0.0001$). Six uterine corpus endometrial adenocarcinoma immune subtypes were identified based on immune cells abundance and their interrelationships. ICI score of D subtype was the highest and tumour purity as the lowest, while E subtype had the lowest score and the highest tumour purity. Seven uterine corpus endometrial adenocarcinoma subtypes were identified based on the differential gene expression profiles between the immune subtypes. GSEA enrichment analysis found the current high and low groups being enriched in the E2F target, G2M checkpoint, mitotic spindle and MFY target V1 pathway, where *MAD2L1*, *EXO1*, *SMC4* and *KIF15* genes were abnormally expressed. Further characterization of somatic mutation patterns of uterine corpus endometrial adenocarcinoma immune subtype depicted that *PTEN* mutation rates were higher in patients of high ICI scores than with low ICI scores. There were differences in *IHH*, *LRRC8D* and *PCSK4* expressions of high and low ICI scoring groups. In recent years, studies have aimed to discover prognostic biomarkers of uterine corpus endometrial adenocarcinoma for prognosis and efficient immunotherapy. High-risk uterine corpus endometrial adenocarcinoma had been analyzed through building models. In this study, uterine corpus endometrial adenocarcinoma was classified based on immune microenvironment which demonstrated good predictive performance for the developed immune-based classification strategy. The study outcomes can improve treatment strategies for patients at high risk of uterine corpus endometrial adenocarcinoma.

ABBREVIATIONS

TCGA: The Cancer Genome Atlas Program; TME: tumour microenvironment; ssGSEA: single-sample Gene Set Enrichment Analysis; KEGG: Kyoto Encyclopaedia of Genes and Genomes; TMB: Tumour mutation burden; TIIC: tumour-infiltrating immune cell; ICI: Immune cell infiltration; CD: cluster of differentiation; MDSC: myeloid-derived suppressor cells; PCSK4: Proprotein Convertase Subtilisin/Kexin Type 4; CDKN2A: Cyclin dependent kinase inhibitor 2A; IHH: Indian hedgehog homolog; LRRC8D: Leucine Rich Repeat Containing 8 VRAC Subunit D; CTSW: Cathepsin W; TNFRSF18: TNF Receptor Superfamily Member 18; DEPDC1: DEP Domain Containing 1; DIAPH3: Diaphanous Related Formin 3; MAP2L1: microtubule associated protein-2; EXO1: Exonuclease 1; SMC4: Structural Maintenance Of Chromosomes 4; KIF15: Kinesin Family Member 15; n.s.: not significant; FoxO1: Forkhead box O1; NCAPH: Non-SMC Condensin I Complex Subunit H; ZSWIM1: Zinc finger, SWIM-type containing 1; NPRL3: Nitrogen permease regulator-like 3; GOLGA7: Golgi autoantigen, golgin subfamily a, 7; ST6GALNAC4: ST6 N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 4; CDC16: cell division cycle 16 homolog; ITPK1: Inositol-tetrakisphosphate 1-kinase; PCSK4: Proprotein Convertase Subtilisin/Kexin Type 4; CORO1B: Coronin 1B.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

XC and XRL—Performed conception and design, conducted data analysis and interpretation with further editing. AWX—Collected and assembled data. MMY—provided critical review. CSL—Conducted data analysis and interpretation. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study data were from public database and did not require IRB approval. Ethical review, evaluation, and guidance of the project by IRB were not taken as no clinical specimens were involved in this study.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by the National Natural Science Foundation of China-Youth Science Foundation Project (82002640), the Natural Science Research Project of Jiangsu Province University (20KJB320028), and the Natural Science Foundation of Nanjing University of Traditional Chinese Medicine (XZR2020070).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.ejgo.net/files/article/1691357835179245568/attachment/Supplementary%20material.docx.

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How to cite this article: Xin Chen, Xuerou Li, Aiwei Xiong, Minmin Yu, Changsong Lin. Identification of immune subtypes of uterine corpus endometrial adenocarcinoma based on tumour microenvironment. European Journal of Gynaecological Oncology. 2023; 44(4): 124-135. doi: 10.22514/ejgo.2023.065.