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Integrated transcriptomics and single-cell analysis identify ITGAX⁺ macrophages as key immunosuppressive factors in the prostate cancer tumor microenvironment

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Abstract

Background Prostate cancer (PCa) is a leading cause of cancer-related death in men worldwide. The tumor microenvironment (TME), particularly its immune and stromal components, has a critical influence on PCa progression and patient outcomes. Identifying novel TME-associated biomarkers is important for better understanding PCa progression and may inform future diagnostic or therapeutic strategies.

Methods Transcriptome data from the TCGA-PRAD cohort (499 tumor and 52 normal prostate samples) were analyzed using ESTIMATE and immune deconvolution algorithms, integrated with clinical and survival data. Candidate genes were identified through differential expression, Cox regression, and PPI network analysis, followed by survival and GSEA functional prediction. Single-cell RNA sequencing further delineated gene expression patterns and immune functions within the TME.

Results Integrin subunit αX (ITGAX) emerged as a prognostic gene closely linked to advanced clinicopathological features and poor progression-free survival. High ITGAX expression correlated with increased M2 macrophages and reduced activated NK cells. Single-cell analysis revealed that ITGAX is enriched in macrophage subsets, consistent with a terminally differentiated phenotype associated with immunosuppressive signatures. Cell–cell communication analysis demonstrated extensive inhibitory interactions between ITGAX⁺ macrophages and CD4⁺, CD8⁺, and Treg cells, suggesting potential inhibitory interactions with T cells and a role in shaping an immunosuppressive TME.

Conclusion ITGAX marks a macrophage population associated with an immunosuppressive TME and poor prognosis in PCa. These findings highlight that ITGAX⁺ macrophages may contribute to immune evasion and represent potential targets for future therapeutic investigation.

Keywords Prostate cancer, ITGAX, Macrophages, Tumor microenvironment, Immune suppression



1 Background

Prostate cancer (PCa) ranks among the most common urological malignancies in men worldwide and is a leading cause of cancer-related mortality in males [1–3]. Epidemiological data indicate persistently high incidence and mortality rates in Western countries, with a rising trend observed annually in China [1]. Although early-stage prostate cancer patients may achieve certain therapeutic effects through surgery or endocrine therapy, existing treatments remain inadequate for significantly improving the prognosis of patients with advanced or castration-resistant prostate cancer (CRPC) [2, 4, 5]. Therefore, in-depth analysis of the molecular mechanisms underlying PCa development and the identification of novel prognostic biomarkers and therapeutic targets hold significant clinical importance.

In recent years, a growing body of research has confirmed that the tumor microenvironment (TME) plays a crucial role in the initiation and progression of prostate cancer [6–8]. Comprising tumor cells, stromal cells, immune cells, and extracellular matrix, the TME's complex cellular composition and signaling networks not only influence tumor growth and metastasis but also determine patient sensitivity to immunotherapy [9, 10]. Tumor-infiltrating immune cells (TICs) play a central role in maintaining immune homeostasis, promoting immune escape, and shaping immunosuppressive environments [11–13]. Therefore, identifying key TME-associated molecules is crucial for understanding the immunoregulatory mechanisms of PCa and improving patient outcomes.

Integrin family members play crucial roles in cell adhesion, migration, and immune responses [14, 15]. Among them, integrin subunit α X (ITGAX, also known as CD11c) serves as a key marker for myeloid cells and has been reported to be associated with immune regulation in multiple tumors [16, 17]. However, its role and clinical significance within the prostate cancer TME remain poorly understood.

This study comprehensively utilized TCGA data, ESTIMATE scores, and immune infiltration analysis, combined with protein interaction networks and Cox regression, to identify potential prognostic-related genes, with a particular focus on ITGAX. Further analysis of its expression distribution and immune function within the TME via single-cell RNA sequencing suggested a potential role of ITGAX⁺ macrophages in immune suppression and T cell interactions. This study aims to elucidate the potential mechanisms and clinical value of ITGAX in PCa, providing a foundation for future exploration of novel diagnostic and therapeutic strategies.

2 Materials and methods

2.1 Data acquisition and preprocessing

We downloaded HTSeq-FPKM format transcriptomic data and corresponding clinical information for prostate cancer (PCa) from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>), including 499 tumor samples and 52 normal control samples. For survival analysis, 498 patient samples with complete follow-up information were included.

Single-cell RNA sequencing (scRNA-seq) data were obtained from the Gene Expression Omnibus (GEO) database under accession number GSE274229, originally published by Lyu et al. (Nature, 2025) [18]. This dataset includes 44 prostate cancer samples, comprising 6 metastatic castration-resistant prostate cancer (mCRPC) samples, 25 metastatic hormone-sensitive prostate cancer (mHSPC) samples, and 13 localized prostate

cancer samples. Raw gene–cell count matrices provided by the original study were used for downstream analyses.

2.2 Calculation of immune and stromal scores

The ESTIMATE R package was used to compute the ImmuneScore, StromalScore, and ESTIMATEScore for each sample, reflecting the infiltration levels of immune cells, stromal cells, and their combined effect within the tumor microenvironment (TME), respectively.

2.3 Survival analysis

Survival analysis was performed using the survival and survminer packages in R. Kaplan–Meier methods were employed to plot survival curves, with log-rank tests used to compare differences. A $P < 0.05$ threshold was set for statistical significance.

2.4 Differentially expressed genes (DEGs) screening

The 499 PCa samples were divided into high- and low-score groups based on the median immune and stroma scores. Differential analysis was performed using the limma package. Screening criteria were $|\log_2FC| > 1$ and $FDR < 0.05$. Visualization of differential genes was performed using the pheatmap package to generate heatmaps, and the VennDiagram package to identify genes common to both immune and stroma groups.

2.5 Functional enrichment analysis

The clusterProfiler, org.Hs.eg.db, enrichplot, and ggplot2 packages were used to perform Gene Ontology (GO) and KEGG pathway enrichment analysis on the intersecting genes. Significant enrichment was defined as $P < 0.05$ and $q < 0.05$.

2.6 Clinical-pathological correlation analysis

The limma and ggpvr packages were used to analyze correlations between immune/stroma scores and patient clinical-pathological characteristics (age, Gleason score, staging, etc.). Significance was assessed using the Wilcoxon signed-rank test or Kruskal–Wallis test.

2.7 Prognosis-related gene screening

Performed univariate Cox regression analysis and Kaplan–Meier survival analysis on the intersecting genes, with a filtering threshold of $P < 0.01$. Forest plots were generated to visualize the results.

2.8 Protein–protein interaction (PPI) network

The PPI network for the intersecting genes was constructed using the STRING database (interaction confidence threshold > 0.95) and visualized with Cytoscape v3.8.0.

2.9 GSEA analysis

Enrichment analysis was performed on the MSigDB hallmark, C7, and KEGG gene sets using GSEA v4.0.3 software, based on ITGAX expression levels (high/low groups). Significance thresholds were set at NOM $P < 0.05$ and FDR $q < 0.05$. For low-expression

group gene sets, the criteria were relaxed to NOM $P < 0.05$ due to limited sample size. Visualization employed the *plyr*, *ggplot2*, *grid*, and *gridExtra* packages.

2.10 Tumor-infiltrating immune cells (TICs) analysis

The CIBERSORT algorithm assessed the relative proportions of 22 immune cell types across all PCa samples. Correlation with ITGAX expression was evaluated using the *corrplot*, *ggplot2*, *ggpubr*, and *ggExtra* packages. Screening threshold: $P < 0.05$. Key immune cells associated with ITGAX were identified by intersecting differential expression and correlation analyses.

2.11 Processing and clustering of single-cell transcriptomic data

Following quality control, low-quality cells exhibiting high mitochondrial gene proportions or insufficient gene counts were removed. Data normalization and scaling were performed using the Seurat R package (v4.3.0). Highly variable genes were selected for principal component analysis (PCA) and dimensionality reduction using t-SNE or UMAP. To account for potential batch effects across different samples, dataset integration was performed using Seurat's *FindIntegrationAnchors* and *IntegrateData* functions prior to clustering. Cells were then clustered using graph-based Louvain algorithms and annotated based on known marker genes.

2.12 Cell communication analysis

To analyze signaling interactions among different cell types within the tumor microenvironment, single-cell data underwent cell communication analysis using the CellChat R package (v1.5.0). Intercellular signaling strength was inferred from known ligand-receptor databases, and activity of major signaling pathways was calculated. Signal networks and bubble plots were generated to identify key intercellular interactions and regulatory nodes.

2.13 Pseudotemporal analysis

Pseudotemporal analysis was performed on specific immune cell or macrophage subpopulations using Monocle3 or Slingshot for cell differentiation trajectory inference. Pseudotime axes were constructed to visualize the dynamic transition from early states to mature or functional states, and to analyze the temporal expression trends of key genes.

2.14 Tissue source and inclusion criteria

Human prostate tissue specimens were obtained from patients who underwent surgical resection at Tianjin Medical University Second Hospital, with written informed consent from all participants. The use of human samples was approved by the Institutional Ethics Committee (Approval No. KY2025K404) and conducted in accordance with the Declaration of Helsinki.

Normal prostate tissues were defined as histologically benign prostate tissues obtained from patients undergoing surgery for non-malignant conditions or from tumor-adjacent regions confirmed to be free of malignant cells. Prostate cancer tissues were defined as specimens with a pathological diagnosis of prostate adenocarcinoma, confirmed independently by at least two experienced pathologists according to standard

histopathological criteria. Only samples with adequate tissue integrity and sufficient material for immunofluorescence analysis were included in this study.

2.15 Tissue immunofluorescence staining

Prostate cancer tissue and control tissue were fixed in formalin, embedded in paraffin, and sectioned (4–5 μm thick). Following dewaxing and rehydration, sections underwent high-temperature antigen retrieval in citrate buffer (pH 6.0) or EDTA buffer (pH 8.0), followed by blocking of non-specific binding sites with a blocking solution containing 5–10% normal serum or BSA. Subsequently, primary antibodies targeting specific proteins (e.g., ITGAX, macrophage markers, or T-cell markers) were selected based on experimental design and incubated overnight. Fluorescently labeled secondary antibodies were then added for incubation. For multiplex immunofluorescence, layer-by-layer staining or Opal staining techniques were employed. After nuclear staining with DAPI, images were captured using confocal or high-resolution fluorescence microscopy. Signal quantification analysis, including cell colocalization, cell proportion, and fluorescence intensity statistics, was performed using software such as ImageJ. Detailed information on antibodies is provided in Table S1.

3 Results

3.1 Overall analysis workflow of this study

This study performed a systematic analysis of transcriptomic data from the TCGA-PRAD cohort, including 499 primary prostate cancer tissues and 52 normal prostate samples. The ESTIMATE algorithm was first applied to calculate stromal and immune scores, which were then integrated with clinicopathological data to assess the association between tumor microenvironment (TME) components and patient prognosis. Differentially expressed genes (DEGs) were identified, followed by Cox regression and protein–protein interaction (PPI) network analyses to screen for potential prognostic candidate genes. ITGAX was identified as a prominent candidate, and its potential biological functions were further explored using gene set enrichment analysis (GSEA). To examine ITGAX distribution at the cellular level, single-cell RNA sequencing datasets were incorporated for cell type annotation and characterization of major immune populations within the TME, with particular emphasis on macrophages and their interactions with T cells. The overall workflow, illustrated in Fig. 1, provides an integrative framework that spans large-scale transcriptomic profiling to single-cell level analysis (Fig. 1).

3.2 Stromal and immune scores in the tumor immune microenvironment correlate with clinicopathological features and prognosis in prostate cancer

To examine the relationship between TME composition and clinical outcomes, stromal, immune, and ESTIMATE composite scores were calculated for each sample (Fig. 2A and B). These scores were positively correlated with the corresponding proportions of stromal and immune components in the TME. Kaplan–Meier analysis revealed that higher immune scores were significantly associated with unfavorable prognosis, whereas stromal and ESTIMATE scores showed no clear prognostic relevance. This suggests that immune cell infiltration may serve as a more reliable predictor of PCa outcomes (Fig. 2C and D). Further clinicopathological correlations demonstrated that stromal scores were positively associated with age, Gleason score, and T stage; immune scores correlated

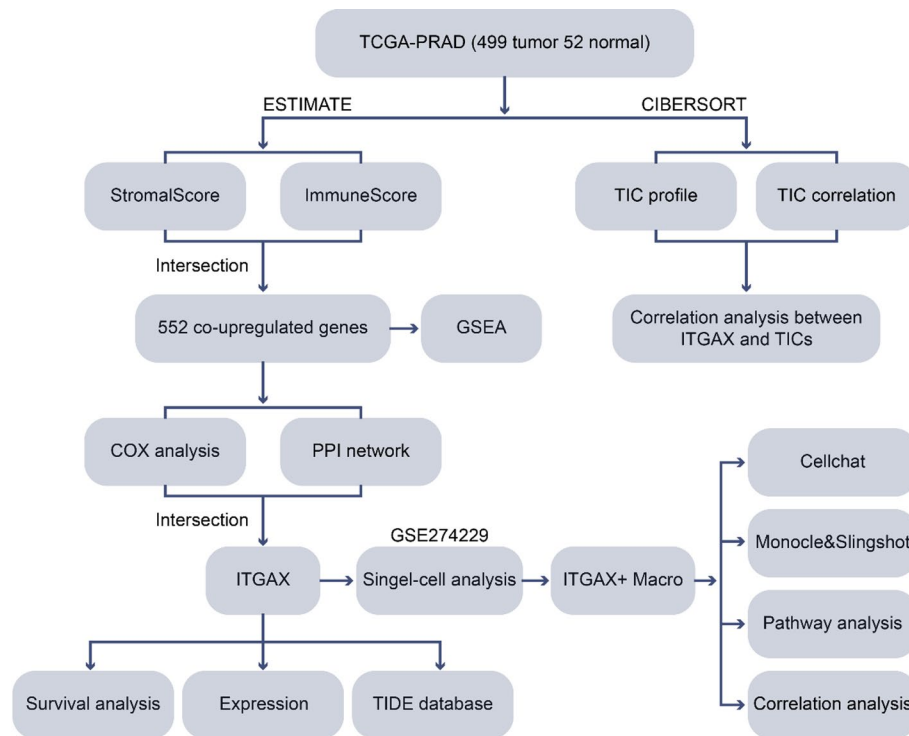


Fig. 1 Overall workflow diagram. Based on TCGA-PRAD transcriptomic and clinical data, we first calculated immune and stromal scores and evaluated their associations with clinical features and prognosis. Subsequently, differentially expressed genes were screened and subjected to functional enrichment and network analysis, with core candidate genes identified via Cox regression. The functional roles of key genes were further assessed through GSEA, immune cell infiltration analysis, and TIDE prediction. Finally, we validated the expression and functions of key genes in the prostate cancer tumor microenvironment using single-cell transcriptomics and immunohistochemistry/immunofluorescence experiments

only with age; while ESTIMATE scores showed patterns similar to stromal scores, also exhibiting positive associations with age, Gleason score, and T stage (Fig. 2E and K). Collectively, these findings indicate that alterations in the balance between stromal and immune components of the TME are associated with patient age, tumor progression, and malignancy in prostate cancer.

3.3 Cross-analysis of stromal- and immune-related differentially expressed genes reveals enrichment in immune pathways

To delineate molecular features associated with TME components, differential expression analysis was performed between high- and low-score groups defined by stromal and immune scores ($|\log_2FC| > 1$, FDR $q < 0.05$). A total of 1,283 genes were upregulated in the high-stromal group and 961 genes in the high-immune group, with 552 genes overlapping between the two groups (Fig. 3A). These shared genes likely represent a core transcriptional program associated with TME-related biological processes in PCa.

Functional enrichment analysis demonstrated that these 552 genes were predominantly involved in immune-related processes. GO analysis revealed enrichment in leukocyte activation, proliferation, adhesion, chemotaxis, and migration, while KEGG analysis highlighted pathways such as hematopoietic cell lineage, cytokine–cytokine receptor interaction, cell adhesion molecules (CAMs), phagosome formation, and leukocyte differentiation (Fig. 3B and C).

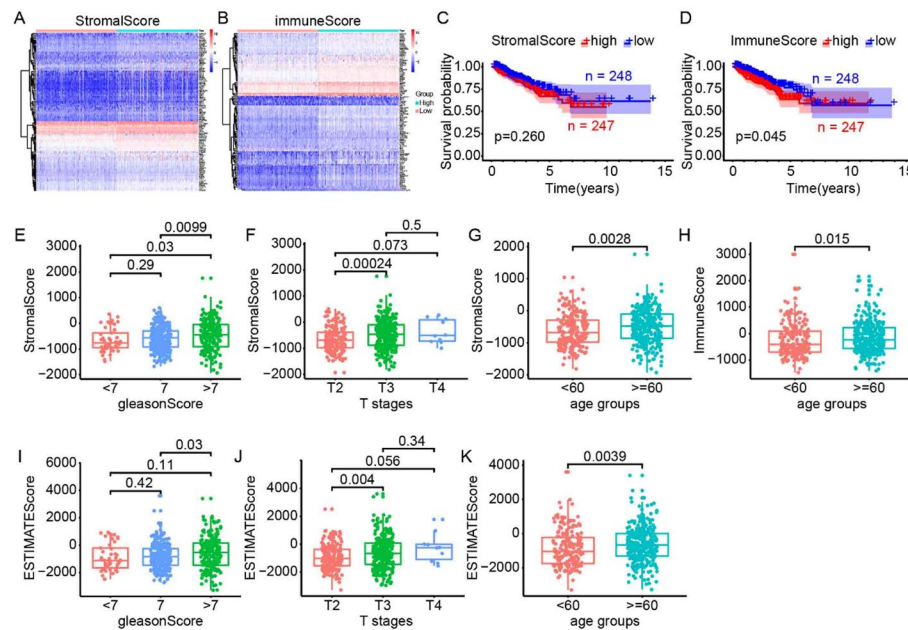


Fig. 2 Association of stromal, immune, and ESTIMATE scores with clinical features and prognosis in TCGA prostate cancer. **A, B** Heatmaps of differentially expressed genes for stromal score (**A**) and immune score (**B**) in TCGA-PRAD data. **C, D** Survival curves for high- and low-stromal-score (**C**) and high- and low-immune-score (**D**) groups in TCGA-PRAD data. **E–G** Distribution of stromal scores across prostate cancer Gleason grades, T stages, and age groups in TCGA-PRAD data. **H** Distribution of immune scores across age groups in TCGA-PRAD prostate cancer data. **I–K** Distribution of ESTIMATE score across Gleason grade, T stage, and age groups in TCGA-PRAD prostate cancer data

As expected, given that ESTIMATE stromal and immune scores are derived from pre-defined gene signatures enriched for immune- and stromal-related biology, the overlapping differentially expressed genes exhibited strong enrichment in immune-associated pathways. Rather than representing an unexpected finding, this result confirms the robustness of the ESTIMATE-based stratification and provides a biologically coherent framework for subsequent analyses focusing on immune regulation within the PCa tumor microenvironment.

3.4 ITGAX is highly expressed in prostate cancer and correlates with clinicopathological features and prognosis

To further evaluate the clinical significance of the 552 candidate genes, univariate Cox regression analysis was performed (threshold: $P < 0.01$, $HR > 1$), identifying 38 genes significantly associated with poor prognosis (Fig. 4A). A protein–protein interaction (PPI) network was then constructed using the STRING database (interaction confidence ≥ 0.95), comprising 200 nodes. The top 35 hub genes were identified based on degree centrality (Fig. 4B, Figure S1). Intersecting the 38 prognosis-related genes with those in the PPI network with degree ≥ 5 yielded only ITGAX and TYROBP as overlapping candidates, both linked to adverse prognosis (Fig. 4C and E). External analyses in independent prostate cancer datasets indicated that ITGAX, but not TYROBP, exhibited a reproducible association with poor prognosis (Figure S2). Expression analysis revealed that ITGAX was significantly upregulated in prostate cancer tissues compared with normal counterparts, whereas TYROBP showed no significant difference (Fig. 4F–I). Immunohistochemical analysis demonstrated that ITGAX expression was markedly higher in prostate cancer tissues than in normal prostate tissues (Fig. 4J). In addition, we examined

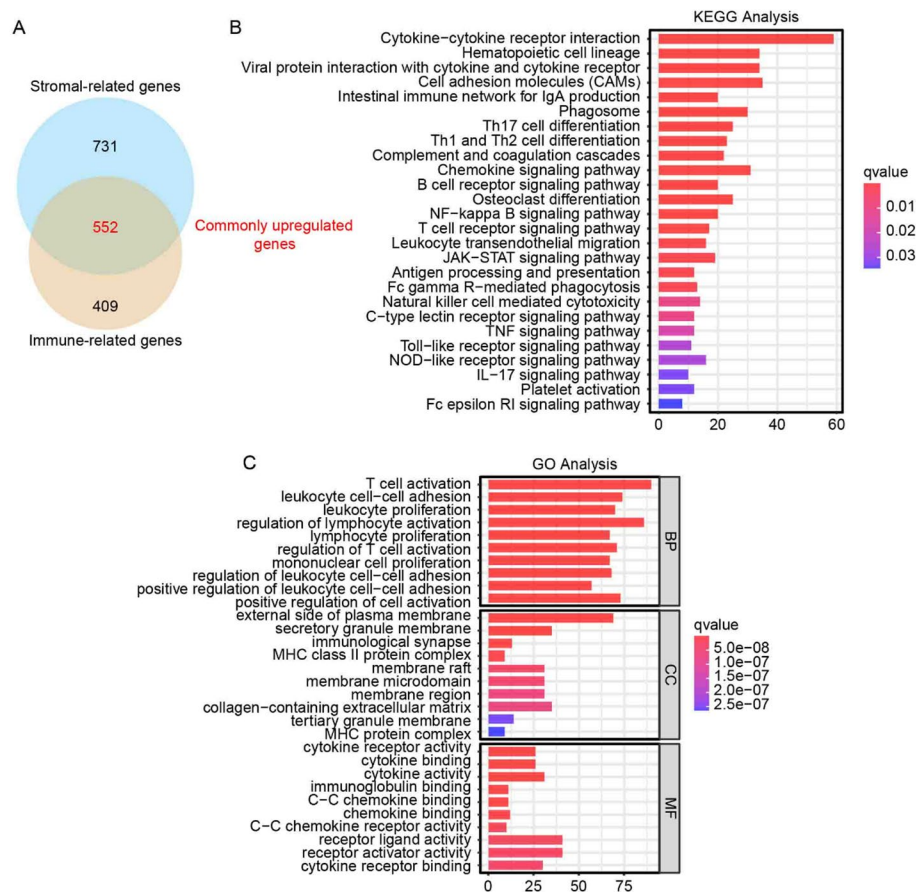


Fig. 3 Identification and enrichment of co-upregulated stromal and immune genes in prostate cancer. **A** Venn diagram of genes co-upregulated by stromal and immune scores in TCGA-PRAD data. **B** KEGG pathway enrichment analysis for the 552 genes in **(A)**. **C** GO pathway enrichment analysis of the 552 genes in **A**

ITGAX expression across different disease stages using the PCTA database. The results revealed significant differences between benign tissue and primary tumors (combined Gleason score) ($P = 0.000164$), as well as between metastatic castration-resistant prostate cancer (mCRPC) and primary tumors with $GS < 7$ ($P = 0.001462$), $GS = 7$ ($P = 0.03906$), and primary tumors with combined GS ($P = 0.026$). In contrast, no significant difference was observed between mCRPC and primary tumors with $GS > 7$ ($P = 0.7287$) (Fig. 4K). Therefore, ITGAX was selected as the prominent candidate gene for subsequent investigations. Multi-database validation, together with immunohistochemical analysis, consistently confirmed ITGAX overexpression in prostate cancer tissues. Moreover, ITGAX expression was positively correlated with patient age, Gleason score, T stage, and N stage, highlighting its potential association with PCa progression and poor prognosis (Fig. 4L and O).

3.5 GSEA analysis indicates ITGAX may drive tumor microenvironment remodeling in prostate cancer

To investigate the potential functions of ITGAX, GSEA was performed between high- and low-expression groups. The ITGAX high-expression group was significantly enriched in immune-related pathways, including allograft rejection, inflammatory response, interferon signaling, chemokine signaling, cytotoxicity, and cytokine-receptor

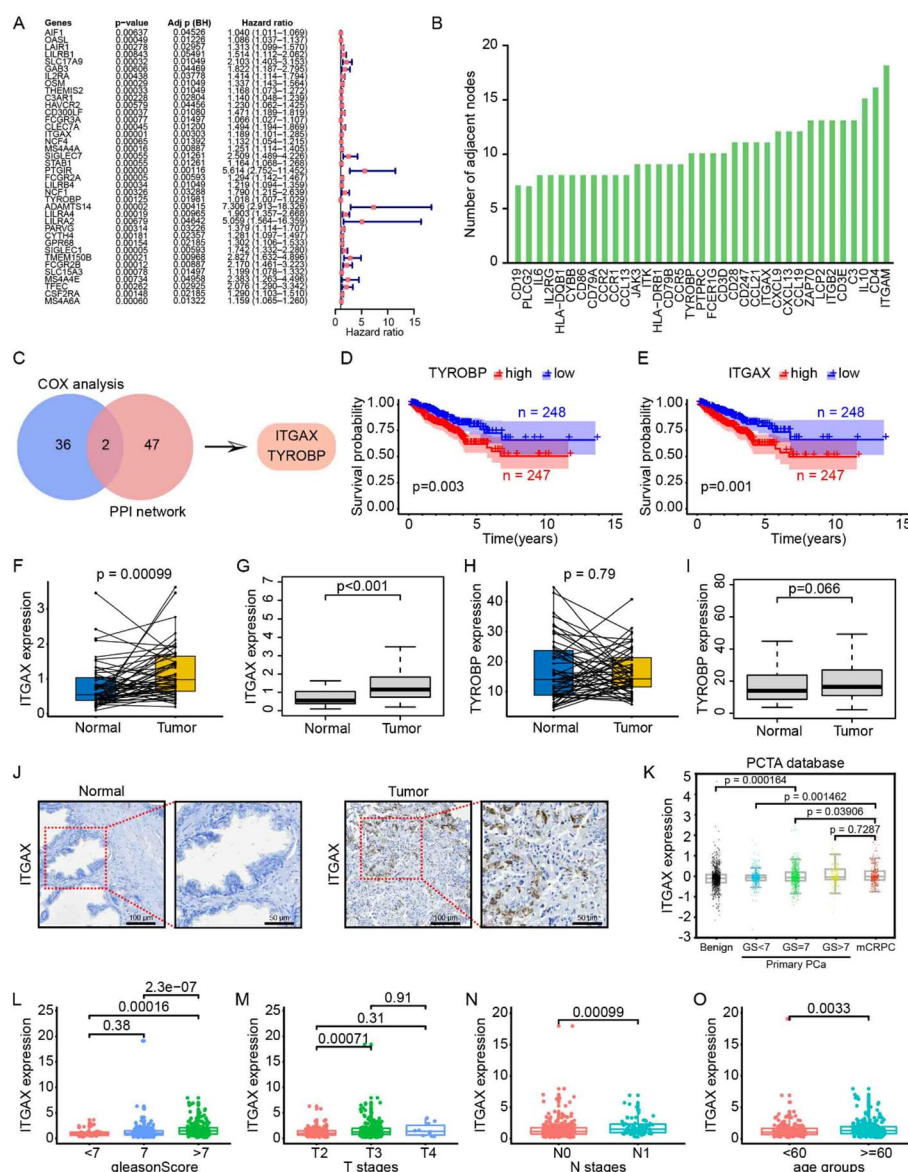


Fig. 4 Prognostic and clinical implications of TYROBP and ITGAX derived from stromal and immune gene signatures in TCGA-PRAD. **A** COX regression analysis of genes co-upregulated by stromal and immune scores ($HR > 1$ and $P < 0.05$). **B** Bar plot showing the top 35 genes ranked by degree centrality identified from the PPI network analysis. **C** Venn diagram showing the intersection of genes significantly identified by COX univariate analysis and those with degree ≥ 5 in the PPI network. **D, E** Survival curves for high- and low-expression groups of TYROBP (**E**) and ITGAX (**F**) in TCGA-PRAD data. **F, G** Boxplots showing paired (**G**) and unpaired (**H**) differences in ITGAX expression between normal prostate and prostate cancer tissues. **H, I** Boxplots showing paired (**I**) and unpaired (**J**) differences in TYROBP expression between normal prostate and prostate cancer tissues. **J** Immunohistochemical detection of ITGAX expression in normal prostate versus prostate cancer tissue. **K** Expression differences of ITGAX across prostate cancer stages in the Prostate Cancer Transcriptome Atlas (PCTA) database (<http://www.thepcta.org>). Significant differences were observed for benign vs. primary tumors (GS combined, $P = 0.000164$), mCRPC vs. GS < 7 ($P = 0.001462$), mCRPC vs. GS $= 7$ ($P = 0.03906$), and mCRPC vs. primary tumors (GS combined, $P = 0.026$), but not for mCRPC vs. GS > 7 ($P = 0.7287$). **L–O** ITGAX expression in TCGA-PRAD data across prostate cancer Gleason scores, T stages, N stages, and age groups

interactions. Notably, fewer enriched pathways were detected in the low-expression group compared with the high-expression group, which may reflect a lower magnitude of transcriptional variation in these samples (Fig. 5A and C, Figure S3A–3 C). These findings suggest that high ITGAX expression is associated with a shift from metabolic to

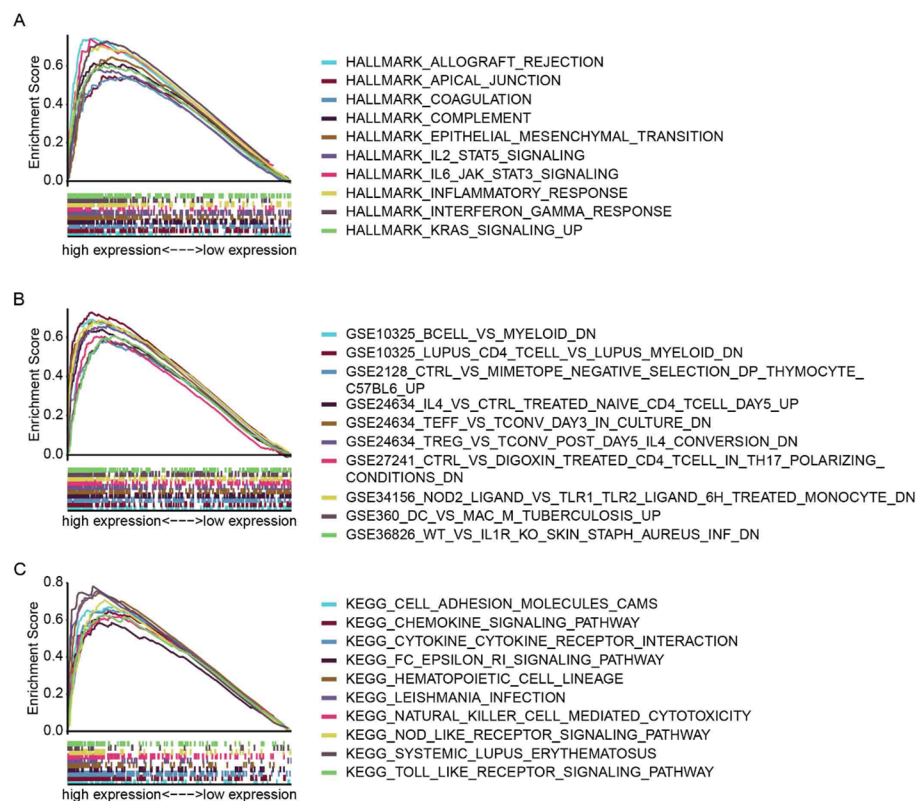


Fig. 5 Gene set enrichment analysis identifies biological and immune programs associated with ITGAX expression in prostate cancer. **A** GSEA showing significantly enriched MSigDB Hallmark pathways in the ITGAX-high versus ITGAX-low group. **B** GSEA showing significantly enriched C7 pathways in the ITGAX-high versus ITGAX-low group. **C** GSEA showing significantly enriched KEGG pathways in the ITGAX-high versus ITGAX-low group

immune-related transcriptional programs, potentially reflecting alterations in the TME of PCa.

3.6 ITGAX expression correlates with immune cell infiltration in prostate cancer

To further examine the relationship between ITGAX and immune infiltration, the CIBERSORT algorithm was applied to TCGA-PCa samples to quantify 22 immune cell types (Fig. 6A and B). Four cell types showed significant associations with ITGAX expression: Th cells, activated NK cells, M2 macrophages, and monocytes (Fig. 6C). Notably, ITGAX levels were positively correlated with M2 macrophages and negatively correlated with activated NK cells, indicating a potential association with an immuno-suppressive TME. These correlations suggest that ITGAX expression may reflect macrophage polarization toward the M2 phenotype and altered NK cell activity (Fig. 6D).

3.7 Single-cell transcriptomic analysis identifies macrophages as the primary ITGAX-expressing population

To resolve the cellular localization of ITGAX, single-cell transcriptomic data from PCa were analyzed using dimensionality reduction and clustering. Thirteen major subpopulations were identified, including myeloid cells, T cells, B cells, and epithelial tumor cells (Fig. 7A and D). ITGAX expression was almost exclusively confined to the myeloid compartment, with marked enrichment in macrophages, while its expression in T cells, B cells, and tumor cells was negligible (Fig. 7E and F). These findings indicate that ITGAX

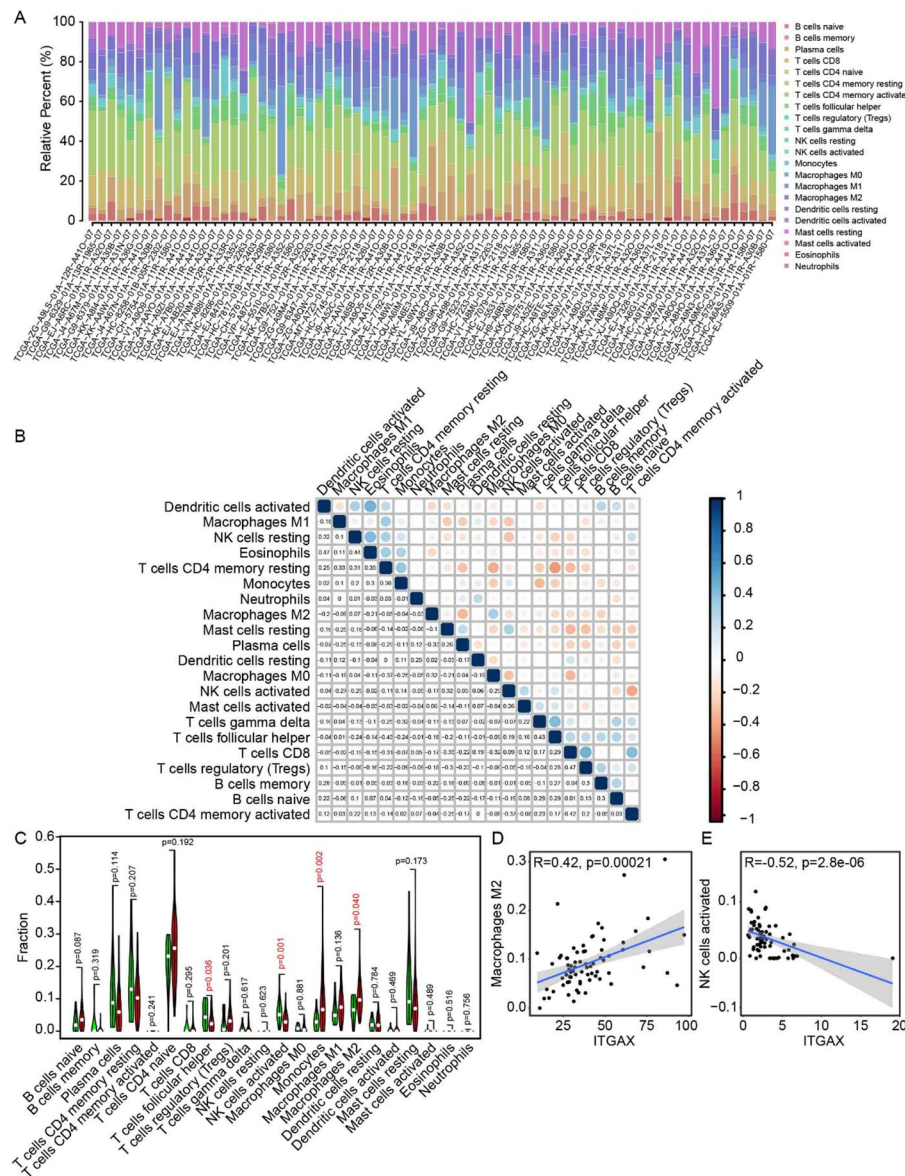


Fig. 6 Association of ITGAX expression with immune cell infiltration in prostate cancer. **A** CIBERSORT analysis of immune cell infiltration proportions and correlations in TCGA-PRAD samples. **B** Violin plot showing immune cell proportions in ITGAX high and low groups. **C**, **D** Scatter plots demonstrating correlations between ITGAX expression and M2 macrophages (**C**) and activated NK cells (**D**)

is primarily expressed by tumor-associated macrophages and is consistent with an immunosuppressive phenotype in the prostate cancer tumor microenvironment.

3.8 ITGAX⁺ macrophages exhibit potent immunosuppressive features

Secondary clustering of myeloid cells in PCa single-cell data identified a distinct ITGAX⁺ macrophage subset (Fig. 8A and C). The infiltration of this population varied markedly across patients, indicating an association with TME heterogeneity (Fig. 8D). Transcriptional profiling showed high expression of multiple immunosuppressive genes, with a transcriptional signature closely aligned with M2 macrophages, suggesting potential immunosuppressive characteristics (Fig. 8E and F). Functional enrichment analysis indicated enrichment in pathways related to T cell regulation, inflammatory response

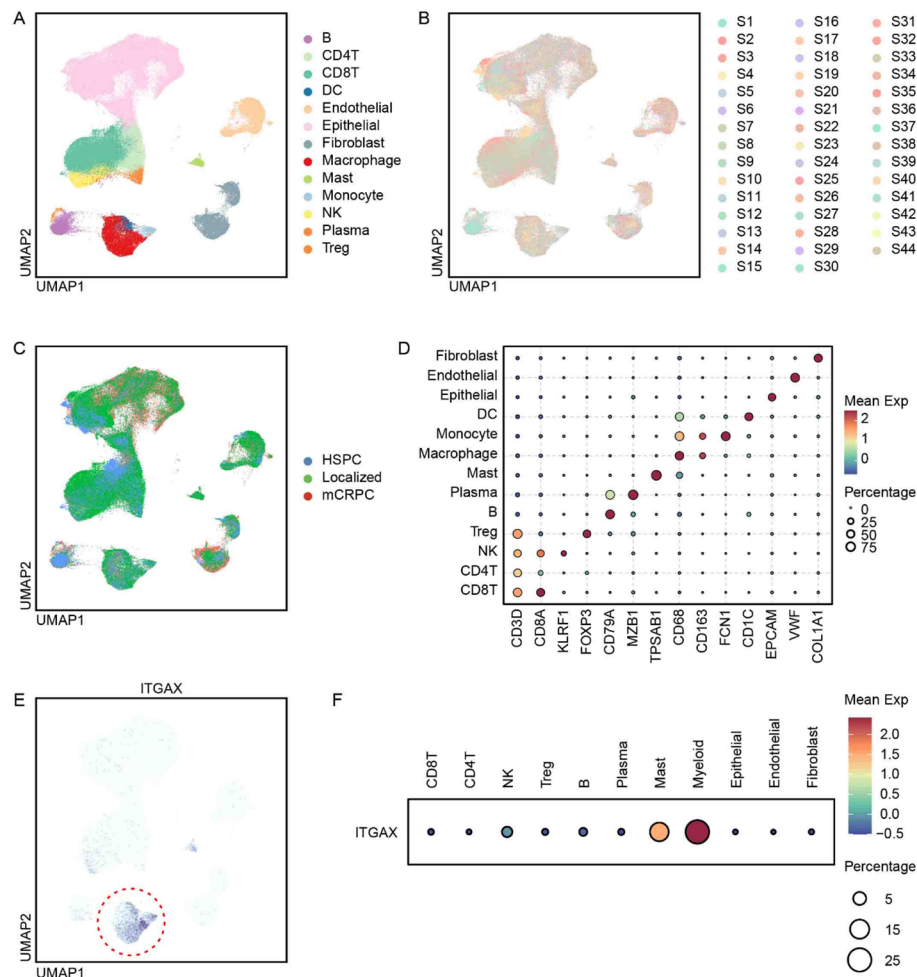


Fig. 7 Single-cell transcriptomic landscape of prostate cancer and ITGAX Expression across major cell types. **A–C** Single-cell transcriptome UMAP visualization colored by major cell types (**A**), sample origin (**B**), and disease group (**C**). **D** Bubble plot showing signature gene expression in major prostate cancer cell types. **E** FeaturePlot displaying ITGAX expression localization across major cell types. **F** Bubble plot showing ITGAX expression levels in major prostate cancer cell types

modulation, and immune signaling associated with tumor progression (Fig. 8G). Given that CD163 and CD206 are well-established markers of M2-like immunosuppressive macrophages, immunofluorescence staining was performed to assess M2 macrophage infiltration in ITGAX-high tumors (Fig. 8H and I). Together, these findings suggest that ITGAX⁺ macrophages may contribute to immunosuppressive features in the PCa microenvironment.

3.9 ITGAX⁺ macrophages occupy the terminal stage of differentiation

To explore their developmental state, Monocle3 and Slingshot trajectory analyses were applied to macrophage subsets in PCa. ITGAX⁺ macrophages were predominantly located at the terminal end of the differentiation trajectory, suggesting they may correspond to a mature, terminally differentiated population (Fig. 9A and C). Along this trajectory, immunosuppressive genes such as CTSD, CCL18, SPP1, and MMP9 were progressively upregulated, reaching peak expression in ITGAX⁺ macrophages (Fig. 9D). These results indicate that ITGAX⁺ macrophages occupy a terminally differentiated state

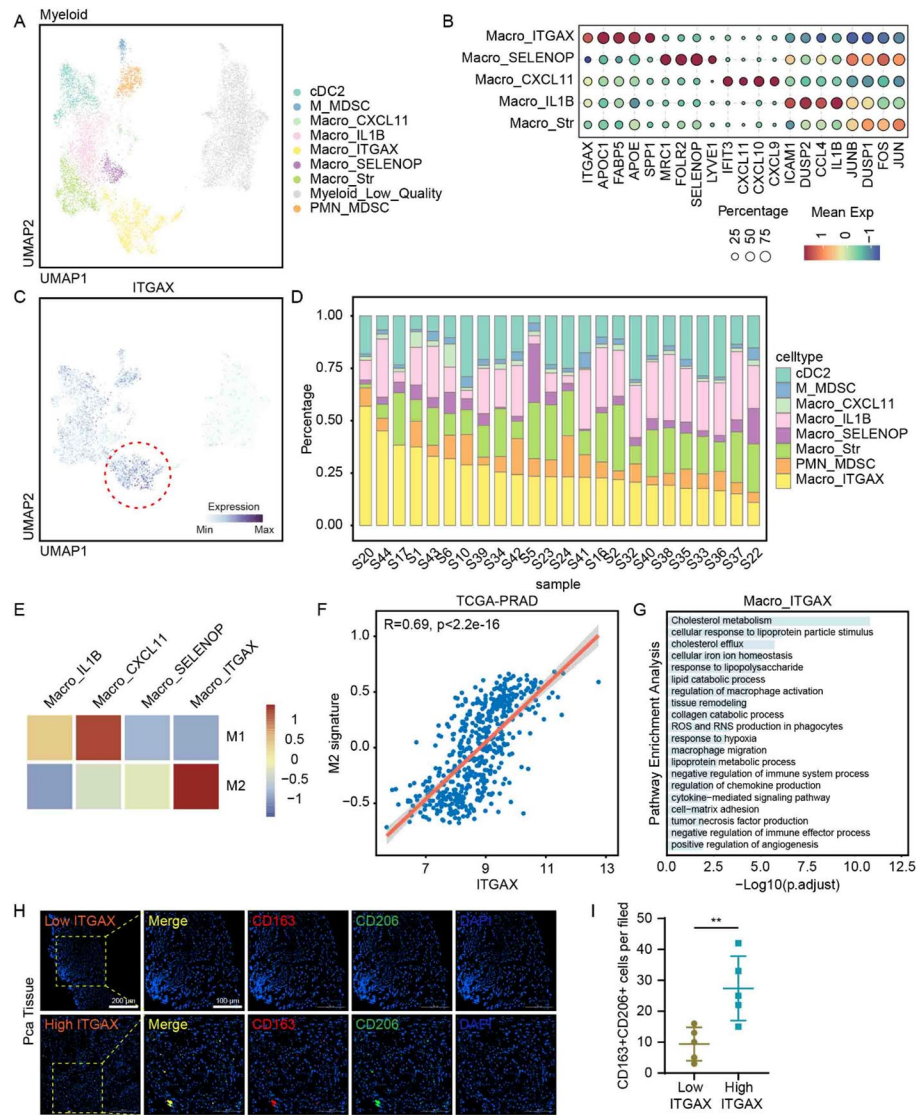


Fig. 8 ITGAX expression in myeloid cell subsets correlates with M2 macrophage signatures and immune infiltration in prostate cancer. **A** UMAP visualization of single-cell transcriptomes showing myeloid cell subpopulation distribution. **B** Bubble plot displaying marker gene expression across myeloid cell subpopulations. **C** FeaturePlot illustrating ITGAX expression localization within myeloid cell subpopulations. **D** Bar chart showing the proportion of myeloid cell subpopulations in different prostate cancer samples. **E** Heatmap illustrating the correlation between four myeloid cell types and M1/M2 macrophage signatures. **F** Scatter plot demonstrating the correlation between ITGAX and M2 signature in the TCGA-PRAD dataset. **G** Pathway enrichment analysis for ITGAX+ macrophages. **H, I** Representative immunofluorescence images (**H**) and quantitative analysis (**I**) demonstrating CD163+ cell infiltration in high- and low-ITGAX prostate cancer tissues

and display transcriptional features associated with immunosuppressive function. Thus, ITGAX marks mature, immunosuppressive macrophages that are likely to contribute to tumor immune evasion and PCa progression.

3.10 CellChat analysis reveals extensive immunosuppressive crosstalk between ITGAX⁺ macrophages and T cells

CellChat analysis demonstrated robust ligand–receptor interactions between ITGAX⁺ macrophages and CD4⁺ T cells, CD8⁺ T cells, and Tregs, with ITGAX expression showing a strong positive correlation with Treg abundance (Fig. 10A and E). These

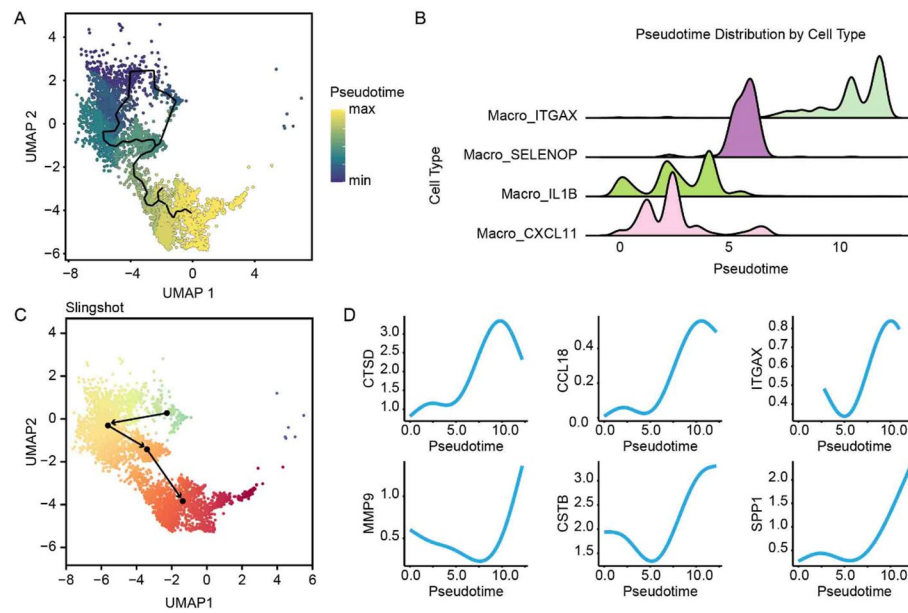


Fig. 9 Pseudo-time analysis of macrophage subsets highlights ITGAX-associated differentiation pathways in prostate cancer. **A, B** Monocle3 pseudo-time analysis: UMAP plot (**A**) and pseudo-time trajectory (**B**) of macrophage subpopulations. **C** Slingshot pseudo-time analysis revealing macrophage subpopulation differentiation trajectories. **D** Pseudo-time dynamics of genes including CTSD, CCL18, SPP1, MMP9, and ITGAX in macrophage subpopulations

interactions were enriched in immunosuppressive and exhaustion-related pathways, including HLA antigen presentation, CD86–CTLA4/CD28, MIF–CD74, SPP1–CD44, NECLP–TIGIT, and LGALS9–HAVCR2 (Fig. 10F). Consistently, immunofluorescence staining revealed increased Treg infiltration in ITGAX-high tumors (Fig. 10G and H). Collectively, these results suggest that ITGAX⁺ macrophages are associated with extensive predicted ligand–receptor interactions with multiple T cell subsets, which may relate to increased Treg abundance and altered effector T cell signatures in the tumor microenvironment.

3.11 TIDE analysis indicates ITGAX impairs T cell function and immunotherapy response

To further assess the functional impact of ITGAX, TIDE database analysis was performed. ITGAX-high tumors exhibited significantly lower T cell function scores, consistent with reduced effector activity (Fig. 11A and D). Correlation analysis further demonstrated strong positive associations between ITGAX expression and key T cell exhaustion markers, including PD-1, CTLA-4, and TIGIT (Fig. 11E). Immunofluorescence confirmed reduced CD8⁺ T cell infiltration and elevated expression of exhaustion-associated molecules in ITGAX-high tumors (Fig. 11F and G). Collectively, these data suggest that high ITGAX expression is associated with features of immune suppression in PCa, including reduced T cell function and increased expression of exhaustion markers, which may relate to immunotherapy response.

4 Discussion

The tumor microenvironment (TME) plays a pivotal role in the initiation, progression, and therapeutic response of Prostate cancer (PCa) [6, 11, 19]. It is increasingly recognized that the accumulation of immunosuppressive cells, such as tumor-associated

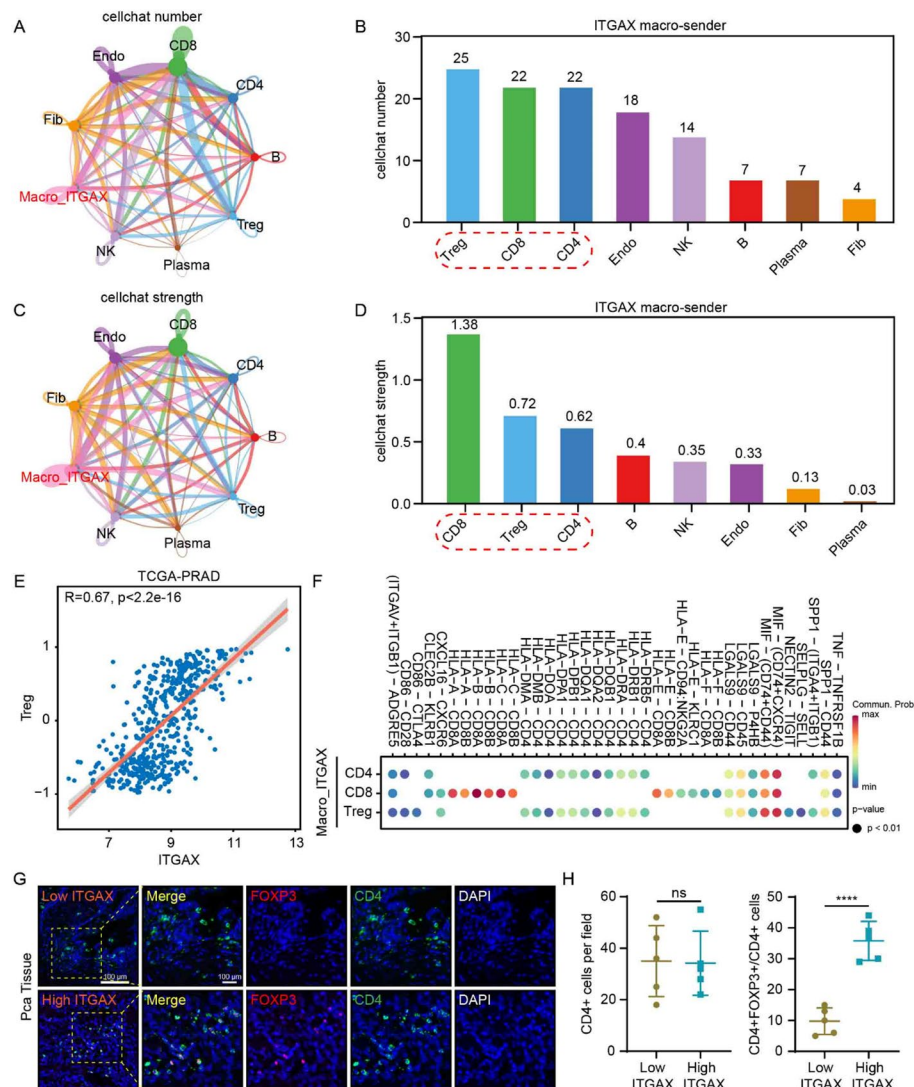


Fig. 10 Cell-cell communication analysis reveals crosstalk between ITGAX+ macrophages and immune cells in prostate cancer. **A, B** CellChat analysis of intercellular communication counts. **C, D** CellChat analysis of intercellular communication strength. **E** Scatter plot showing correlation between ITGAX and Treg signature in the TCGA-PRAD dataset. **F** Bubble plot illustrating interaction pathways between ITGAX+ macrophages and CD4+T, CD8+T, and Treg cells. **G, H** Representative immunofluorescence images (**G**) and quantitative analysis (**H**) showing CD4+FOXP3+ cell infiltration in prostate cancer tissues from high- and low-ITGAX groups

macrophages (TAMs) and regulatory T cells (Tregs), together with extensive stromal remodeling, is associated with a permissive microenvironment that may support tumor growth and progression [20, 21]. Several studies have shown that immunologically “cold” TMEs with low effector T cell infiltration and high suppressive cell content are associated with aggressive clinical behavior and poor prognosis in PCa [22–24]. These observations underscore the need to dissect the molecular drivers underpinning TME composition to identify new biomarkers and therapeutic targets.

In this context, our study identified ITGAX as a TME-associated gene that is markedly upregulated in PCa tissues and positively correlated with adverse clinical features, including higher Gleason score, advanced TNM stage, and older patient age. Previous studies have reported ITGAX as a marker of myeloid-lineage cells, particularly dendritic cells and macrophages [25–27], but its prognostic relevance in PCa has remained largely

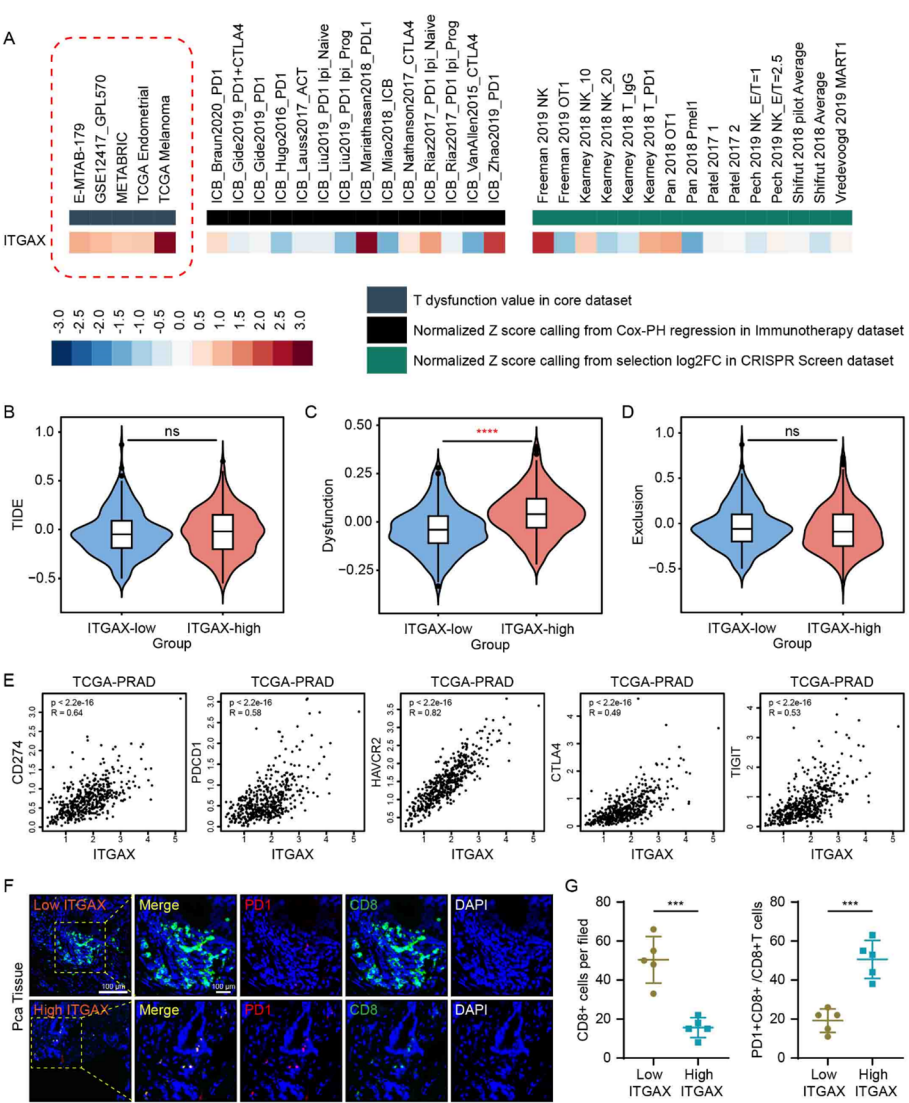


Fig. 11 ITGAX associates with immune suppression and T cell exhaustion in prostate cancer. **A** Heatmap of ITGAX gene scores in the TIDE database. **B–D** Prediction of ITGAX’s immune-suppressive functions based on the TIDE melanoma model, including TIDE score (**B**), T cell dysfunction score (**C**), and T cell exclusion score (**D**). **E** Scatter plot showing correlation between ITGAX and TEX-related markers in the TCGA-PRAD dataset. **F, G** Representative immunofluorescence images (**F**) and quantitative analysis (**G**) demonstrating PD1 + CD8+ cell infiltration in prostate cancer tissues from high- and low-ITGAX groups

unexplored. The strong association between ITGAX expression and aggressive clinicopathological parameters in our analysis suggests that ITGAX may serve as a surrogate indicator of an immunosuppressive TME, offering potential utility for risk stratification in PCa.

Single-cell transcriptomic analysis revealed that ITGAX expression was predominantly confined to macrophages exhibiting gene signatures consistent with M2-like polarization. These ITGAX⁺ macrophages localized at the terminal end of macrophage differentiation trajectories, suggesting a terminally differentiated state with transcriptional features associated with immunosuppressive function. Functional enrichment analysis further showed that ITGAX-high tumors were enriched in immune regulatory pathways, such as cytokine–receptor interactions and chemokine signaling, while

ITGAX-low tumors were enriched in metabolic pathways, including oxidative phosphorylation and protein metabolism. This immune–metabolic shift may reflect differences in macrophage functional states associated with ITGAX expression. Moreover, ligand–receptor analysis identified predicted interactions between ITGAX⁺ macrophages and CD4⁺, CD8⁺, and regulatory T cells, which were consistent with associations from CIBERSORT and TIDE analyses suggesting reduced effector T cell signatures and increased Treg abundance in ITGAX-high tumors.

Notably, fewer significantly enriched pathways were observed in ITGAX-low tumors compared with the high-expression group, which may reflect a lower magnitude of transcriptional variation and fewer pronounced signaling changes. While ITGAX-high tumors were associated with multiple immune-related pathways, ITGAX-low tumors were primarily linked to metabolic processes, indicating differences in signaling patterns within the tumor microenvironment. Collectively, these findings suggest that ITGAX is associated with macrophages exhibiting features of immunosuppressive function, which may relate to altered T cell signatures and immunosuppressive TME characteristics. Given its association with tumor aggressiveness and TME features, ITGAX may serve as a potential prognostic biomarker and a candidate for future studies exploring therapeutic strategies targeting macrophages or T cell responses.

Nevertheless, several limitations should be acknowledged. This study relied largely on publicly available datasets, which may introduce selection bias, and direct functional validation of ITGAX in vitro and in vivo is still required. External validation analyses were primarily focused on ITGAX, the core candidate identified in this study, whereas other stromal and immune genes prioritized through computational screening should be regarded as exploratory due to less consistent prognostic associations across datasets. Furthermore, most TCGA data are derived predominantly from patients of European ancestry, raising concerns regarding limited population diversity. The relatively small sample size of available single-cell datasets also warrants confirmation in larger and more ethnically diverse cohorts. Finally, immune infiltration, cell–cell communication, and immune evasion analyses were based on computational inference using predefined gene signatures and algorithms, reflecting relative rather than absolute immune or stromal abundance and not establishing causal or directional relationships.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s12672-026-04737-3>.

Supplementary Material 1.

Author contributions

Y.S., P.Z. and Z.S. conceived and designed the study. Y.S., S.S. W.W. performed bioinformatics analyses. L.L. and J.T. carried out validation experiments. Z.S., L.L. and J.T. contributed to data curation and interpretation. P.Z. and Z.S. supervised the project. Y.S. and S.S. wrote the main manuscript text, and all authors reviewed and approved the final manuscript.

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Data availability

The datasets analyzed in this study are publicly available. Transcriptome and clinical data of prostate cancer patients were obtained from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>). The single-cell RNA sequencing dataset was retrieved from the Gene Expression Omnibus (GEO) under accession number GSE274229, GSE54460 (<https://www.ncbi.nlm.nih.gov/geo/>).

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Second Hospital of Tianjin Medical University approved the study. All human tissue samples used in this study were obtained with informed consent from patients. All methods were performed in accordance with the relevant guidelines and regulations of the Ethics Committee of the Second Hospital of Tianjin Medical University. All human tissue samples used in this study were obtained with written informed consent from the patients prior to sample collection.

Consent for publication

No individual personal data (such as images or details) are included in this manuscript. Consent to Publish: not applicable.

Competing interests

The authors declare no competing interests.

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