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Integrated single-cell and bulk transcriptomics reveals an IGF2BP3-driven metabolic circuitry in endometrial cancer

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Abstract

Background This study investigates the epigenetic-metabolic interplay in endometrial cancer (EC) through integrated single-cell and bulk multi-omics analyses, aiming to identify prognostic biomarkers and elucidate mechanisms driving metabolic reprogramming.

Methods Single-cell RNA sequencing (scRNA-seq) data from five EC patients and bulk RNA-seq data from TCGA/GTEX cohorts were analyzed. Non-negative matrix factorization (NMF) clustering, CellChat, and SCENIC were employed for subtyping, intercellular communication, and transcriptional network analysis. Prognostic models were constructed using LASSO-Cox regression. Functional validation included RIP-seq, ChIP-qPCR, luciferase assays, and in vivo tumor models. Metabolic activity was assessed via glucose/lactate measurements and extracellular acidification rate (ECAR).

Results Our analysis identified 68 epigenetics-related genes, with 20 prognostic biomarkers. Analysis of scRNA-seq data from five EC patients identified major cell populations, including B cells, ciliated cells, endothelial cells, epithelial cells, fibroblasts, macrophages, monocytes, smooth muscle cells, and T cells. NMF clustering of malignant epithelial cells identified four epigenetically distinct subpopulations (Epigen-C1–C4) within tumors, rather than patient-level molecular subtypes. Bulk TCGA/GTEX analyses further supported prognostic modeling at the cohort level. Epigen-C2, characterized by hypermetabolic activity, exhibited advanced differentiation and poor prognosis. A six-gene prognostic signature (SDF2L1, ASPM, MUC1, MCM7, PHGDH, MSX1) derived from Epigen-C2-associated differentially expressed genes demonstrated robust risk stratification across training, testing, and full cohorts. Mechanistically, IGF2BP3, an m6A reader overexpressed in EC, may stabilize MUC1 mRNA by binding to m6A modification sites, thereby enhancing MUC1 protein expression. Upregulated MUC1 amplified HIF-1 α levels, which transcriptionally activated the glycolytic enzyme ENO1 through promoter binding. Concurrently, IGF2BP3 stabilized ENO1 mRNA, forming a feed-forward loop that accelerated glycolysis, evidenced by increased glucose consumption, lactate production, and ECAR. In vivo, IGF2BP3 knockdown suppressed tumor growth, while overexpression exacerbated

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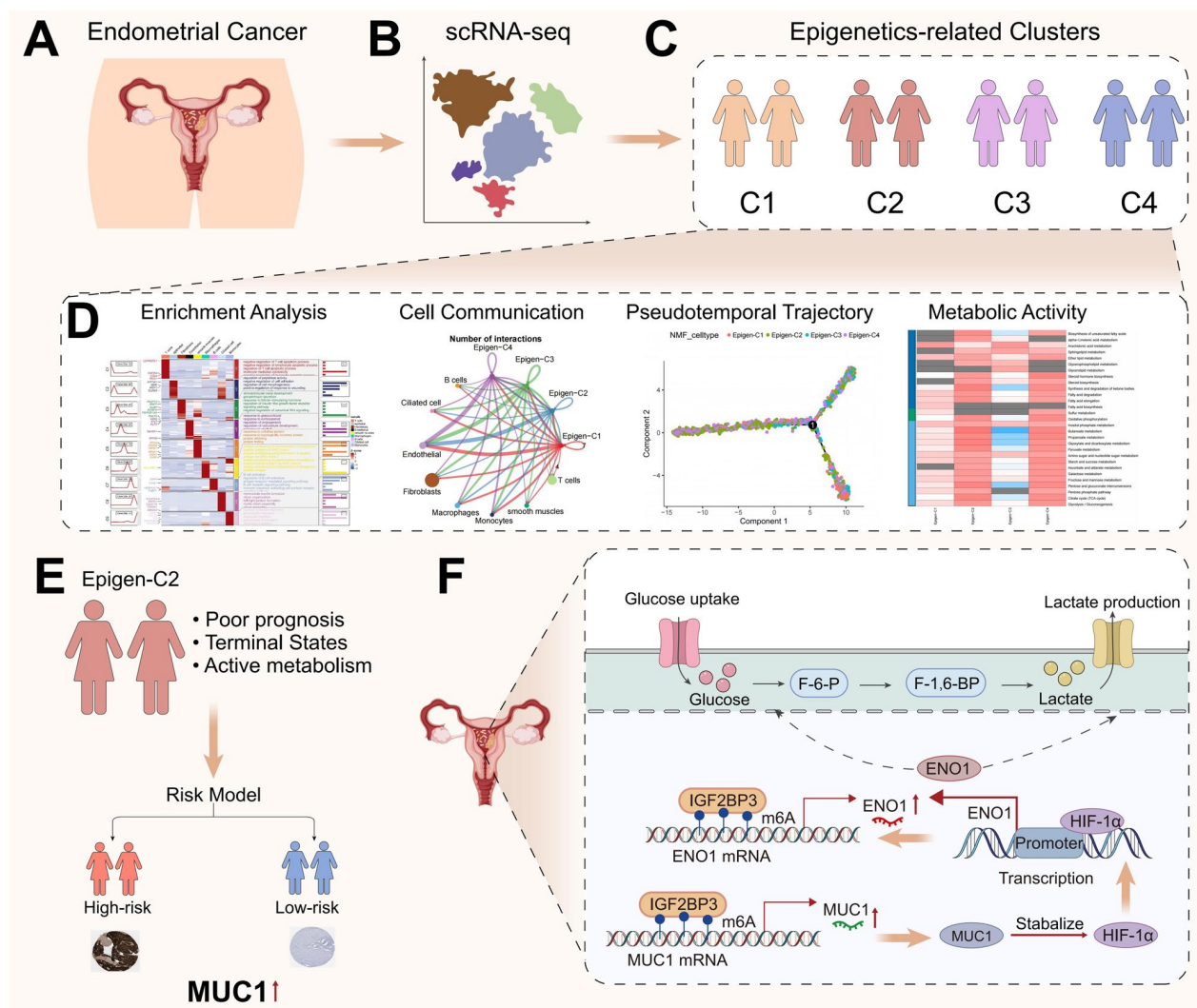
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progression. These findings unveil the IGF2BP3/MUC1/HIF-1 α /ENO1 axis as a critical driver of m6A-associated metabolic reprogramming in EC.

Conclusions This study characterizes intra-tumoral malignant epithelial heterogeneity in EC using single-cell epigenetics, informing potential precision therapy hypotheses without establishing a stable patient-level molecular classification. It defines the IGF2BP3/MUC1/HIF-1 α /ENO1 axis as a critical driver of m6A-associated metabolic reprogramming in EC.

Keywords Endometrial Cancer, IGF2BP3, Metabolic Reprogramming, Epigenetic Modification, Tumor Microenvironment

Graphical Abstract



Background

Endometrial cancer (EC) is recognized as the sixth most prevalent female malignancy worldwide, with notable geographic variations in its incidence [1, 2]. The underlying mechanisms governing the genetic and epigenetic alterations that drive EC progression and influence its response to cancer therapy remain incompletely understood [3]. The continued exploration of biomarker-driven

strategies, novel targeted therapies, and chemotherapy-free regimens remains essential to address existing unmet clinical needs and enhance oncologic outcomes across various molecular subtypes.

Epigenetic regulation, a mechanism that modulates gene expression through heritable changes in chromatin structure without altering the underlying DNA sequence, plays a critical role in the initiation, progression, and

therapeutic resistance of cancer [4]. Key mechanisms encompass dynamic histone modifications [5], DNA hypermethylation at the promoters of tumor suppressor genes [6], dysregulation of non-coding RNAs [7], as well as epitranscriptomic alterations including m6A methylation that influence RNA stability and translational efficiency [8]. Recent studies highlight the clinical significance of epigenetic dysregulation. Research has demonstrated that METTL3-mediated m6A modifications contribute to the stabilization of oncogenic transcripts in hepatocellular carcinoma [9]. Advances in targeting epigenetic regulators, such as EZH2 inhibitors, show promise in reactivating silenced tumor suppressor genes [10]. Furthermore, emerging evidence underscores the diagnostic potential of circulating cell-free DNA methylation patterns [11]. Although significant progress has been made, several challenges persist in addressing off-target effects and adaptive resistance. To overcome these limitations, integrated multi-omics approaches are essential for deciphering context-dependent epigenetic vulnerabilities and advancing the development of precision therapies.

Recent advances in RNA sequencing technologies, particularly single-cell RNA sequencing (scRNA-seq) and bulk RNA sequencing (bulk RNA-seq), have revolutionized our understanding of tumor biology and therapeutic responses in immuno-oncology [12]. scRNA-seq allows high-resolution analysis of tumor heterogeneity by profiling individual cell transcriptomes, revealing distinct immune cell subpopulations and their functional states within the tumor microenvironment (TME) [13]. This high-resolution analysis has been instrumental in identifying rare cell populations, delineating cellular trajectories during the development of therapeutic resistance, and elucidating cell-type-specific signaling pathways that contribute to immune evasion [13]. In contrast, bulk RNA-seq remains an indispensable tool for large-scale cohort analyses, offering a cost-effective approach to tumor classification, prognostic stratification based on gene signatures, and the quantification of global transcriptomic alterations associated with therapeutic interventions [14, 15]. The integration of both approaches has significantly enhanced their clinical applicability. Deconvolution algorithms utilize scRNA-seq-derived reference profiles to infer immune cell composition from bulk sequencing data, whereas machine learning models integrate multi-omics features to predict responses to immunotherapy [12]. Despite the technical limitations of scRNA-seq, such as dropout events and batch variability, as well as the relatively low cellular resolution of bulk RNA-seq, the combined application of these technologies in clinical trials is facilitating the advancement of precision immunotherapy. By correlating immune contexture with treatment outcomes, this integrative approach

contributes to the identification of novel biomarkers and the development of effective combination therapy strategies.

This study explores the epigenetic-metabolic interactions in EC using integrated single-cell and bulk multi-omics analyses, aiming to identify prognostic biomarkers and understand metabolic reprogramming mechanisms. It identifies the IGF2BP3/MUC1/HIF-1 α /ENO1 axis as a key driver of m6A-dependent metabolic changes in EC. The findings link molecular mechanisms to clinical outcomes, providing insights for prognosis and RNA-targeted therapies.

Methods

RNA Sequencing

Total RNA was extracted and subjected to rigorous quality control. RNA integrity was evaluated using the Qsep400 Bioanalyzer, and RNA concentration was quantified using the Qubit 4.0 Fluorometer. Following mRNA enrichment, complementary DNA (cDNA) libraries were generated through fragmentation, end repair, poly(A) tailing, adapter ligation, and PCR amplification. Library quality was confirmed by Qubit quantification (Invitrogen), insert size analysis on an Agilent 2100 Bioanalyzer, and quantitative PCR (Q-PCR)-based determination of effective concentration (>2 nM). Libraries that met the quality criteria were converted into DNA nanoballs (DNBs) and sequenced on a high-throughput platform. For single-cell RNA sequencing (scRNA-seq), cell suspensions were processed using the 10 $\times$ Genomics Chromium Single Cell 3' platform. Libraries were prepared according to the manufacturer's protocol using the Chromium Single Cell 3' GEM, Library & Gel Bead Kit v3, Chromium Chip B, and i7 Multiplex Kit. Sequencing was performed on an Illumina NextSeq 500 using the recommended read lengths and sequencing depth specified by 10 $\times$ Genomics [16].

RNA stability assay

EC cells were transfected with the appropriate plasmid and treated with 5 μ g/ml actinomycin D for 0 h, 2 h, 4 h, 6 h, and 8 h. Total RNA was then isolated and analyzed by qRT-PCR.

RNA Immunoprecipitation assay

RIP assays were performed using the Beyotime RIP Assay Kit (P1801S) with Protein A/G Agarose beads pre-incubated with specific antibodies or IgG control in NT2 Buffer. Cell lysates prepared in cold Lysis Buffer containing 1 mM DTT, 100 U/ml RNase inhibitor, and 0.2 mM PMSF were incubated with antibody-coated beads at 4 $^{\circ}$ C for 4 h. After four NT2 Buffer washes, RNA-protein complexes were eluted at 55 $^{\circ}$ C for 30 min, with 10% lysate

reserved as input control. All centrifugation steps were performed at $14,000\times g$ (4°C).

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assay was performed using the ChIP Assay Kit (Beyotime, China) following the manufacturer's protocol. Briefly, cells were harvested and cross-linked with 1% formaldehyde, and the reaction was quenched with 125 mM glycine. Genomic DNA was fragmented into 200–500 bp by ultrasonication [17]. The cell lysates were incubated with specific antibodies to capture the protein–DNA complexes. An anti-IgG antibody was used as a negative control to account for nonspecific binding.

Dual-luciferase reporter assay

A predicted DNA fragment located upstream of the transcription start site, along with its associated mutations, was cloned into vectors derived from pGL3. Following transfection, the activities of both Firefly and Renilla luciferases were quantified 24 h later using the Dual-Luciferase Reporter Assay Kit (Promega) in accordance with the manufacturer's instructions. Firefly luciferase activity was normalized to the Renilla luciferase signal to control for the transfection efficiency.

Glucose consumption and lactate production

Glucose and lactate concentrations in the EC cell culture medium were quantified by spectrophotometric analysis using a glucose assay kit (F006-1-1) and a lactate detection kit (A019-2-1) from Nanjing Jiancheng Bioengineering Institute (China) [18]. All assays were performed according to the manufacturer's instructions, and the results were normalized to the protein content of each sample [19, 20].

Seahorse analysis

The glycolytic activity of distinct cell populations was assessed using a Seahorse XF-96 extracellular flux analyzer (Agilent, Santa Clara, CA, USA) in accordance with the manufacturer's protocol. Cells from each experimental group were seeded in triplicate in a defined medium and incubated in a CO_2 -free environment for 1 h prior to analysis. Glucose (10 mM), oligomycin (1 μM), and 2-deoxy-D-glucose (2-DG, 50 mM) were sequentially injected, and the extracellular acidification rate (ECAR) was recorded. The data were normalized to the cell count to account for differences in cell density.

Mouse models of EC

Four-week-old female athymic nude mice were obtained from GemPharmatech and housed in a specific pathogen-free (SPF) facility. All animal procedures were conducted in accordance with the protocols approved by the

Institutional Animal Ethics Committee of Clinical Oncology School of Fujian Medical University. Each mouse was subcutaneously injected with 1×10^6 cells suspended in 100 μL of medium. After four weeks, the mice were euthanized with CO_2 , and the tumors were excised, weighed, and measured. Tumor volume was calculated using the formula: $(\text{width}^2 \times \text{length})/2$.

Statistical analysis

Statistical analysis was conducted using R or GraphPad Prism. Student's t-test and one-way ANOVA were used to evaluate differences between groups. Results are expressed as mean $\pm$ standard deviation (SD) from at least three independent experiments. Data from various databases were batch-removed before merging. A p -value of ≤ 0.05 was considered statistically significant. Unless otherwise specified, p -values were adjusted for multiple testing using the Benjamini–Hochberg (BH) false discovery rate (FDR) procedure.

Other methodologies used in this study are described in the Supplementary Methods section.

Results

Differentially expressed and prognostic epigenetics-related genes in EC

Epigenetics-related factors were obtained from the EpiFactors 2022 database [21]. Comprehensive profiling of epigenetics-related genes in the TCGA-UCEC cohort revealed significant dysregulation patterns associated with EC. Differential expression analysis identified 68 epigenetically regulated genes ($|\log_2\text{FC}| > 1$, BH-adjusted p -values < 0.05 ; Fig. 1A), including 28 significantly down-regulated genes and 40 significantly upregulated genes in EC tissues. Univariate Cox Regression analysis of these 68 genes identified 20 prognostic biomarkers significantly correlated with overall survival (Fig. 1B). Notably, genes involved in DNA replication and mitotic regulation, such as BUB1, CDK1, and TTK, showed hazard ratios (HR) greater than 1, indicating that higher expression was associated with increased risk and poorer survival. In contrast, genes including GADD45G, RBM24, and ZNF516 exhibited hazard ratios below 1, suggesting an association with reduced risk and better survival. To quantify the prognostic epigenetics-related expression pattern, we calculated a ssGSEA-based signature score using the 20 epigenetics-related genes significantly associated with overall survival in univariate Cox analysis. The results indicated that tumor specimens exhibited significantly higher scores compared to the normal controls (Fig. 1C). Stratifying patients based on the optimal cutoff value of the epigenetics-related ssGSEA score revealed a significant divergence in survival outcomes, with high-score cases exhibiting markedly decreased survival rates, thereby confirming its prognostic value (Fig. 1D).

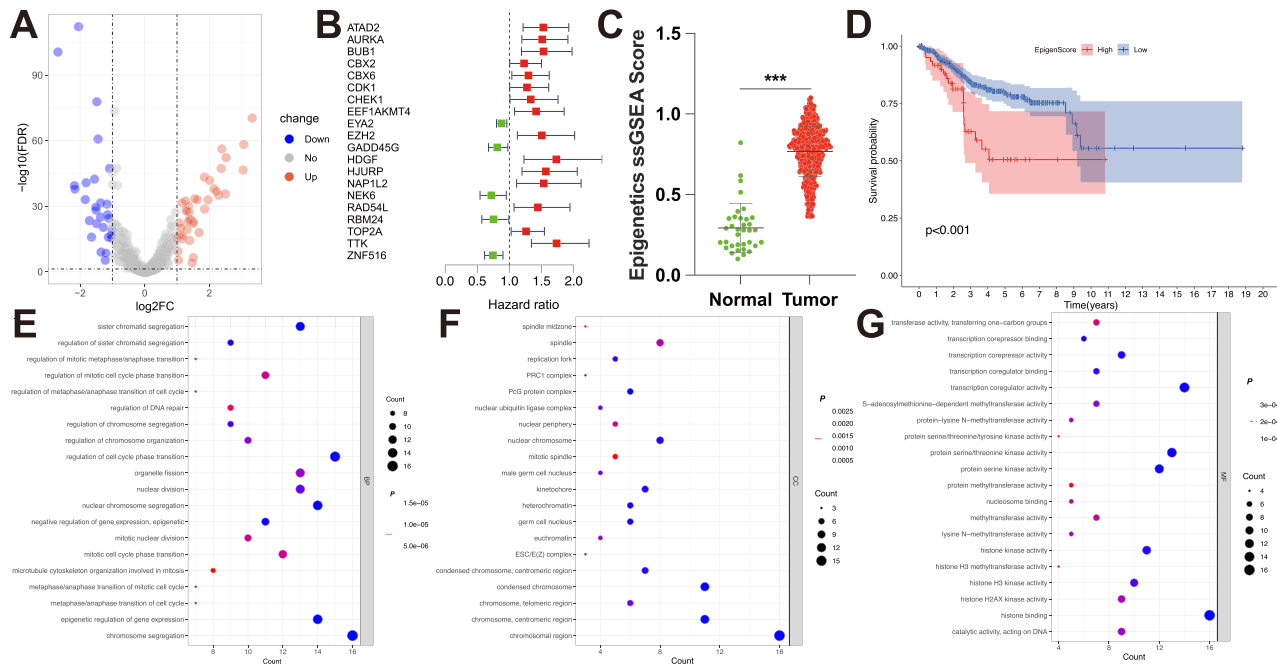


Fig. 1 Differentially Expressed and Prognostic Epigenetics-Related Genes in EC. **A** Volcano plot of 68 differentially expressed epigenetics-related genes in TCGA-UCEC cohort. Red dots indicate significantly upregulated genes; blue dots represent downregulated genes. **B** Forest plot of univariate Cox regression analysis showing 20 prognostic genes. Red squares denote risk factors ($\text{HR} > 1$), green squares indicate protective factors ($\text{HR} < 1$). **C** Comparative boxplot of ssGSEA-based signature scores between tumor (red) and normal tissues (green). **D** Kaplan-Meier survival curves stratified by optimal ssGSEA score cutoff. **E–G** GO enrichment analysis of the 68-gene signature: **E** Biological Processes (BP); **F** Cellular Components (CC); **G** Molecular Functions (MF). P-values were adjusted for multiple testing using the Benjamini–Hochberg method

Functional annotation of the 68-gene signature through Gene Ontology (GO) enrichment analysis revealed coordinated dysregulation across multiple biological domains (BH-adjusted p -values < 0.05). In terms of Biological Processes (Fig. 1E), significant alterations were observed in chromosome segregation, mitotic nuclear division, and epigenetic regulation of gene expression. Regarding Cellular Components (Fig. 1F), key affected structures included condensed chromosomes, kinetochores, nuclear chromosomes, and PRC1 complexes. Within Molecular Functions (Fig. 1G), notable changes were identified in methyltransferase activity, histone H3 kinase activity, and histone binding. The results of KEGG enrichment analysis are shown in Supplementary Figure S1 (BH-adjusted p -values < 0.05). These findings indicate extensive epigenetic reprogramming during the progression of EC.

Identification of epigenetics-related malignant epithelial subpopulations via NMF clustering

Based on scRNA-seq data from five EC samples, we conducted dimensionality reduction and clustering analyses to identify distinct cellular subpopulations. Comprehensive cell annotation identified major cell populations, including B cells, ciliated cells, endothelial cells, epithelial cells, fibroblasts, macrophages, monocytes, smooth muscle cells, and T cells (Fig. 2A). By applying infercnv, an algorithm designed to infer somatic copy number

alterations (CNAs) from scRNA-seq data to identify malignant cells, we found that epithelial cells exhibited pronounced CNA signals, suggesting that the epithelial compartment was enriched for malignant cells. Enrichment analyses confirmed biologically relevant functions associated with each cell type (BH-adjusted P values < 0.05), such as T cell-related processes in T cell clusters, and MHC class II-related processes in macrophage populations, which were visualized using the ClusterGVis, a package for comprehensive visualization of gene expression and clustering patterns (Fig. 2B). To quantify malignant transformation, we employed five scoring methods to assess epigenetics-related scores. Following normalization and integration, malignant cells demonstrated a significantly elevated epigenetics-related score (Fig. 2C), underscoring the presence of aberrant epigenetic regulation during tumorigenesis. To further investigate the heterogeneity of EC, we employed NMF algorithm to analyze 20 prognostic epigenetics-related genes within malignant cells. This analysis identified distinct epigenetics-related clusters, which stratified the malignant cells into four subgroups: Epigen-C1, Epigen-C2, Epigen-C3, and Epigen-C4 (Fig. 2D). We note that Epigen-C1–C4 represent malignant epithelial states/subpopulations identified within tumors and do not necessarily correspond directly to established patient-level classifications (e.g., TCGA categories).

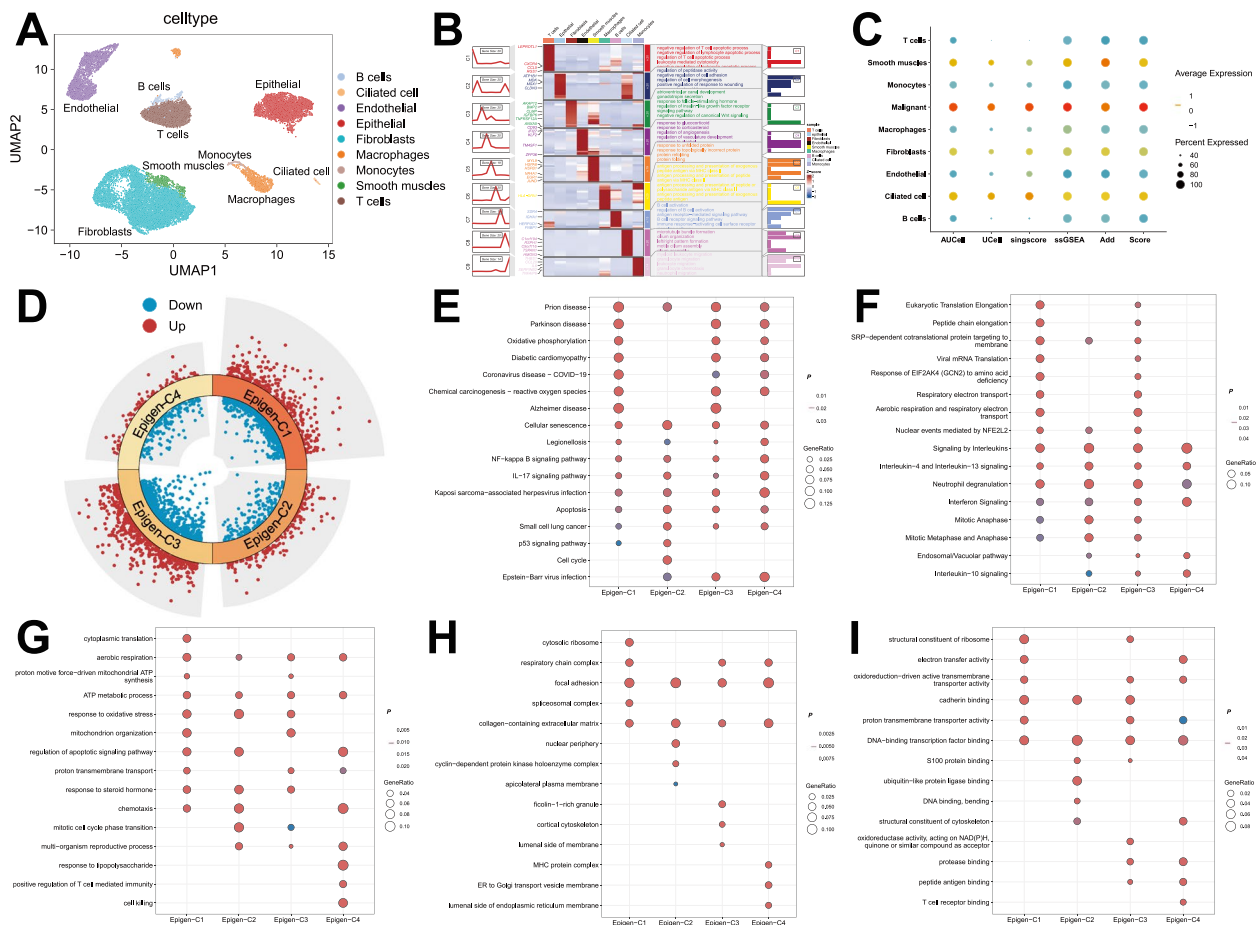


Fig. 2 Identification of Epigenetics-Related Malignant Epithelial Subpopulations via NMF Clustering. **A** Uniform Manifold Approximation and Projection (UMAP) visualization of scRNA-seq data from five EC specimens, annotated with nine major cell populations. **B** Functional annotation heatmap generated by ClusterGVis. **C** Bubble plot comparing integrated epigenetics scores among cell populations. **D** Differential expression of genes across various epigenetics-related clusters. **E** KEGG enrichment analysis of various epigenetics-related clusters. **F** ReactomePA enrichment analysis of various epigenetics-related clusters. **G-I** GO enrichment analysis of various epigenetics-related clusters: **G** Biological Processes (BP); **H** Cellular Components (CC); **I** Molecular Functions (MF). *P*-values were adjusted for multiple testing using the Benjamini-Hochberg method

Differential gene expression profiling combined with functional enrichment analysis identified four distinct molecular subpopulations of EC (BH-adjusted *P* values < 0.05), each marked by unique pathway activations (Fig. 2E-I). Epigen-C1 showed elevated protein synthesis activity through enriched cytoplasmic translation and ribosome components. Epigen-C2 exhibited activation of nuclear periphery organization, CDK complex formation, and apicolateral membrane functions, along with enhanced DNA-binding and ubiquitin-like ligase activities. Epigen-C3 was characterized by granule formation, cortical cytoskeleton remodeling, and oxidoreductase activity targeting NAD(P)H-quinone systems. Epigen-C4 was linked to immunomodulatory pathways such as LPS response, T-cell immunity, and cytotoxic killing, with specific expression of MHC components, ER-Golgi transport proteins, and ER membrane markers, along with enriched T-cell receptor binding. This single-cell, epigenetics-based characterization delineates intra-tumoral

malignant epithelial heterogeneity in EC and may inform precision therapy hypotheses, while not constituting a stable, patient-level molecular classification.

Cell-cell communication analysis reveals subpopulation-specific signaling dynamics

To elucidate the intercellular crosstalk between epigenetics-related EC clusters (Epigen-C1–C4) and components of the TME, we performed a cell-cell communication analysis using CellChat. A strong intercellular correlation was identified among the four epigenetics-related cell clusters and other cellular components within the TME, suggesting significant interactions within the EC ecosystem (Fig. 3A). Based on the ligand-receptor interaction data for each cell within the EC ecosystem, we identified that the four epigenetics-associated EC clusters predominantly engaged in cellular communication through the MK signaling pathway (Fig. 3B). To further elucidate

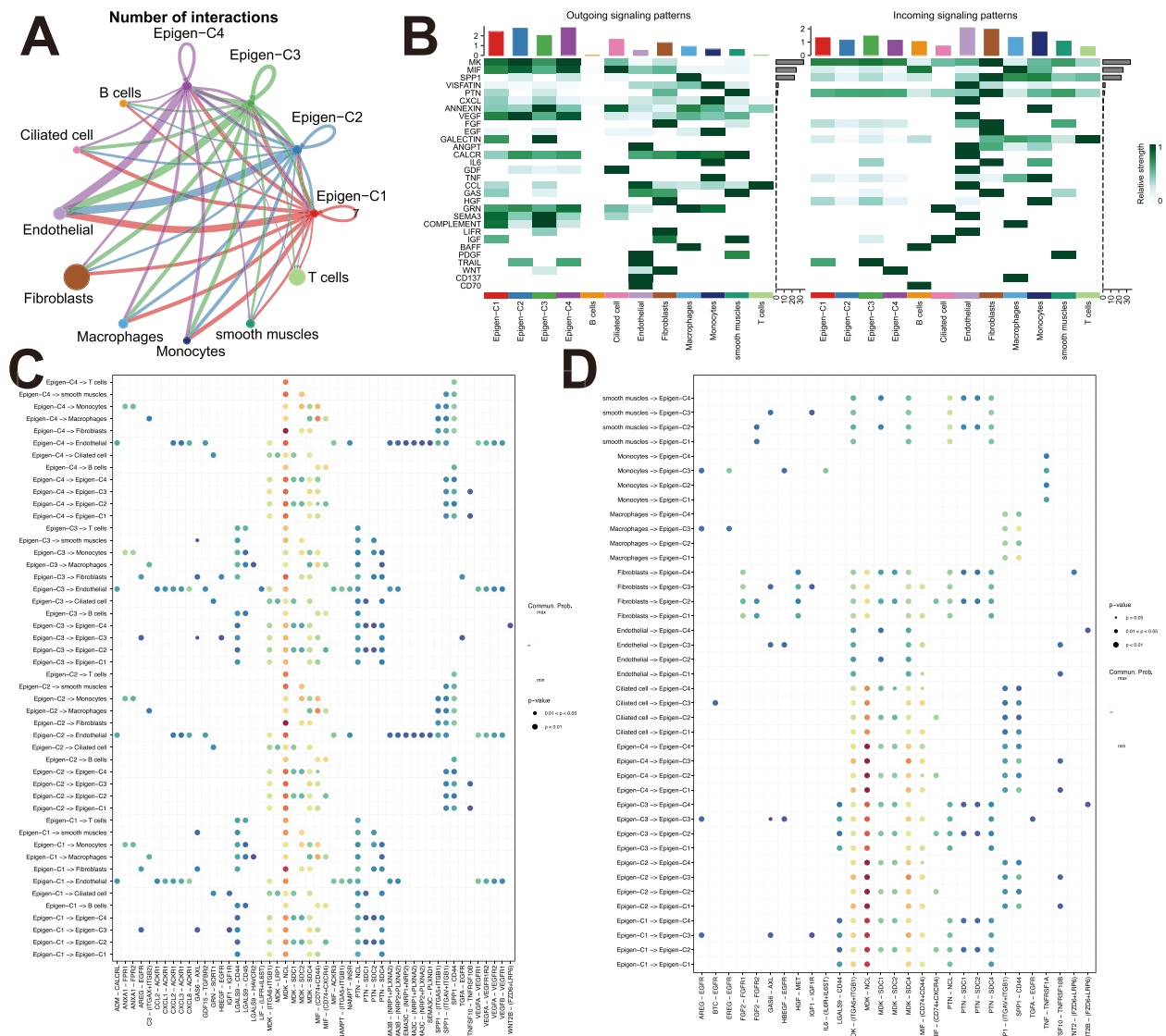


Fig. 3 Cell-Cell Communication Analysis Reveals Subpopulation-Specific Signaling Dynamics. **A** CellChat-derived interaction network showing robust crosstalk between epigenetics-related clusters and tumor microenvironment components. **B** Heatmap illustrating the patterns of outgoing and incoming signaling within various subpopulations of cells. **C-D** The interactions between ligands and receptors were visualized in the bubble chart, where epigenetics-related clusters functioned either as ligand-expressing **C** or receptor-expressing **D** cells

differential interactions, pathway-specific heatmaps revealed distinct signaling biases (Fig. 3C-D), highlighting the complex associations between epigenetics-related EC clusters and other cell types, and illustrating intricate cell–cell interaction networks. The predominant outgoing and incoming signaling pathways in the four EC clusters involved the MDK–NCL axis (Fig. 3C-D). These findings suggest that Midkine signaling [22] may play a significant role in the epigenetic reprogramming of EC malignant subpopulations, contributing to the formation of bidirectional communication networks.

Integrated characterization of transcriptional, metabolic, and developmental heterogeneity in epigenetics-related malignant subpopulations

SCENIC analysis identified distinct TF regulon activation patterns across EC epigenetic subpopulations. A systematic examination of TF-transcriptional program relationships revealed key regulatory networks linked to biological processes in specific cell clusters (Fig. 4A). Integrative analysis using regulatory specificity scores (RSS) pinpointed master regulators for each subpopulation: SOX17 was associated with tumor infiltration features in Epigen-C1; IRF1, POU5F1, and MAFF were predominantly active in Epigen-C2, Epigen-C3, and Epigen-C4 respectively (Fig. 4B). This comprehensive regulatory mapping confirms functional

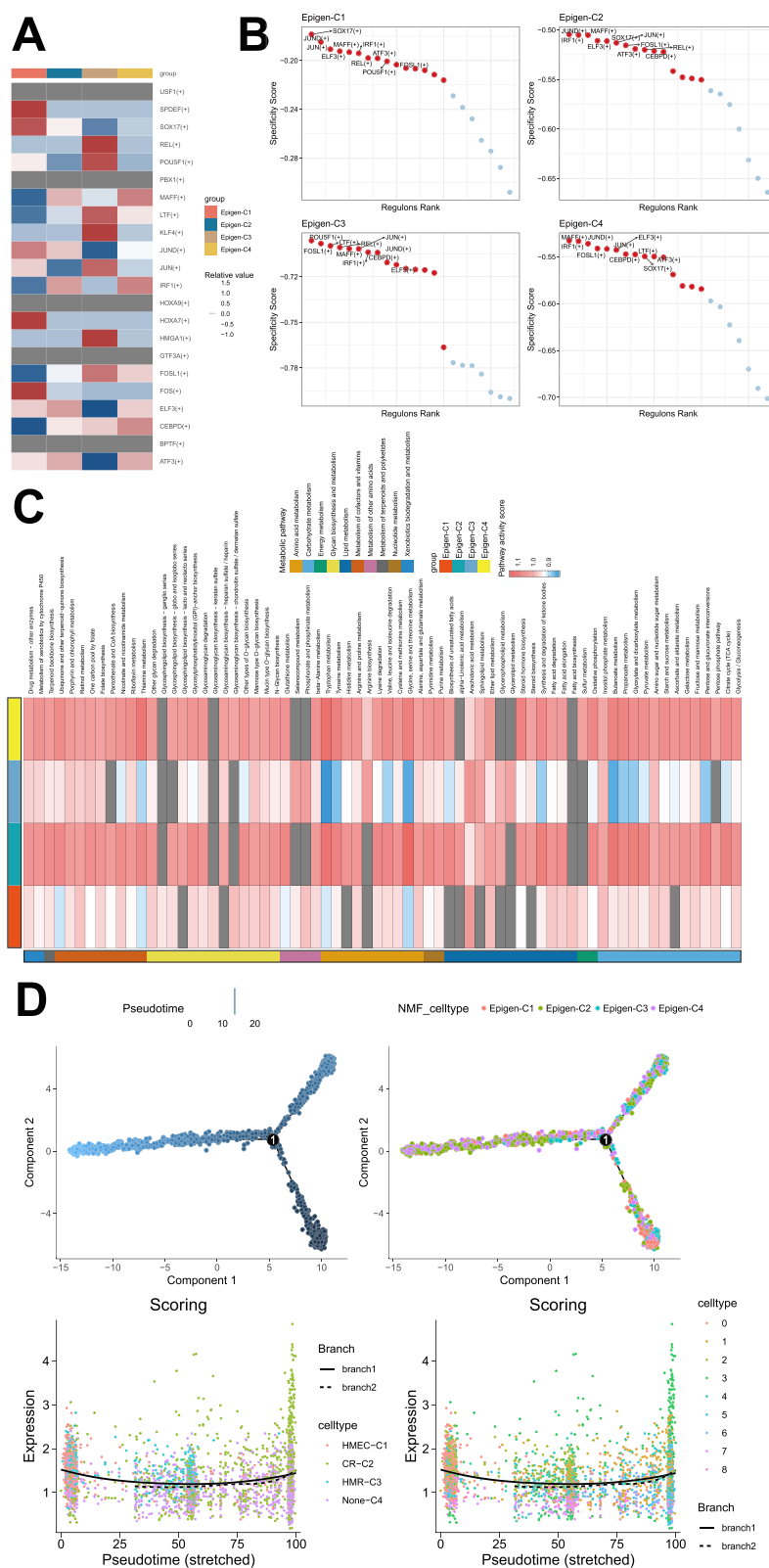


Fig. 4 Integrated Characterization of Transcriptional, Metabolic, and Developmental Heterogeneity in Epigenetics-Related Malignant Subpopulations. **A** Heatmap illustrating the regulon activity of enriched transcription factors across distinct epigenetics-related clusters as identified by SCENIC. **B** Regulon specificity score (RSS) of transcription factor regulatory networks within epigenetics-related clusters. **C** Heatmap illustrating the metabolic profiles across four epigenetics-related clusters. **D** The analysis of pseudotime trajectories in epigenetics-associated cell subpopulations

differences among epigenetic subpopulations and highlights candidate TFs that may drive subpopulation-specific oncogenic processes through unique transcriptional networks.

Metabolic profiling identified Epigen-C2 as the most metabolically active subpopulation, exhibiting significant enrichment in pathways related to energy metabolism, lipid processing, and redox regulation (Fig. 4C). Subsequently, we performed pseudotime trajectory analysis on epigenetics-related cell subpopulations. The results revealed that Epigen-C1 was predominantly positioned at the initial stage of development, whereas Epigen-C2 was primarily located at the terminal stage (Fig. 4D). Together, these integrative analyses reveal that epigenetic subpopulations occupy distinct positions within both transcriptional profiles and developmental trajectories. Notably, Epigen-C2 represents a state marked by elevated metabolic activity and terminal differentiation, potentially driven by progressive epigenetic remodeling.

Prognostic stratification and validation of an epigen-C2-based risk prediction model

We calculated ssGSEA scores in bulk transcriptomic datasets (TCGA; with normal controls supplemented by GTEx where indicated) using DEGs defining the four malignant epithelial states (Epigen-C1–C4) derived from scRNA-seq, and observed significant differences between EC and normal samples for each state signature (Fig. 5A). Importantly, Epigen-C1–C4 represent intra-tumoral malignant epithelial states inferred from scRNA-seq ($n=5$ patients), not stable patient-level subpopulations; bulk TCGA/GTEx analyses provided complementary cohort-level support for prognostic modeling. Subsequent survival analyses of state-specific ssGSEA scores showed that a high Epigen-C2 score was significantly associated with poorer clinical outcomes, whereas the other state signatures showed weaker or non-significant associations (Fig. 5B–E). To leverage the prognostic relevance of Epigen-C2, we intersected Epigen-C2 DEGs with EC tumor–normal DEGs to construct a candidate gene set for model development. Through Lasso-Cox regression analysis (Supplementary Figure S2A–B), the overlapping gene set was refined into a six-gene signature: SDF2L1, ASPM, MUC1, MCM7, PHGDH, and MSX1. Stratifying patients based on median risk scores, the high-risk groups demonstrated significantly poorer survival outcomes in the training cohort (Fig. 5F), testing cohort (Fig. 5G), and full cohort (Fig. 5H). Time-dependent ROC analysis further confirmed the model's predictive accuracy across all cohorts—the training cohort (Fig. 5I), testing cohort (Fig. 5J), and full cohort (Fig. 5K)—thereby validating its applicability for prognostic evaluation in EC.

Among the six genes incorporated into the model described above, MUC1 was consistently found to be significantly upregulated in EC samples compared to normal controls, irrespective of the inclusion of normal

samples from GTEx. Furthermore, the magnitude of its fold change exceeded that of the other five genes (Supplementary Figure S3). Therefore, the following discussion will focus on exploring the influence of MUC1 on the malignant progression of EC.

The expression characteristics of IGF2BP3 in EC and its transfection efficiency in EC cells

MUC1 is a highly glycosylated transmembrane protein overexpressed in various solid and hematological malignancies, playing critical roles in tumor progression, metastasis, and therapy resistance through activation of oncogenic pathways [23]. Although the downstream signaling pathways and therapeutic targeting of MUC1 have been extensively studied, its upstream regulatory mechanisms remain largely unclear, especially epigenetic regulatory elements such as m6A RNA modification, which has yet to be examined in the context of MUC1 expression [23]. To identify potential trans-regulators (Supplementary Figure S4), we integrated RNA-binding proteins (RBPs) predicted by ENCORI that target MUC1 with transcriptomic data exhibiting significant EC overexpression (upregulated) and prognostic significance ($HR>1$). This tripartite screening yielded five high-confidence candidates (Supplementary Figure S4), among which IGF2BP3 emerged as the lead contender due to its established roles in mRNA stabilization via m6A RNA modification.

Compared with control tissues, both the RNA (Fig. 6A) and protein (Fig. 6B) levels of IGF2BP3 were significantly elevated in EC. High expression of IGF2BP3 was also correlated with poorer prognosis in EC patients (Fig. 6C). We assessed IGF2BP3 mRNA and protein expression across four EC cell lines (KLE, Ishikawa, RL95-2, and HEC1A) (Fig. 6D) and selected HEC1A cells, which exhibited the highest IGF2BP3 expression, for subsequent knockdown experiments. Knockdown efficiency was evaluated by measuring IGF2BP3 mRNA and protein levels after transfection (Fig. 6E). Conversely, Ishikawa cells, which exhibited the lowest IGF2BP3 expression, were selected for overexpression studies, and overexpression efficiency was confirmed by assessing IGF2BP3 mRNA and protein levels (Fig. 6F).

Multi-omics analysis reveals IGF2BP3 regulates hypoxia pathways and targets

Following the knockdown of IGF2BP3, RNA-seq analysis revealed 1678 differentially expressed genes, consisting of 769 downregulated and 909 upregulated transcripts (Fig. 7A). Functional enrichment analysis of these DEGs revealed widespread biological impacts, with significant enrichment (BH-adjusted P values <0.05) in Biological Processes (Fig. 7B), Cellular Components (Fig. 7C), Molecular Functions (Fig. 7D), and KEGG pathways (Fig. 7E). To

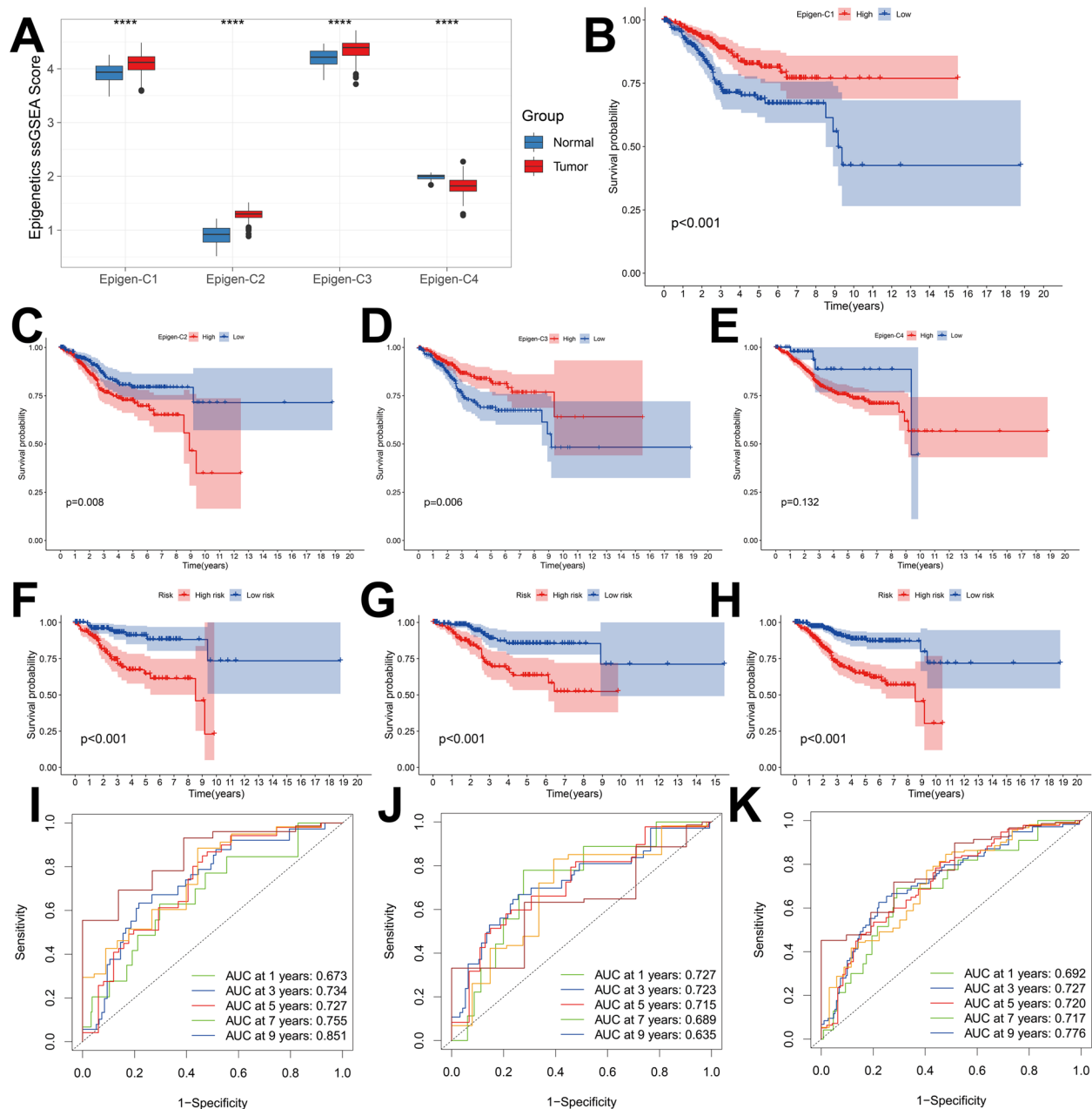


Fig. 5 Prognostic Stratification and Validation of an Epigen-C2-Based Risk Prediction Model. **A** ssGSEA score comparison of four epigenetic clusters between tumor (red) and normal (blue) samples. **B-E** Survival landscape stratified by epigenetic clusters: Epigen-C1 (**B**); Epigen-C2 (**C**); Epigen-C3 (**D**); Epigen-C4 (**E**). **F-H** Risk stratification performance in survival divergence across Training (**F**), Testing (**G**), and Full (**H**) cohorts. **I-K** Time-dependent ROC validation Training (**I**), Testing (**J**), and Full (**K**) cohorts. Bulk cohort analyses test associations of state-derived signatures with outcomes, but do not define patient-level subtypes

identify key mechanisms, we applied strict criteria: for BP terms, the top 20 pathways included response to oxygen levels, hypoxia response and so on (Fig. 7B). Filtered CC terms showed membrane raft organization and contractile fiber structures (Fig. 7C) and MF terms showed enrichment in growth factor binding and extracellular matrix interactions (Fig. 7D). KEGG analysis, excluding disease-related terms, identified metabolic reprogramming pathways such

as HIF-1 signaling as highly enriched (Fig. 7E). We identified six high-confidence candidate genes—SCD, ENO1, SLC7A5, DDIT4, MUC1, and HOXB9—by intersecting transcripts that were downregulated following IGF2BP3 knockdown with those overexpressed in EC and confirmed as direct IGF2BP3 binding targets via RIP-seq analysis (Fig. 7F). This observation aligns with the aforementioned

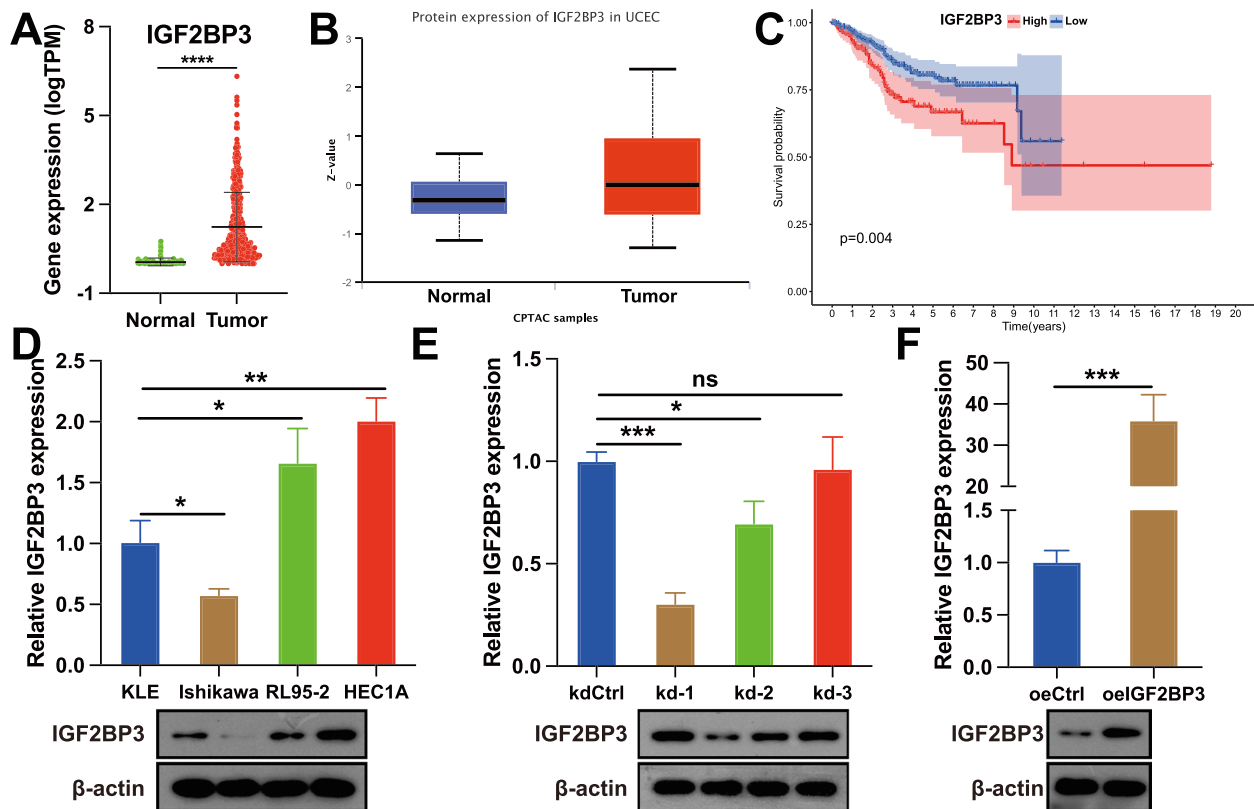


Fig. 6 The expression characteristics of IGF2BP3 in EC and its transfection efficiency in EC cells. **A** IGF2BP3 mRNA expression in tumor tissues compared to normal tissues. **B** Validation of IGF2BP3 protein expression in tumor tissues compared to normal tissues. **C** Kaplan-Meier survival analysis stratified by IGF2BP3 expression. **D** Basal IGF2BP3 expression across EC cell lines. **E** Knockdown efficiency in HEC1A cells with three sequences. **F** Overexpression validation in Ishikawa cells

hypothesis that IGF2BP3 may play a regulatory role in the expression of MUC1.

IGF2BP3 Regulates the MUC1/HIF-1 α /ENO1 pathway in an m6A-associated manner

It is known that the IGF2BP3 protein recognizes a specific m6A consensus motif to stabilize m6A-modified RNA [24], leading to increased expression of the target mRNA. To investigate whether IGF2BP3 regulates MUC1 via the m6A pathway, we used the SRAMP web tool to predict MUC1 m6A modification sites based on sequence-derived features and identified two sites with “Very high confidence” at single-base resolution (Supplementary Figure S5A). RNA immunoprecipitation (RIP) assays showed that IGF2BP3 binds to MUC1 mRNA (Fig. 8A). Subsequent RNA stability assays indicated that IGF2BP3 knockdown increased the decay rate of MUC1 mRNA after actinomycin D treatment, consistent with functional destabilization (Fig. 8B). The Western Blot experiment further confirmed that IGF2BP3 positively regulates MUC1 expression (Fig. 8C). Transcriptome analysis after IGF2BP3 knockdown revealed enrichment of hypoxia/HIF-1 signaling downstream target gene programs (Fig. 7B and E). Coupled with Western blotting (Fig. 8D) and prior evidence that MUC1 stabilizes HIF-1 α

protein [25], these data support a model in which IGF2BP3-MUC1 primarily modulates HIF-1 α protein stability (at the post-translational level), thereby altering HIF-1 α transcriptional activity and downstream target gene expression. In this study, we found no evidence to suggest that IGF2BP3 directly regulates HIF1A mRNA levels.

The transcription factor HIF-1 α is capable of inducing an increase in the expression of glycolytic enzymes at the transcriptional level [26]. Based on the cumulative evidence presented above, it is hypothesized that the transcription factor HIF-1 α may regulate the glycolytic enzyme ENO1 (2-phospho-D-glycerate hydrolase) at the transcriptional level. The promoter region of ENO1 was identified as potentially containing high-confidence HIF-1 α binding sites based on sequence analysis using the JASPAR database (Fig. 8E). We then focus on the site with the highest score. Results from ChIP-qPCR assays demonstrated that HIF-1 α was enriched at the identified region of ENO1 gene (Fig. 8F). Overexpression of HIF-1 α led to a notable increase in luciferase activity in cells with the wild-type ENO1 binding site, whereas no such effect was observed in cells carrying the mutated binding site (Fig. 8G). Furthermore, Western Blot results supported the conclusion that HIF-1 α enhances the expression of

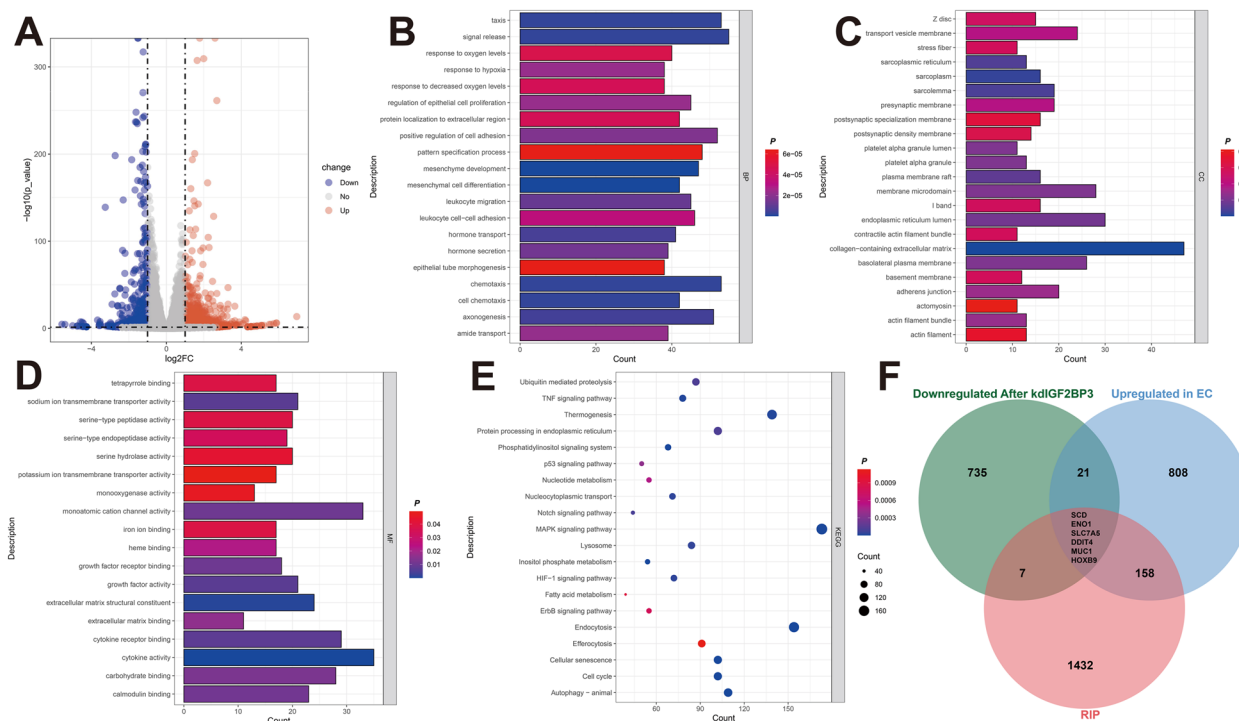


Fig. 7 Multi-omics Analysis Reveals IGF2BP3 Regulates Hypoxia Pathways and Targets. **A** Volcano plot of differentially expressed genes (DEGs) following IGF2BP3 knockdown. **B–D** Functional enrichment analysis of DEGs: Top 20 Biological Processes (BP) (**B**); Cellular Component (CC) terms (**C**); Molecular Function (MF) categories (**D**); KEGG pathways (**E**). **F** Tripartite integration identifies six core targets: Intersection of IGF2BP3 knockdown-downregulated genes, EC-upregulated transcripts, and RIP-seq confirmed binders. P -values were adjusted for multiple testing using the Benjamini–Hochberg method

ENO1 (Fig. 8H). To examine whether IGF2BP3 regulates ENO1 in an m6A-associated manner, we used SRAMP to predict putative m6A sites on the ENO1 transcript and identified two high-confidence sites (Supplementary Figure S5B). RIP assays showed that IGF2BP3 binds to ENO1 mRNA (Fig. 8I). Further experiments indicated that silencing IGF2BP3 speeds up ENO1 mRNA degradation after actinomycin D treatment, suggesting it stabilizes this transcript (Fig. 8J). The subsequent Western Blot experiment further validated the upstream-downstream relationship demonstrated in the preceding analysis (Fig. 8K). Mechanistically, IGF2BP3 stabilizes MUC1 mRNA (presumably through m6A recognition), which increases HIF-1 α protein abundance via enhanced stability—a post-translational event—without necessitating changes in HIF1A mRNA. Subsequently, the elevated HIF-1 α protein may enhance transcriptional activity at target promoters such as ENO1. Meanwhile, IGF2BP3 also stabilizes ENO1 mRNA, collectively establishing a feed - forward loop.

IGF2BP3 Promotes malignant phenotypes of EC by Enhancing Glycolysis

IGF2BP3 knockdown markedly suppressed EC cell proliferation (Fig. 9A), whereas IGF2BP3 overexpression increased proliferation (Fig. 9B). Consistently, IGF2BP3 silencing reduced colony-forming capacity (Fig. 9C,

Supplementary Figure S6A), while IGF2BP3 overexpression enhanced colony formation (Fig. 9D, Supplementary Figure S6B). Transwell and wound-healing assays further showed that IGF2BP3 knockdown inhibited cell migration and invasion (Fig. 9E–F, Supplementary Figure S6C–E), whereas IGF2BP3 overexpression exerted the opposite effects (Fig. 9G–H, Supplementary Figure S6F–H). Metabolic profiling revealed attenuated glycolysis upon IGF2BP3 knockdown, as evidenced by decreased glucose consumption (Fig. 9I), lactate production (Fig. 9J), and extracellular acidification rate (Fig. 9K). Conversely, IGF2BP3 overexpression increased glucose consumption (Supplementary Figure S6I), lactate production (Supplementary Figure S6J), and ECAR (Supplementary Figure S6K). Western blotting indicated that the IGF2BP3/MUC1/HIF-1 α /ENO1 axis was downregulated in IGF2BP3-silenced group (Supplementary Figure S6L) but upregulated upon IGF2BP3 overexpression (Supplementary Figure S6M). In vivo, IGF2BP3 knockdown reduced xenograft tumor growth (Fig. 9L), reflected by lower tumor volume (Fig. 9M) and weight (Fig. 9N). Complementary overexpression experiments produced the opposite phenotype in vivo (Supplementary Figure S6N–P). Collectively, these data support a pro-tumorigenic role of IGF2BP3 in EC by promoting metastatic traits and glycolytic reprogramming.

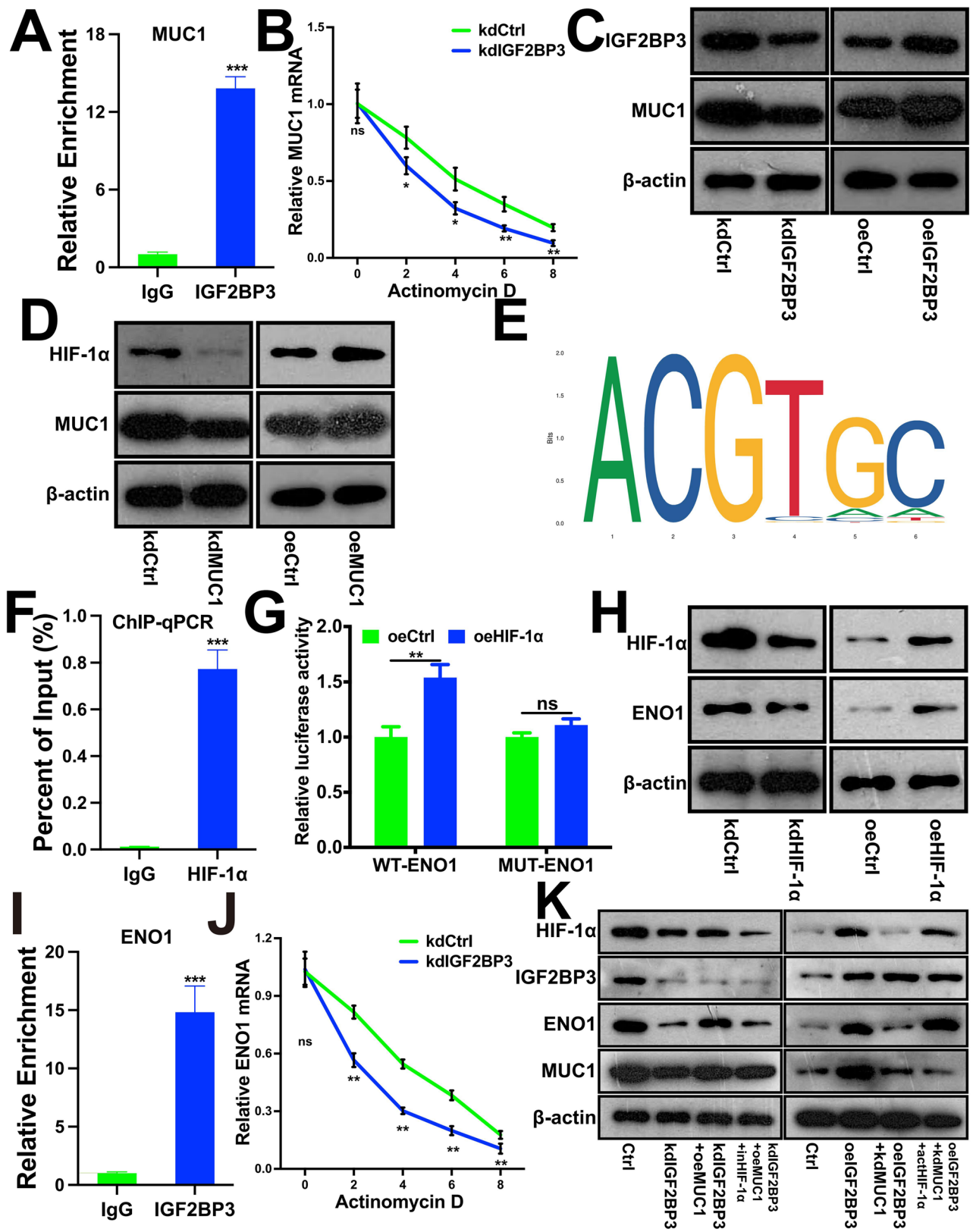


Fig. 8 (See legend on next page.)

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Fig. 8 IGF2BP3 Regulates the MUC1/HIF-1 α /ENO1 Pathway in an m6A-Associated Manner. **A** RIP-qPCR confirms IGF2BP3 binding to MUC1 mRNA. **B** RNA stability assay shows accelerated MUC1 mRNA decay after IGF2BP3 knockdown. **C** Western blot demonstrates IGF2BP3-dependent MUC1 protein regulation. **D** Western blot demonstrating that MUC1 stabilizes HIF-1 α protein. **E** JASPAR-predicted HIF-1 α binding motif in ENO1 promoter. **F** ChIP-qPCR validates HIF-1 α enrichment at ENO1 promoter. **G** Luciferase reporter assay confirms HIF-1 α -mediated ENO1 transcriptional regulation. **H** Western blot demonstrating that HIF-1 α upregulates ENO1 protein. **I** RIP-qPCR confirms IGF2BP3 binding to ENO1 mRNA. **J** RNA stability assay shows accelerated ENO1 mRNA decay after IGF2BP3 knockdown. **K** Western blotting analysis for rescue experiments. inHIF-1 α : inhibitor of HIF-1 α . actHIF-1 α : activator of HIF-1 α

Discussion

Our study demonstrates the combined power of scRNA-seq and bulk RNA-seq in unraveling the epigenetic-metabolic interactions in EC. Using high-resolution scRNA-seq along with bulk RNA-seq, we identified molecular differences in the EC microenvironment and discovered potential biomarkers for clinical use. This approach reflects current trends in multi-omics cancer research: bulk RNA-seq captures overall gene expression patterns useful for prognosis, while scRNA-seq reveals distinct cell populations and their functional changes [12, 27]. Through NMF clustering of scRNA-seq data, we identified four epigenetically distinct malignant subpopulations (Epigen-C1–C4), highlighting the ability of single-cell methods to detect tumor diversity. Among them, Epigen-C2 is the most metabolically active and linked to advanced disease stages and worse clinical outcomes. These details may be missed by bulk RNA-seq due to its averaging effect across cell types. Our result aligns with findings that scRNA-seq can detect rare but clinically significant cell populations [28, 29]. In contrast, bulk RNA-seq helped us develop a reliable six-gene prognostic signature, validated across multiple cohorts, showing its value for risk assessment in clinical practice.

Our study establishes a robust epigenetics-driven prognostic stratification system through integrative analysis of single-cell and bulk transcriptomic data. The six-gene signature (SDF2L1, ASPM, MUC1, MCM7, PHGDH, MSX1) derived from Epigen-C2-associated DEGs demonstrated superior prognostic performance compared to conventional clinicopathological parameters, achieving high AUC values across validation cohorts. This level of precision is consistent with the emerging multi-omics approaches currently being applied in the prognostication of EC, as demonstrated by our recent studies integrating metabolic profiles and mitochondrial gene expression data [30, 31]. Notably, our model uniquely integrates both epigenetic regulatory mechanisms (MCM7-DNA replication licensing) [32] and metabolic reprogramming pathways (PHGDH-serine biosynthesis) [33], thereby reflecting the biological interplay characteristic of Epigen-C2's hypermetabolic phenotype. Our study also identifies MUC1 as a key signature component. Unlike previous studies highlighting its established role in protecting epithelial cells from adverse environmental factors [34], our mechanistic validation of IGF2BP3-mediated m6A regulation extends the existing findings on epitranscriptomic control in EC progression.

Clinically, this stratification system has the potential to enhance existing molecular classification frameworks by identifying high-risk patients and addressing current gaps in personalized treatment approaches [35]. Future validation in prospective cohorts that incorporate the relevant gene profiling may further improve risk prediction accuracy and help overcome the limitations associated with the retrospective nature of the TCGA data used in the current study.

Our study delineates a novel IGF2BP3-mediated metabolic cascade in EC, where IGF2BP3 orchestrates the MUC1/HIF-1 α /ENO1 axis to drive glycolytic reprogramming (Fig. 10). Mechanistically, IGF2BP3 may stabilize MUC1 mRNA by binding to its m6A modification sites, thereby enhancing MUC1 protein expression. Upregulated MUC1 subsequently amplifies HIF-1 α protein stability (post-translational). Moreover, stabilized HIF-1 α may transcriptionally activate ENO1 through promoter binding, as confirmed by ChIP-qPCR and Dual-luciferase reporter assays. Simultaneously, IGF2BP3 also stabilizes ENO1 mRNA, forming a feed-forward regulatory loop that accelerates glycolytic flux, as evidenced by increased glucose consumption, lactate production, and extracellular acidification rate (ECAR). This regulatory axis integrates epigenetic regulation (m6A modification), oncogenic signaling (MUC1 pathway), and metabolic reprogramming (ENO1 function), shedding light on the metabolic heterogeneity within the tumor microenvironment of EC [36, 37]. MUC1, a transmembrane mucin overexpressed in EC, drives tumor progression through aberrant glycosylation and immune evasion [34, 38, 39]. Our findings reveal its novel post-transcriptional regulation by IGF2BP3 via m6A modification. The downstream effector ENO1, a glycolytic enzyme catalyzing 2-phosphoglycerate-to-phosphoenolpyruvate conversion, is critical for Warburg effect induction [36]. HIF-1 α , a hypoxia-responsive transcription factor, directly promotes the transcription of ENO1. Concurrently, IGF2BP3 enhances the stability of both MUC1 and ENO1 mRNA, thereby establishing a self-reinforcing regulatory loop that sustains hypermetabolism in the malignant Epigen-C2 subpopulation of EC. This mechanism further reinforces glycolytic dependency and accelerates malignant progression of EC.

In conclusion, our study highlights the critical importance of integrating bulk and single-cell analytical approaches to effectively link molecular mechanisms

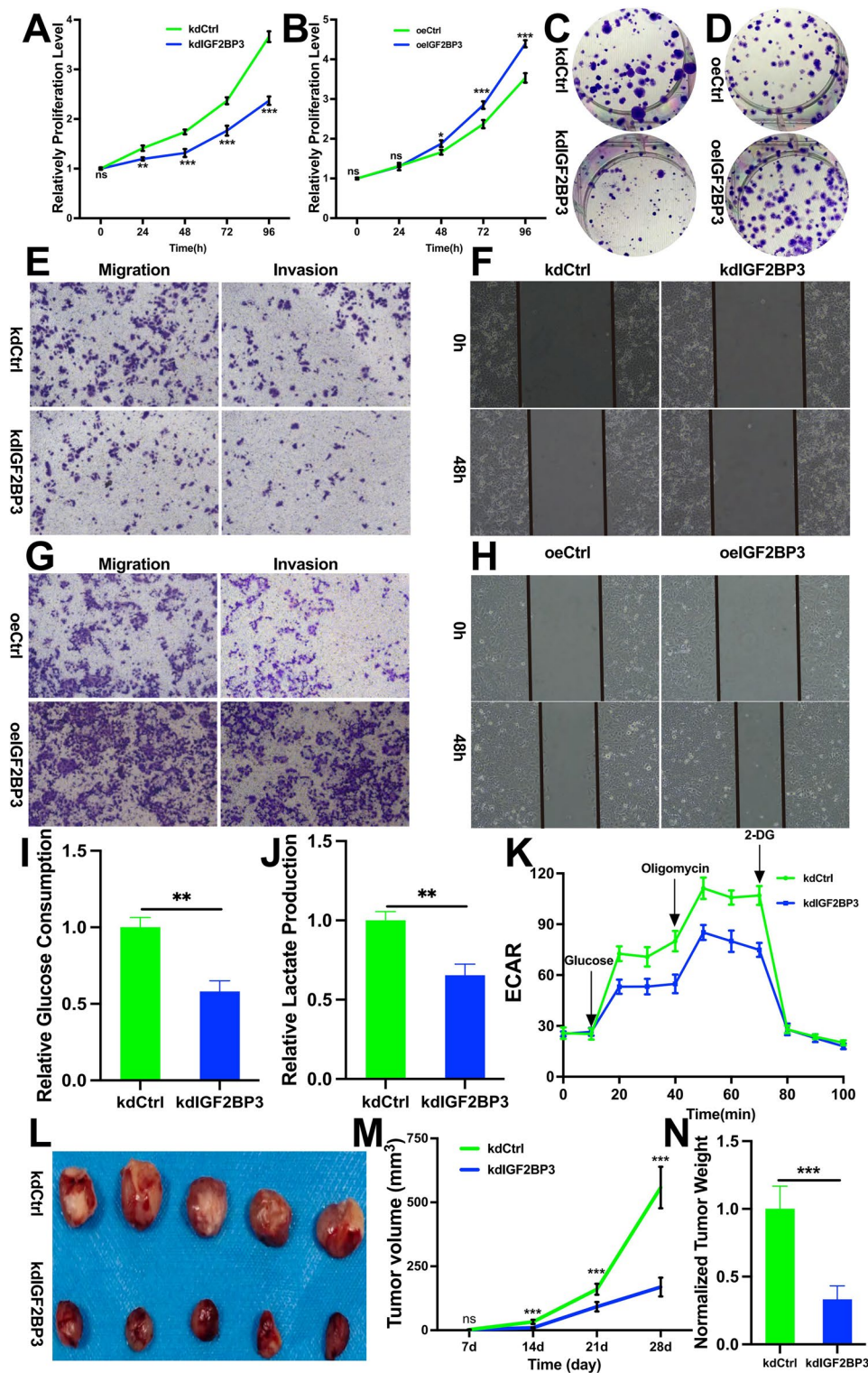


Fig. 9 IGF2BP3 Promotes Malignant Phenotypes of EC by Enhancing Glycolysis. **A–B** Cell proliferation determined by CCK-8 assay in EC cells with IGF2BP3 knockdown **A** or IGF2BP3 overexpression **(B)**. **C–D** Representative images of colony formation assays following IGF2BP3 knockdown **C** or overexpression **(D)**. **E–H** Representative images of Transwell migration/invasion assays **E, G** and wound-healing assays **F, H** in cells with IGF2BP3 knockdown **E–F** or overexpression **(G–H)**. **I–K** Glycolytic activity assessed by glucose consumption **(I)**, lactate production **(J)**, and extracellular acidification rate (ECAR) **K** after IGF2BP3 knockdown. **L–N** Xenograft assays in nude mice showing representative tumor images **L** and quantification of tumor volume **M** and tumor weight **N** in the IGF2BP3 knockdown group versus control

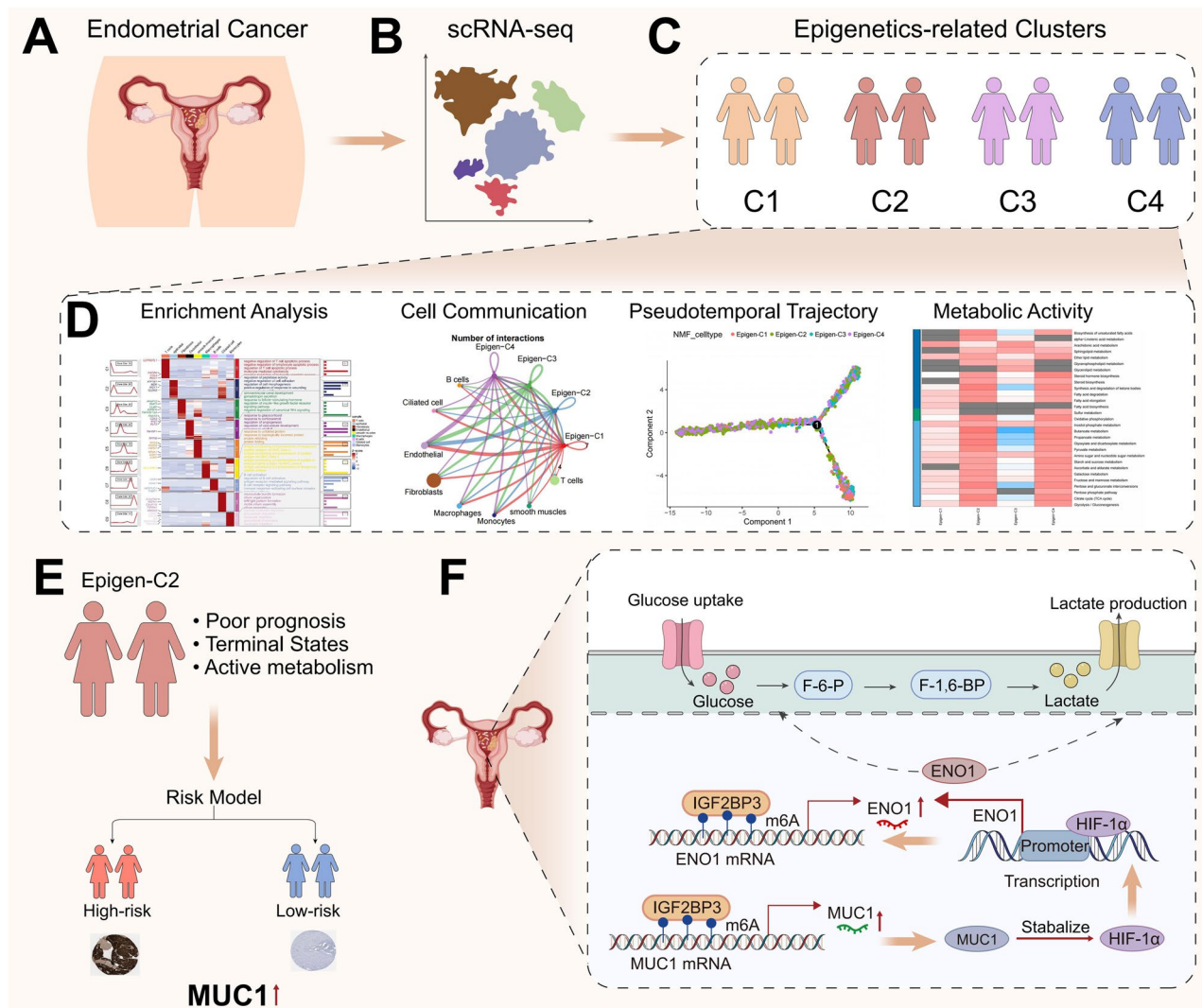


Fig. 10 The framework and mechanism model diagram of the entire research

with clinical outcomes. This integrative paradigm, which aligns with the principles of precision oncology, not only enhances the accuracy of prognostic modeling but also facilitates the discovery of novel therapeutic targets, such as the IGF2BP3/MUC1 axis, thereby opening new avenues for the development of RNA modification-based therapies in EC.

Nevertheless, certain limitations remain. First, the scRNA-seq cohort was relatively small ($n=5$), which may not adequately represent inter-patient variability or the true prevalence of Epigen-C2-like hypermetabolic states; therefore, larger and preferably prospective single-cell cohorts—particularly in patients receiving targeted therapies—are needed to validate subpopulation-specific metabolic dependencies and to confirm the clinical relevance of the Epigen-C2 subpopulation. Second, because standard scRNA-seq lacks spatial resolution, we could not localize the IGF2BP3-driven metabolic cascade

within distinct tumor microenvironments or resolve potential paracrine circuitry; integrating spatial transcriptomics and spatial proteomics in future studies will be essential to map the IGF2BP3/MUC1/HIF-1 α /ENO1 axis across anatomical niches and cellular neighborhoods. Third, although SRAMP-based motif prediction and RIP-based binding/stability assays support an m6A-dependent mechanism on MUC1 and ENO1, we did not perform site-directed mutagenesis of predicted m6A motifs or orthogonal MeRIP-qPCR/CLIP validations to directly abrogate IGF2BP3 binding; future work will employ point mutations at consensus sites, methylation perturbation, and MeRIP-qPCR/CLIP to formally test the necessity and sufficiency of specific m6A marks for IGF2BP3-mediated transcript stabilization. Finally, while xenograft models support a tumor-promoting role for IGF2BP3, the absence of genetically engineered mouse models and orthotopic EC systems limits our ability to

define spatial–temporal dynamics during tumor evolution and to pinpoint therapeutic windows; addressing this gap will also facilitate rigorous evaluation of co-targeting strategies along this pathway, including the development of small-molecule inhibitors against IGF2BP3 (given structural similarities across RNA-binding proteins) and potential combinations with MUC1-directed immunotherapies (e.g., vaccines or CAR-T approaches) [40] and metabolic interventions that suppress ENO1-driven glycolysis.

Conclusions

This study identifies the IGF2BP3/MUC1/HIF-1α/ENO1 axis as a key driver of m6A-dependent metabolic reprogramming in endometrial cancer (EC). Using single-cell and bulk multi-omics analyses, we identified four epigenetically distinct malignant subpopulations (Epigen-C1–C4), with Epigen-C2 showing hypermetabolic activity, advanced differentiation, and worse prognosis. A six-gene prognostic signature (SDF2L1, ASPM, MUC1, MCM7, PHGDH, MSX1) effectively stratified patient risk across cohorts, demonstrating clinical value. Mechanistically, IGF2BP3 stabilizes MUC1 mRNA through m6A modification, boosting HIF-1α-driven ENO1 expression and further stabilizing ENO1 mRNA to create a self-sustaining metabolic loop. In vivo experiments confirmed IGF2BP3’s role in tumor promotion, as its knockdown reduced tumor growth and overexpression accelerated progression. These findings link molecular mechanisms to clinical outcomes and highlight the value of combining bulk and single-cell data in precision oncology. The study improves prognostic modeling by integrating epigenetic and metabolic features and suggests RNA modification-targeted therapies as a promising approach. Limitations include the need for larger single-cell datasets and spatial transcriptomics validation. Future research should explore combining IGF2BP3/MUC1 inhibition with metabolic blockers to disrupt glycolytic dependency, offering new therapeutic options for high-risk EC patients.

Abbreviations

AUC	Area Under the Curve
CCK-8	Cell Counting Kit-8
CDK1	Cyclin-dependent kinase 1
ChIP-qPCR	Chromatin Immunoprecipitation quantitative PCR
CPTAC	Clinical Proteomic Tumor Analysis Consortium
DEGs	Differentially Expressed Genes
EC	Endometrial Cancer
ECAR	Extracellular Acidification Rate
ECM	Extracellular Matrix
ENO1	Enolase 1
Epigen-C1–C4	Epigenetic Clusters 1–4
FBS	Fetal Bovine Serum
GEO	Gene Expression Omnibus
GO	Gene Ontology
GTEx	Genotype-Tissue Expression
HIF-1α	Hypoxia-Inducible Factor 1-alpha
HR	Hazard Ratio
IGF2BP3	Insulin-like Growth Factor 2 mRNA-Binding Protein 3
KEGG	Kyoto Encyclopedia of Genes and Genomes

LASSO	Least Absolute Shrinkage and Selection Operator
logFC	Log Fold Change
m6A	N6-methyladenosine
NMF	Non-negative Matrix Factorization
OD	Optical Density
PBS	Phosphate-Buffered Saline
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
qRT-PCR	Quantitative Real-time PCR
RIP-seq	RNA Immunoprecipitation Sequencing
ROC	Receiver Operating Characteristic
RPMI	Roswell Park Memorial Institute medium
RIPA	Radioimmunoprecipitation Assay buffer
scRNA-seq	Single-Cell RNA Sequencing
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
ssGSEA	Single-sample Gene Set Enrichment Analysis
TCGA	The Cancer Genome Atlas
TF	Transcription Factor
TME	Tumor Microenvironment
UCEC	Uterine Corpus Endometrial Carcinoma

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-026-15858-x>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.

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Not applicable.

Authors' contributions

Yang Sun conceived, designed, and supervised the study. Ruina Jiang conducted data analysis, organized the figures, and drafted the manuscript. Jianfeng Zheng and Xuefen Lin assisted in data analysis and manuscript revision. Ning Xie, Yanhong Li, Feishuang Lin, Siping Wang, and Rongrong Zhang contributed to experimental studies.

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Data availability

Data is provided within the manuscript or supplementary information files. Further inquiries can be directed to the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The animal experiments were conducted in accordance with the Basel Declaration and received approval from the Institutional Animal Ethics Committee at the Clinical Oncology School of Fujian Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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