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Advancements in single-cell sequencing for cervical cancer research

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Abstract

Single-cell sequencing has revolutionized our understanding of cervical cancer (CC), revealing unprecedented cellular heterogeneity, tumor microenvironment (TME) dynamics, and molecular mechanisms underlying progression and therapy resistance. These technologies have identified distinct molecular subtypes (hypoxic, proliferative, and immunoreactive) and epithelial states (cytokeratin$^+$, immune-interacting, and senescent), while uncovering HPV-driven oncogenic mechanisms, including viral integration hotspots (e.g., 8q24.21) and immune evasion strategies (e.g., SPP1$^+$ TAMs and GALNT3-mediated immunosuppression). Metabolic reprogramming further stratifies tumors into spatially organized Warburg effect and OXPHOS-dominant niches, each associated with unique immune infiltration patterns. The TME exhibits a complex interplay between exhausted PD-1$^+$LAG3$^+$TIM3$^+$ T cells, immunosuppressive stromal cells (MYH9$^+$ CAFs, PODXL$^+$ ECs), and rare but potent effector populations (FGFBP2$^+$ NK cells, CXCL13$^+$ TRMs). Despite these advances, clinical translation faces challenges, including resistance mechanisms (NFKB1 mutations, BCL10$^+$ Treg suppression) and a lack of inhibitors for key targets (PCLAF$^+$ TAEpis, MYH9$^+$ CAFs). Promising therapeutic strategies include epigenetic modulation (SALL4), sialylation inhibition (GALNT3/12), and immune-stromal co-targeting (PD-1 + LAG3/TIM3, NRG1-ERBB3 blockade). Future efforts must prioritize functional validation of novel targets (DKK2, ELF3), spatial multi-omics to resolve CAF-immune-metabolic crosstalk, and biomarker-driven clinical trials integrating single-cell classifiers. By bridging single-cell insights with mechanistic and translational studies, the field can overcome stromal-mediated resistance and usher in an era of precision immunotherapy for CC.

Graphical Abstract

This illustration captures how single-cell sequencing unveils cervical cancer's complex landscape, revealing HPV-driven oncogenesis (viral integration at 8q24.21, GALNT3-mediated immune evasion), metabolic heterogeneity (Warburg/OXPHOS niches), and an immunosuppressive microenvironment (exhausted PD-1 + LAG3 + TIM3 + T cells, MYH9 + CAFs). Key resistance mechanisms (NFKB1 mutations, BCL10 + Treg suppression) and actionable targets (SALL4, NRG1-ERBB3) are highlighted, along with emerging strategies like immune-stromal co-targeting. The visual integrates multi-omics data with a

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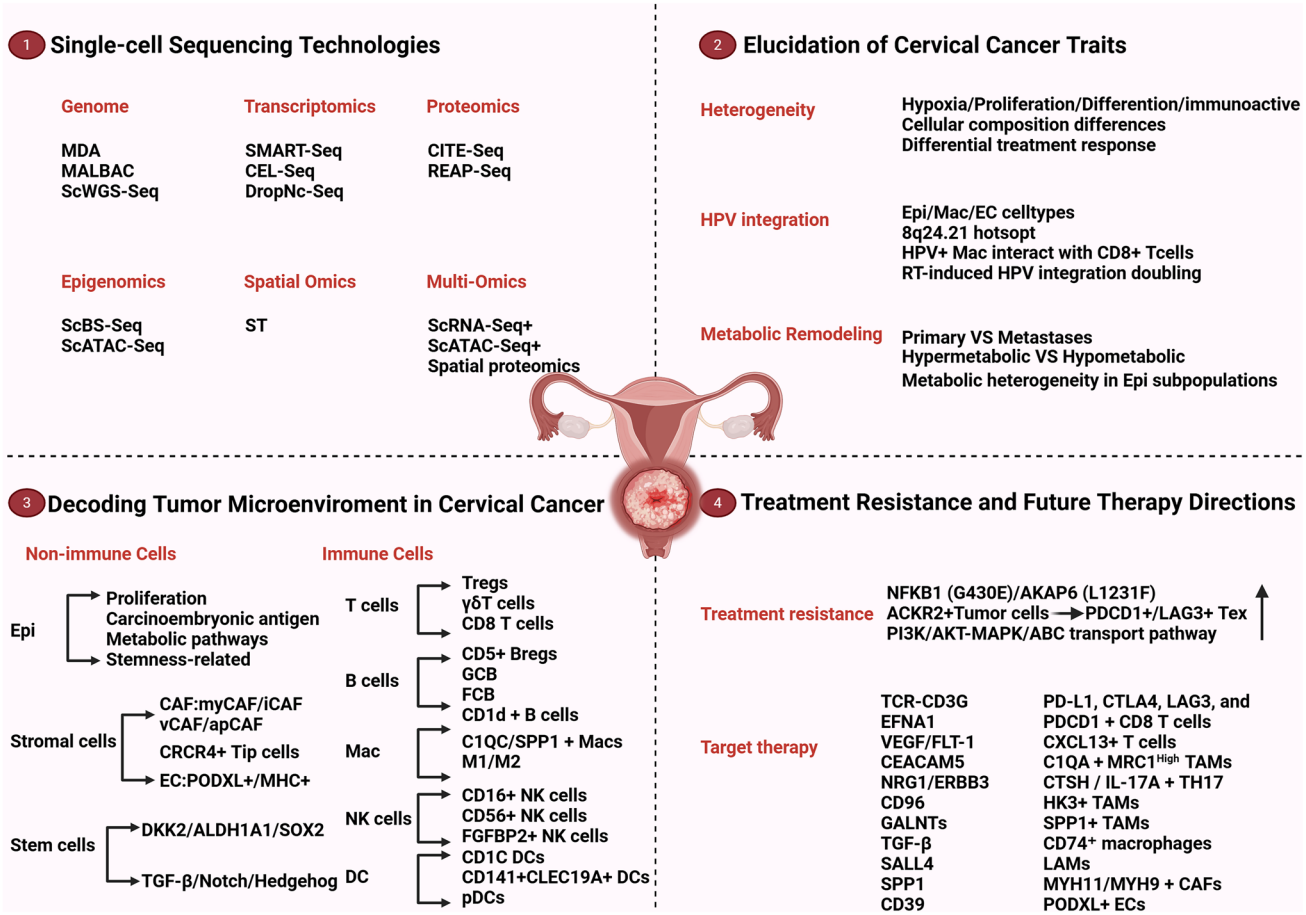
translational pipeline, emphasizing challenges in overcoming stromal resistance and clinical implementation, while pointing toward precision immunotherapy solutions



Keywords Cervical cancer · Single-Cell Sequencing · Biomarkers · Immunotherapy · Precision medicine

Abbreviations

CC	Cervical cancer
RT	Radiotherapy
HPV	Human papillomavirus
MDA	Multiple Displacement Amplification
MALBAC	Multiple Annealing and Looping-Based Amplification Cycles
SMART-seq	Switching mechanism at the 5' end of the RNA transcript
CEL-seq	Cell expression by linear amplification and sequencing
CITE-seq	Cellular indexing of transcriptomes and epitopes by sequencing
REAP-seq	RNA end-associated purification
scWGS	Single-cell whole-genome sequencing
scBS-seq	Single-cell methylation sequencing
scATAC-seq	Single-cell ATAC-seq
FACS	Fluorescence-activated cell sorting
LIANTI	Ligase-Assisted Inverse PCR for Genome-Wide Mapping of Transposon Integration Sites
META-CS	Multiplexed Editing of Target Arrays using Cas9n-Modified Transposases
STRT-seq	Single-cell Transcriptome and Epigenome RNA sequencing
MS	Mass spectrometry
CA	Carcinoma
CE	Cervicitis
CNV	Copy Number Variation
GECs	Glandular epithelial cells
WAS	Wiskott–Aldrich Syndrome Protein
IQCB1	IQ Motif Containing B1
MYO1F	Myosin IF
PDZD11	PDZ Domain-Containing 11
ANXA10	Annexin A10
CXCL5	C-X-C Motif Chemokine Ligand 5

CLDN18	Claudin 18
POU5F1B	POU Class 5 Homeobox 1B
TCA cycle	Tricarboxylic Acid Cycle
EMT	Epithelial–mesenchymal transition
TECs	Tumor-associated endothelial cells
GSVA	Gene set variation analysis
NK cells	Natural Killer cells
DC cells	Dendritic Cell
HIF1A	Hypoxia-Inducible Factor 1 Alpha Subunit
TFDP1	Transcription Factor Dp-1
GRHL1	Grainyhead-Like Transcription Factor 1
STAT1	Signal Transducer and Activator of Transcription 1
MYC	MYC Proto-Oncogene
TP63	Tumor Protein P63
SSECs	Stratified squamous epithelial cells
GECs	Gastrointestinal glandular epithelial cells
MGECs	Mucinous glandular epithelial cells
UGECs	Usual-type glandular epithelial cells
DDIT3	DNA Damage Inducible Transcript 3
EGR4	Early Growth Response 4
PPARG	Peroxisome Proliferator-Activated Receptor Gamma
CESC	Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma
CEAD	Cervical Endocervical Adenocarcinoma
CAF	Cancer-associated fibroblast
CXCL8	C-X-C Motif Chemokine Ligand 8
IGF1	Insulin-like Growth Factor 1
WNT5A	Wingless-Type MMTV Integration Site Family, Member 5A
NRG1	Neuregulin 1
ACTA2	Actin Alpha 2
POSTN	Periostin
ITGB4	Integrin Subunit Beta 4
FAP	Fibroblast Activation Protein
MIA	Multimodal intersection analysis
ESM1	Endothelial Cell-Specific Molecule 1
COL4A1	Collagen Type IV Alpha 1 Chain
ADAMTSL2	ADAMTS Like 2
ANGPTL2	Angiopoietin-Like 2
APLN	Apelin
HPSG2	Heparan sulfate proteoglycan 2
PLVAP	Plasmalemmal vesicle-associated protein
TECs	Tumor-associated endothelial cells
CXCR	C-X-C Chemokine Receptor Type
PDCD1	Programmed Cell Death 1
MKI67	Marker of Proliferation Ki-67
LAG3	Lymphocyte activation Gene 3
TIM3	T-cell Immunoglobulin and Mucin Domain-containing Protein 3
GZMK	Granzyme K
GZMB	Granzyme B
Th17	Elevated T-helper 17
TNFRSF9	Tumor Necrosis Factor Receptor Superfamily Member 9
CIR	Immune reaction
IIR	Inactive immune reaction
IBIR	Imbalanced immune reaction
CD81	Cluster of Differentiation 81
MMP2	Matrix Metallopeptidase 2
LTB	Lymphotoxin Beta
ITK	IL2-Inducible T-Cell Kinase
CX3CL1	C-X3-C Motif Chemokine Ligand 1
TSLP	The Thymic stromal lymphopoietin
FYN	FYN Proto-Oncogene
IL7R	Interleukin 7 Receptor
STAT1	Signal Transducer and Activator of Transcription 1
LACC	Locally advanced cervical cancer
ICMs	Immune checkpoint molecules
KLRD1	Killer Cell Lectin Like Receptor D
KLRC1	Killer Cell Lectin Like Receptor C1
NKG7	Natural Killer Cell Group 7
TRAF4	TNF Receptor-Associated Factor 4
FOSB	FBJ Murine Osteosarcoma Viral Oncogene Homolog B
CXCL1	C-X-C Motif Chemokine Ligand 1
FOS	FBJ Murine Osteosarcoma Viral Oncogene Homolog
CCL20	C–C Motif Chemokine Ligand 20
APOE	Apolipoprotein E
HLA	Human Leukocyte Antigen
CLEC9A	C-Type Lectin Domain Family 9 Member
DKK2	Dickkopf-2
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
PROM1	Prominin 1
CD55	Cluster of Differentiation 55
RHEX	Regulator of Hematopoietic Expansion
ALDH1A1	Aldehyde Dehydrogenase 1 Family Member A1
SOX2	SRY-Box Transcription Factor 2
TCGA	The Cancer Genome Atlas

Introduction

CC is a common malignancy affecting the female reproductive system, and its most frequent cause is HPV infection. HPV has a long latency period and is challenging to detect early [1]. In 2020, around 606,000 new CC cases and 342,000 related deaths were reported globally [2, 3]. The treatment for early-stage CC is primarily surgical removal, whereas that for mid-to-late-stage is RT and chemotherapy [4, 5]. Immunotherapy has promising potential in

treating CC [6]. However, the immunotherapeutic efficacy in advanced-stage cancer patients has limitations [7]. The treatment failures are mainly attributed to the heterogeneity of the tumor [8], which is characterized by the existence of diverse subtypes or cellular clusters within a tumor, genetic disparities, epigenetic, molecular attributes, and biological behaviors. This overview presents the recent advancements in single-cell sequencing research aimed at unraveling the complexity of the CC microenvironment, specifically during tumor initiation, progression, and treatment. The acquired data will offer a comprehensive understanding of CC, facilitating the discovery of potential therapeutic targets and immunotherapy biomarkers.

Single-cell sequencing classifications, technical limitations, and cervical cancer applications

Traditional methods give average genomic information for cell populations, whereas single-cell sequencing provides high-resolution insights into intercellular differences [10] and can analyze the genome sequence of an individual cell, cellular genotypic variations, copy number variations (CNV), chromosomal translocations, etc. Common single-cell DNA sequencing technologies include Multiple Displacement Amplification (MDA) [9] and Multiple Annealing and Looping-Based Amplification Cycles (MALBAC) [10]. Furthermore, single-cell transcriptome sequencing can analyze an individual cell's transcriptome profile, including transcript expression levels, alternative splicing, exon usage, gene expression regulation, etc. [11]. Frequently employed single-cell transcriptome sequencing technologies include the Switching Mechanism at the 5' end of the RNA Transcript (SMART-seq) [12], Cell Expression by Linear amplification and sequencing (CEL-seq) [13], and DroNc-seq [14]. Another technique called single-cell proteomics [15] can elucidate the expression and modification status of proteins in a single cell, including protein types, subtypes, phosphorylation, methylation, etc. The techniques involved include cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) [16] and RNA End-Associated Purification (REAP-seq) [17]. Moreover, single-cell epigenetic sequencing [18] can determine each cell's epigenetic modification status, such as DNA methylation, histone modifications, etc., mainly via single-cell Whole-Genome Sequencing (scWGS) [19], single-cell methylation sequencing (scBS-seq) [20], and Single-cell Assay for Transposase-Accessible Chromatin using sequencing (scATAC-seq) [21]. These techniques have different principles and implementation methods; however, they can determine the epigenetic features of an individual cell. In addition, there are also some single-cell multi-omics integration techniques, like the combination of single-cell RNA sequencing and ATAC sequencing (scRNA-seq + ATAC-seq) [22]. Single-cell spatial genomics [23] is a rapidly developing field that can simultaneously evaluate transcriptome and acquire chromatin accessibility information while preserving spatial context [24, 25]. Single-cell sequencing technology follows a standardized protocol [26], which involves single-cell isolation[27], lysis, biomolecule extraction [28], library preparation, sequencing [29], and subsequent data analysis [30, 31]. While this technology has revolutionized the study of CC, providing unprecedented resolution at the individual cell level, it is not without limitations and challenges [32]. A major technical hurdle is amplification bias and noise, arising from the minute quantities of RNA in single cells [33]. Furthermore, cell selection criteria and viability thresholds critically influence data quality, necessitating stringent experimental controls and rigorous bioinformatic validation [34]. The generation of high-dimensional single-cell datasets further demands robust computational infrastructure and specialized analytical pipelines, presenting significant challenges in data processing and interpretation. Importantly, we have refined the discussion to better distinguish between biological variation and technical artifacts in CC studies [35]. Finally, beyond current technical limitations, significant consideration should be given to clinical scalability challenges and cost-effectiveness in translational applications. Equally crucial is optimizing their implementation for precision medicine. Despite these obstacles, single-cell sequencing has unlocked profound biological insights, enabling researchers to dissect cellular heterogeneity, developmental trajectories, and disease mechanisms at an unprecedented scale [36]. As the technology continues to evolve, its role in basic and translational biology is expected to expand, further cementing its importance in biomedical research [37].

The rapid advancement of single-cell technologies has profoundly transformed CC research, enabling multidimensional analyses of cellular heterogeneity (e.g., normal vs. HeLa cells, differentiation states) [38–40], disease progression (normal adjacent tumor–tumor [41], ectocervix-Cervical intraepithelial neoplasia(CIN)-early CC-advanced CC [42], normal-High-grade Squamous Intraepithelial Lesion(HSIL)-CC-lymph node metastasis) [43], histopathological subtypes (Squamous cell carcinoma(SCC) vs. Adenocarcinoma(ADC)), HPV infection status (HPV association adenocarcinoma (HPVA) vs. Non-HPV association adenocarcinoma(NHPVA))[44], treatment response (pre- vs. post-chemoradiotherapy), integrated approaches combining scRNA-seq with spatial transcriptomics, proteomics, and TCR sequencing have further elucidated the tumor ecosystem [44–46]. These studies have revealed molecular signatures distinguishing CC from other malignancies, mapped its progression trajectory, and characterized HPV-driven remodeling of tumor and immune compartments.

Key discoveries include spatially structured intratumoral heterogeneity, immunosuppressive stromal-immune crosstalk, and treatment-induced molecular shifts. Notably, CC demonstrates distinct radiosensitivity linked to its cellular landscape. Identified subpopulations and molecular features hold prognostic value and inform precision therapy. Collectively, this single-cell atlas offers a comprehensive framework for deciphering CC pathogenesis and guiding targeted interventions, as summarized in Fig. 1 and Table 1.

Single-cell sequencing technology for the interpretation of cervical cancer

Heterogeneity in cervical cancer composition

Single-cell sequencing overcomes the limitations of bulk sequencing by enabling a high-resolution dissection of tissue complexity and disease pathogenesis, particularly cellular heterogeneity [67, 68]. In CC, single-cell transcriptomic profiling identified four distinct tumor subtypes: hypoxic, proliferative, differentiated, and immunoreactive [41]. Tumor cells exhibited three distinct transcriptional states, epithelial-cytokeratin, epithelial-immune, and epithelial-senescence, each corresponding to specific differentiation stages and associated immune microenvironments [46]. Gu et al. applied single-cell RNA sequencing to multiple CC types and found fibroblasts to be the dominant population in carcinoma (CA) and cervicitis (CE), accounting for 84.7% and 63.4% of cells, respectively. In contrast, primary resistant tumors were enriched for epithelial and immune cells [58]. Notably, epithelial subpopulations exhibited varying levels of copy number variations (CNVs) [60], while immune cell types remained relatively conserved across tumors, suggesting a shared Tumor Microenvironment (TME) architecture despite epithelial heterogeneity [44]. However, marked differences in cell composition were observed across CC stages and histological subtypes (SCC vs. ADC) [53]. Stage-specific variation in cell cycle distribution was also noted, with Stage I (CCI) tumors enriched in G1/S-phase cells, and Stage II (CCII) tumors predominated by non-cycling cells [64]. Following RT, epithelial cells decreased, while myeloid-derived suppressor cells (MDSCs) and CD16^{+} NK cells increased, indicating immune remodeling [55]. Moreover, transcriptomic heterogeneity among malignant epithelial cells helped identify subtype-specific

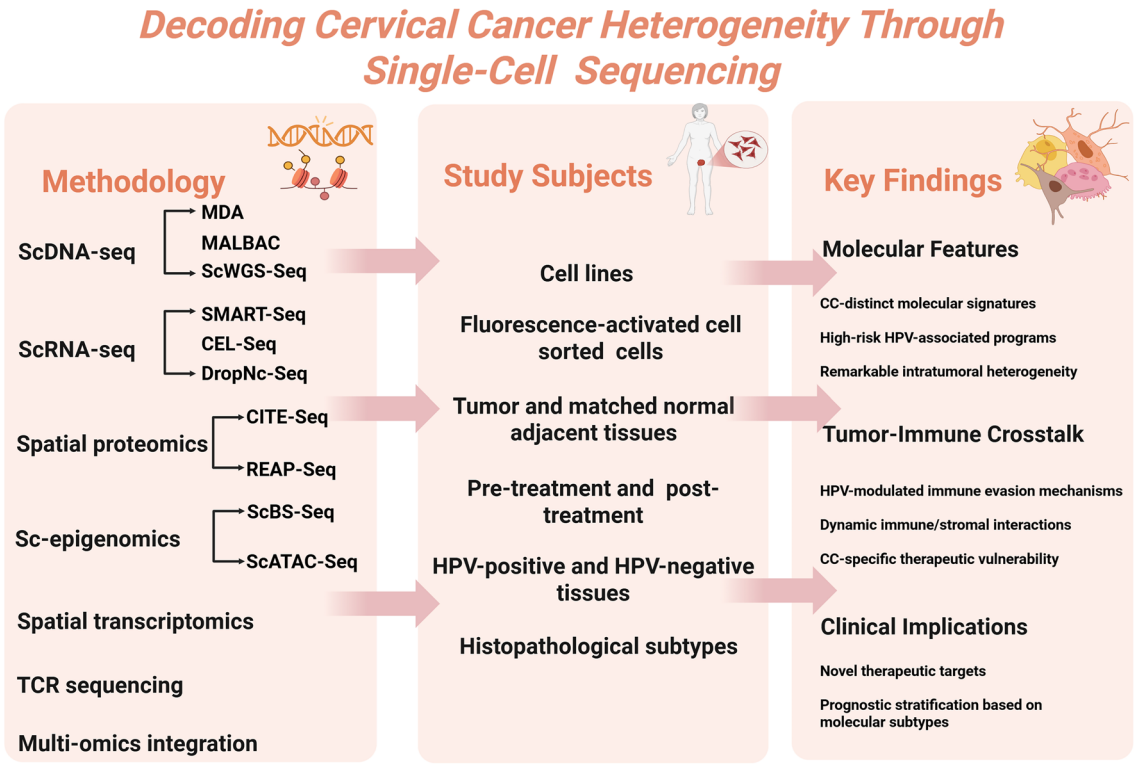


Fig. 1 This framework systematically organizes cervical cancer single-cell research across three dimensions: (1) technological platforms (scDNA/RNA-seq, proteomics, and epigenetics); (2) biological applications spanning cellular heterogeneity, disease progression (normal → CIN → metastasis), histopathological subtypes (SCC/ADC), and HPV pathogenesis; and (3) key discoveries including molecular signatures, spatial architecture, and clinical biomarkers. The diagram demonstrates how multi-omics integration decodes tumor biology from single cells to ecosystem-level interactions, bridging fundamental research to precision medicine

Table 1 Single-cell sequencing technology applied in cervical cancer. This table summarizes the key methodological and analytical components of single-cell studies in cervical cancer, including: sequencing technologies employed, sample sources, cell capture methods, amplification techniques, sequencing platforms utilized, and major research findings

Technology	Sample sources	Cell capture	Amplification methods	Platforms	Results	Reference
Genome sequencing	20 HeLa-CCL2 cells and 10 normal human leukocytes	A mouth pipette under the stereomicroscope	MALBAC	Illumina HiSeq 4000	Constructed a heteroploidy map by CNV detection	[38]
Genome sequencing	13 cases of pre-radiotherapy CC and 12 cases of post-radiotherapy samples	A manually controlled pipetting system	MDA	Illumina X10 instruments	Genomic changes, specifically in the context of HPV infection, before and after radiation therapy	[47]
Transcriptome sequencing	40 single HeLa S3 cells	Microwell chip	SMART-seq2	Illumina HiSeq 2000	Identified through single-cell transcriptome analysis were 283 co-regulated genes by E6 and E7, including known interactors with HPV viral proteins such as CDC25, PCNA, PLK4, BUB1B, and IRF1	[39, 40]
Transcriptome sequencing	720 HeLa-CCL2 cells derived from distinct passages (the 9th, 14th, and 20th)	A mouth pipette under the stereomicroscope	Smart-Seq2 RT	Illumina HiSeq 4000	Build subtype classification of HeLa-CCL2 cells	[38]
Transcriptome sequencing	WT and NFX1-123 KO CaSki/SiHa	Functionalized polydimethylsiloxane	Seq-Well	Illumina NextSeq 550	NFX1-123 interacts with HPV 16 oncogenes in driving and maintaining RNA, cell cycle, and carcinogenesis pathways, and specifically regulates hTERT, telomerase, and CENP-F	[48]
Transcriptome sequencing	Three patients with CC before treatment-naive (TN) and after chemoradiotherapy (CCRT)	-	10×Genomics	Illumina	CCRT-resistant $ACKR2^{+}$ tumor cells secrete TGF-β to drive $CD8^{+}$ T-cell senescence	[49]
Transcriptome sequencing	Tumor and adjacent normal tissues obtained from 2 treatment-naïve patients who were diagnosed with small-cell neuroendocrine carcinoma (SCNEC)	-	10×Genomics	Illumina NovaSeq	Elevated SOX2 expression levels combined with high-risk HPV infection in cervical squamous epithelium could potentially facilitate the malignant transformation toward neuroendocrine carcinoma	[50]

Table 1 (continued)

Technology	Sample sources	Cell capture	Amplification methods	Platforms	Results	Reference
Transcriptome sequencing	Three pairs of primary tumors (PTs) and normal adjacent tissues (NATs) from 3 treatment-naive SCNECC patients	-	10 × Genomics		Four epithelial cells molecular subtypes defined by ASCL1, NEUROD1, POU2F3, and YAP1 TF signatures	[51]
Transcriptome sequencing	Three Pre-RT CC and three post-RT CC	-	10 × Genomics		Single-cell analyses reveal that radiation-activated mast cells orchestrate immunosuppressive niches and fibrotic stroma in CC through paracrine crosstalk	[52]
Transcriptome sequencing	Three patients with HPV-positive SCC and three with HPV-positive AD	-	10 × Genomics	Hiseq X Ten	SCC showed EMT/hypoxia activation and cytotoxic immunity, while AD exhibited cell cycle proliferation and naive/Treg dominance	[53]
Transcriptome sequencing	Three recurrence vs three recurrence-free at 3 years from CSCC	-	10 × Genomics		CXCL13 + T-cell subsets were significantly increased in non-recurrent tumors and acted as major signal senders. In recurrent tumors, FOXP3 + and IL2RA + Tregs were the primary mediators of cell communications	[54]
Transcriptome sequencing	10 sets of paired biopsies from CC collected before and after radiochemotherapy (RCT), along with three samples of neighboring normal cervical tissues	FACS (CD45)	10 × Genomics	Illumina NovaSeq 6000	Intracellular heterogeneity changes of CC before and after RCT	[55]
Transcriptome sequencing	Three samples from the ectocervix, two from CIN, three from early-stage CESC (FIGO stage IB2), and five from advanced-stage CESC (FIGO stage IIB to IIIC)	FACS sorting was performed to separate immune cells (CD45 +), endothelial cells (CD45 − CD31 +), epithelial cells (CD45 − EpCAM +), and fibroblastic cells (CD45 − EpCAM − CD31 −)	10 × Genomics	Illumina NovaSeq 6000	The diversity within CC across various stages	[42]

Table 1 (continued)

Technology	Sample sources	Cell capture	Amplification methods	Platforms	Results	Reference
Transcriptome sequencing	Sequencing of mouse normal cervix, epithelial transition zone, and dysplastic region tissues	Microfluidic chip	10 × Genomics	Illumina NovaSeq 6000	DKK2 upregulation in cervical stromal cells activates stem cell invasion of columnar epithelium or metaplastic lesion formation, promoting carcinogenesis	[56]
Transcriptome sequencing	Three CC tumors were analyzed alongside their corresponding paired NAT samples	Microfluidic chip	Full-length mRNA amplification and library construction system	Illumina NextSeq500	Cellular heterogeneity and molecular stratification of CC	[41]
Transcriptome sequencing	Three Normal cervix, two HSIL, four cervical tumors , and 1 metastatic lymph node	-	10 × Genomics	Illumina NextSeq500	Dynamic change of TME during CC progression	[43]
Transcriptome sequencing	Three samples of SCC with positive HPV status, three samples of ADC with HPVA, and two samples of NHPVA	-	10 × Genomics	NovelBrain Cloud Analysis Platform	Novel diagnostic biomarkers were discovered (CLDN14 for ADC and KRT1 for SCC), along with prognostic cell clusters such as malignant epithelial cells, basal cells, and SPP1 + / C96QC + macrophages	[44]
Transcriptome sequencing	Three tumor samples and one positive lymph node (P-LN) and one negative lymph node (N-LN) from four patients	-	10 × Genomics	Illumina HiSeq × 10	Tumor ecosystems underlying CC metastasis	[57]
Transcriptome sequencing	Three SCC, one ADC, and one chronic cervicitis	-	10x Genomics	-	Single-cell atlas of chemoresistance in CC	[58]
Transcriptome sequencing	CC tissue and the neighboring normal tissue	-	10 × Genomics	Illumina NextSeq500	Tumoral heterogeneity and transcriptional activities of endothelial cells in CC	[59]
Transcriptome sequencing	Two tumor samples and two adjacent samples	FASC(immune cells (CD45 +) and epithelial cells (CD326 +))	10 × Genomics	Illumina NextSeq500	Heterogeneity of HPV-related cervical adenosquamous carcinoma	[60]

Table 1 (continued)

Technology	Sample sources	Cell capture	Amplification methods	Platforms	Results	Reference
Transcriptome sequencing	NC($n=4$), LSIL($n=3$), HSIL($n=5$) and CC($n=3$)	-	10 × Genomics	Illumina NovaSeq	A lipid-associated macrophage (LAM) subset was identified as a pro-tumorigenic component and validated as a predictive biomarker for LSIL-to-HSIL progression	[61]
Transcriptome sequencing	Samples from three patients (T1-T3) before (T1-T3) and after (T1a-T3a) neoadjuvant therapy, with additional post-PD-1 blockade samples from T2/T3 (T2b/c, T3b)	Microwell chip	10 × Genomics	Illumina NovaSeq 6000	CD74 upregulation limited phagocytosis and stimulated M2 polarization, whereas CD74 blockade enhanced macrophage phagocytosis, decreasing CC cell viability in vitro and in vivo	[62]
Transcriptome sequencing	B cells from naive mice and tumor-draining lymph nodes in AT-84-E7-OVA-bearing mice treated with anti-PD-L1, RT, or combination therapy, plus controls	FACS: B cells CD3-CD11b-Gr-1-Ter-199-CD19 +	10 × Genomics	Illumina HiSeq 4000	B-cell markers such as CD19 and IGJ can significantly predict the immunotherapeutic response in HPV-related squamous cell carcinoma	[63]
Sn-RNA seq	Four tumor tissues of stage I and three of stage II Five CSCC fresh frozen tissue	Nucleus isolation	10 × Genomics	-	Decoding the progression of CC	[64]
Sn-RNA seq	Five CSCC and three adenocarcinoma (Cade) samples	The Chromium Next GEM Single-Cell Multiome ATAC + Gene Expression User Guide	10 × Genomics	NovaSeq platform	CD8 + T cells in CSCC were more cytotoxic but less exhausted compared to those in CAde, and phagocytic MRC1 + macrophages were specifically enriched in CSCC	[65]
Spatial transcriptome sequencing	Three NC samples and 15 CSCC samples	10 μm sections were cut from the OCT-embedded samples	10 × Genomics	MGI DNBSEQ sequencer	Revealed the spatial heterogeneity of CC	[45]
TCR sequencing	Three samples of SCC with positive HPV status, three samples of ADC with HPVA, and two samples of NHPVA	Microfluidic chip	5′ gene expression libraries	Illumina sequencer	A thorough single-cell transcriptomic atlas of CC encompassing structural cells, immune cells, and cellular interactions in both squamous cell carcinoma (SCC) and adenocarcinoma (ADC)	[44]

Table 1 (continued)

Technology	Sample sources	Cell capture	Amplification methods	Platforms	Results	Reference
Multiomics (sc RNA sequencing, spatial transcriptomics, and spatial proteomics)	14 treatment-naïve Cervical squamous cell carcinoma and three normal cervical samples	-	10 × Genomics	DNBSEQ-T7	FABP5-driven TGF-β signaling between epithelial keratin tumor cells and CAFs creates an immune-excluded phenotype	[46]
Multiomics (scRNA-seq and spatial transcriptomics)	Two HPV-negative normal, two HPV-positive normal, two HPV-positive HSIL, and three HPV-positive cancer samples	-	10 × Genomics	Illumina sequencer	Identified three HPV-associated epithelial cell states specific to normal cervix, HSIL, and CC	[66]

ADC Adenocarcinoma, *CNV* Copy number variations, *Cade* Cervical adenocarcinoma, *CCRT* Concurrent chemoradiotherapy, *HSIL* High-grade squamous intraepithelial lesion, *LSIL* Low-grade squamous intraepithelial lesion, *MDA* Multiple Displacement Amplification, *MALBAC* Multiple Annealing and Looping-Based Amplification Cycles, *NATs* Normal adjacent tissues, *P-LN* Positive lymph node, *N-LN* Negative lymph node, *PTs* Primary tumors, *RCT* Radiochemotherapy, *SCNEC* Small-cell neuroendocrine carcinoma, *SCC* Squamous cell carcinoma, *SMART-seq* Switching Mechanism at the 5' end of the RNA Transcript, *TN* Treatment-naïve

therapeutic vulnerabilities. For instance, dasatinib and doramapimod were effective in squamous cell carcinoma (CSCC), while pyrimethamine and lapatinib targeted adenocarcinoma (CAde), findings further validated in cell lines and CC-derived organoids [65]. We summarize and present these findings in Fig. 2, demonstrating how single-cell sequencing uncovers cellular heterogeneity in CC, delineates distinct tumor subtypes, reveals stage-specific variations, characterizes treatment-induced immune remodeling, and identifies subtype-specific therapeutic vulnerabilities.

Single-cell sequencing study of HPV infection in the development of cervical cancer

HPV causes the development of various tumors, with HPV16 and 18 subtypes being the most frequently linked with CC [69, 70]. The HPV-CC samples exhibited a higher immune cell infiltration level, while epithelial cells and myofibroblasts had a higher proportion in the HPV + CC samples with extensive heterogeneity [71]. HPV genes from various HPV types exhibited integration preferences in various samples and disease stages [72]. Wu et al. performed single-cell sequencing on 40 HeLa S3 cells and revealed that about one-third of genes had more than one alternative splicing isoform, mostly with only one alternative splicing isoform, and only in a few cells. Moreover, a large number of fusion transcript events were discovered, with a much higher probability than that assessed in bulk sequencing data. Single-cell sequencing also detected more HPV18-infected genome integration sites, frequently at the 8q24.21 site. Additionally, among the 16 identified sites, 11 were validated by polymerase chain reaction and Sanger sequencing [39]. HPV genes were differentially expressed across normal patients and the three disease stages. Notably, HPV integration events were more common in squamous epithelial cells at the single-cell level. The ratio of HPV-integrated cells increased as the disease progressed from normal tissue to CSCC, eventually stabilizing. The TCR analysis revealed that HPV + squamous cell carcinoma had higher clonal expansion than HPV cases; however, non-HPV-related squamous cell carcinoma also indicated elevated clonality [73]. The previous studies have shown that HPV only infects epithelial cells [45]; however, Li et al. discovered that HPV infection targets specific subsets of epithelial cells, endothelial cells (ECs), and macrophages [59]. Wang et al. compared the expression of HPV genes, such as E1, E6, and E7, in the single-cell dataset (GSE168652) via tools including Unique Molecular Identifier and Viral-Track and proved that the HPV genome was expressed in epithelial cells, macrophages, and T cells. Furthermore, the differentially expressed genes in HPV + than HPV- macrophages were predominantly associated with cell adhesion-related

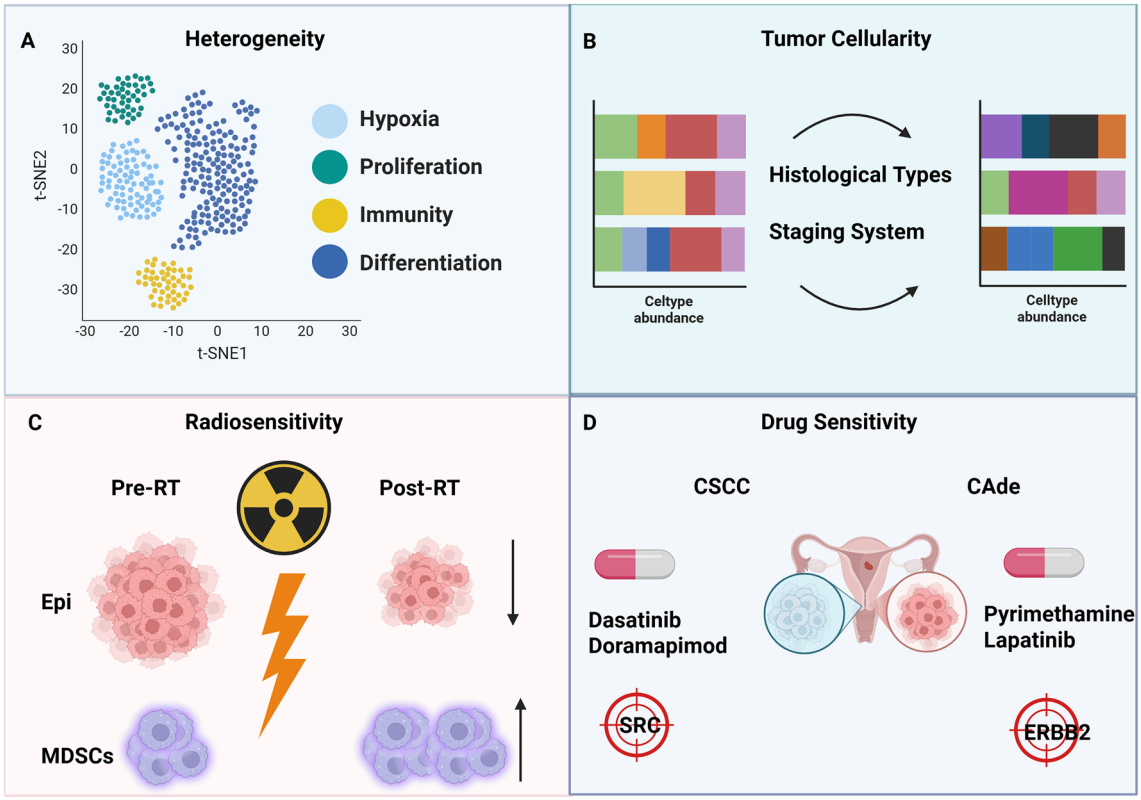


Fig. 2 Single-cell profiling reveals cervical cancer's multidimensional heterogeneity: **A** UMAP identifies hypoxic, proliferative, differentiated, and immunoreactive molecular subtypes; **B** histopathology- and stage-dependent cellular composition; **C** radiotherapy-induced epithelial reduction with MDSC expansion; and **D** subtype-specific drug vulnerabilities (SRC inhibition in CSCC vs ERBB2 targeting in CAde)

pathways. Genes such as WAS, IQCB1, MYO1F, and PDZD11 were highly expressed in HPV + macrophages and were associated with better prognosis in CC patients. Moreover, there was an increased interaction between HPV16 + macrophages/CD8 + T cells and epithelial cells compared with HPV16- macrophages/CD8 + T cells and epithelial cells [74]. HPV infection promotes the malignant transformation of epithelial cells, which has been validated by Qiu et al. via inferCNV analysis. It has also been indicated that some non-HPV-infected metastatic cells express specific genes, such as ANXA10, CXCL5, and CLDN18. These genes also stimulate the differentiation pathway in cervical glandular epithelial cells (GECs) by influencing signal transduction and disrupting cellular polarity, morphology, and growth [44]. Single-cell RNA sequencing of HPV16-infected compared to uninfected organoids identifies twelve distinct keratinocyte populations, with a subset of an HPV-reprogrammed keratinocyte subpopulation (HIDDEN cells) that forms the surface compartment and requires overexpression of the ELF3/ESE-1 transcription factor. Mapped to reconstruct their respective 3D geography in stratified squamous epithelium. Instead of conventionally terminally differentiated cells [75]. It was also discovered that after RT, HPV integration sites doubled compared with the pre-therapy samples. Subsequently, comparative analysis revealed a key driver gene, POU5F1B, causing radiation therapy resistance [47]. In HPV 16 E6 (16E6)-expressing epithelial cells, NFX1-123 augments and is required for full hTERT expression, leading to a growth advantage, that NFX1-123 has with HPV 16 oncogenes in driving and maintaining RNA, cell cycle, and carcinogenesis pathways, and specifically regulating hTERT, telomerase, and CENP-F[48]. Moreover, high-risk HPV infection and amplification of SOX2 in the squamous epithelium may contribute to the progression of small-cell neuroendocrine tumorigenesis in the cervix[50]. We systematically summarize these findings in Fig. 3, illustrating how HPV infection drives cervical carcinogenesis through three key mechanisms: (1) epithelial cell malignant transformation, (2) viral genome integration events, and (3) dynamic immune microenvironment modulation. Single-cell resolution analyses further delineate the viral subtype-specific cellular tropism, host transcriptional reprogramming networks, and the emergence of therapy-resistant clonal populations.

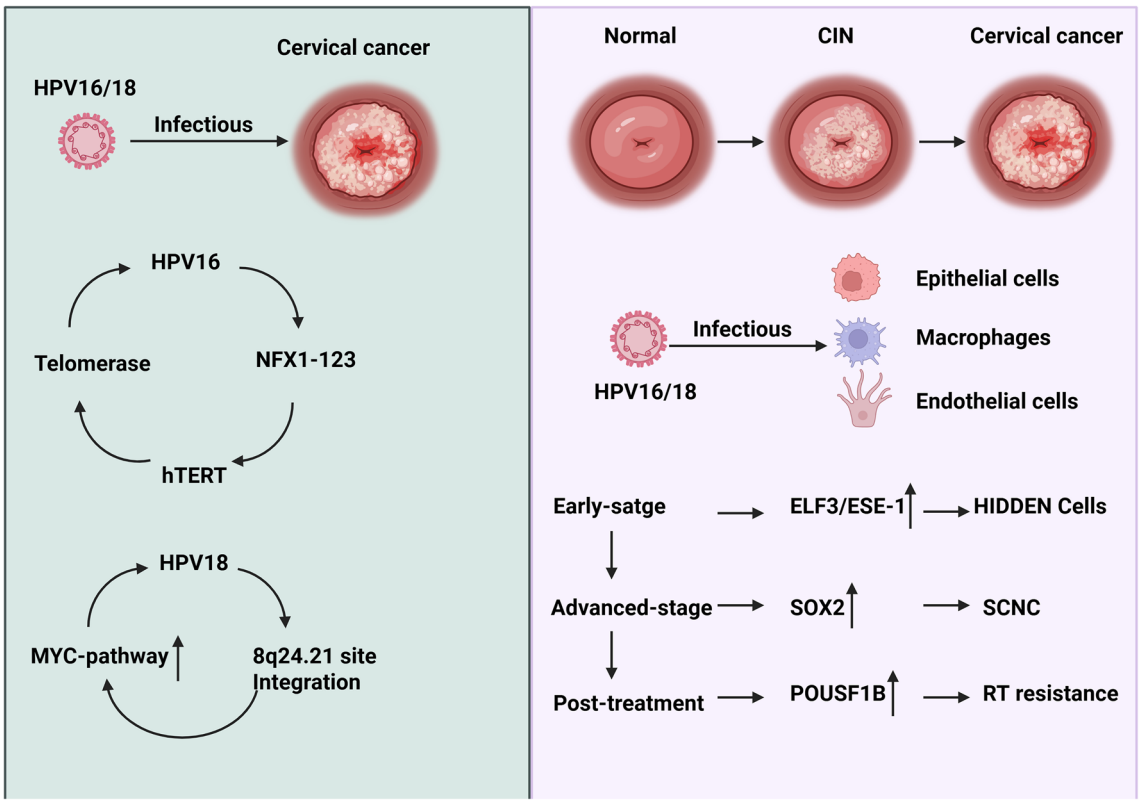


Fig. 3 The schematic delineates HPV-host dynamics during carcinogenesis: (1) Molecular interplay via HPV16 E6-NFX1-123-hTERT (telomerase activation) and HPV18-8q24.21-MYC oncogenic axes. (2) Spatially restricted infection of epithelial cells, macrophages, and endothelium. (3) Temporal progression from normal tissue to invasive CSCC (HPV integration increasing 10–60%) through key transitions—ELF3/ESE-1 + HIDDEN cell formation, SOX2-driven neuroendocrine transformation, and POU5F1B-mediated radioresistance

Single-cell sequencing reveals metabolic heterogeneity within cervical cancer

CC is a common gynecologic malignancy closely linked to metabolic dysregulation [76, 77]. Metabolic biomarkers—such as fatty acids, cholesterol, glucose, and triglycerides, promise for early detection and diagnosis [78, 79]. Recent single-cell analyses have revealed substantial metabolic heterogeneity among epithelial cells in CC. Wang et al. identified six distinct epithelial clusters, each exhibiting unique metabolic profiles. Some subsets were enriched in glycolysis to meet heightened energy demands, others in lipid metabolism for membrane synthesis, and certain clusters showed glutamine pathway activation, potentially associated with treatment resistance [80]. Epithelial cells were further classified into seven clusters based on metabolic and transcriptomic features. For instance, Epi1 showed prominent hypoxic signaling, Epi2-4 were enriched for glycolysis and oxidative phosphorylation(OXPHOS), while Epi5 was characterized by p53 pathway activation and epithelial–mesenchymal transition (EMT) signatures [60]. Moreover, the metabolic landscape of epithelial cells varied by pathological stage and location. In metastatic lymph nodes, epithelial cells were enriched in cell cycle-related pathways and immune response signaling. In contrast, primary tumor-derived epithelial cells were enriched for EMT, oxidative phosphorylation, and interferon-α responses [57]. Beyond epithelial cells, tumor-associated endothelial cells (TECs) also exhibited metabolic activity, particularly in xenobiotic metabolism, peroxisome proliferator-activated receptor (PPAR) signaling, amino acid biosynthesis, and carbon metabolism pathways linked to therapy resistance[59]. Spatial transcriptomic profiling further demonstrated region-specific metabolic heterogeneity within tumors, which significantly influenced immune infiltration. Tumors were stratified into hypermetabolic and hypometabolic regions based on Gene Set Variation Analysis (GSVA) scores. Hypermetabolic regions exhibited enhanced Warburg effect signatures—suggestive of hypoxia and nutrient stress—and were enriched in $CD56^{+}$ NK cells and immature dendritic cells (DCs). In contrast, hypometabolic regions were more populated by eosinophils, immature B cells, and regulatory T cells (Tregs), potentially reflecting immune suppression or dysfunction [45]. Furthermore, Yin et al. reported a negative correlation between intratumoral hypoxia and tumor-infiltrating T cells, a phenomenon especially pronounced

in tumor cores compared to peripheral margins [81]. These findings are comprehensively integrated in Fig. 4, demonstrating how single-cell analyses elucidate metabolic heterogeneity in CC by identifying: (1) tumor cell-intrinsic metabolic reprogramming (glycolytic/OXPHOS/lipogenic subpopulations), (2) their functional crosstalk with immune evasion mechanisms, and (3) the emergence of metabolically-driven therapeutic resistance phenotypes.

Single-cell sequencing reveals the characteristics of tumor cells and microenvironment

Epithelial cells

Genomic and transcriptional heterogeneity in epithelial cells

Single-cell genome sequencing has revealed pronounced chromosomal instability, particularly at chromosome 19, in CC cell lines such as HeLa CCL2, which segregates into six clusters with varying proliferation rates and interferon response signatures [38]. CNV analysis further classified epithelial cells into malignant (C1-C4) and near-normal (C5-C7) clusters, where malignant subsets exhibited enrichment in hypoxia, angiogenesis, and mesenchymal migration pathways, driven by transcription factors such as HIF1A, TFDP1, GRHL1, and STAT1 [41]. Similarly, inferCNV-based clustering distinguished metastatic from non-metastatic epithelial cells, with the former showing keratinization and squamous differentiation regulated by MYC and TP63, while the latter displayed antiviral responses [44]. Pseudotime trajectory analysis has traced the evolution of epithelial subpopulations (Epi1-Epi8), revealing malignant transformation trajectories and differentiation states [42]. Tumor epithelial cells can be stratified into functional clusters: Proliferative (Cluster 3), Metabolic (Cluster 0), Stem-like (Cluster 8), and Carcinoembryonic antigen-expressing (Cluster 1). These clusters correlate with TCGA transcriptomic subtypes (A-D), where Cluster D (matching scRNA-seq Cluster 1) predicts better survival, while Cluster B (matching Cluster 0) is associated with poor prognosis [59]. Single-cell sequencing unveils genomic heterogeneity and malignant transformation trajectories in CC epithelial cells, with hypoxic/

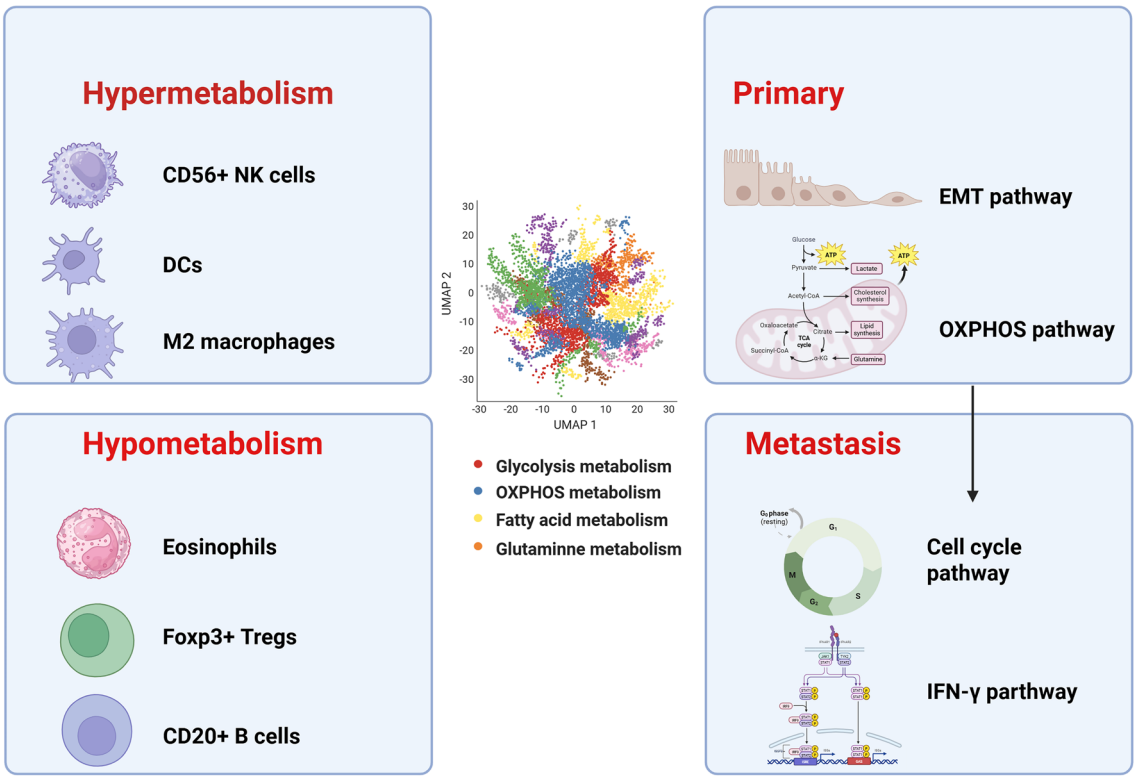


Fig. 4 Metabolic heterogeneity underlies therapeutic resistance: (1) Single-cell analysis defines glycolytic, OXPHOS, lipogenic, and glutaminolytic epithelial subtypes; (2) spatial immuno-metabolic zonation shows CD56 + NK enrichment in active regions versus FOXP3 + Tregs/CD20 + B cells in quiescent zones; and (3) primary-metastatic divergence (OXPHOS/EMT-dominant primaries vs cell cycle/interferon-active metastases) reveals resistance mechanisms

angiogenic malignant subsets linked to distinct clinical outcomes.

Subtype-specific heterogeneity and therapeutic implications

Single-cell analysis reveals distinct epithelial subpopulations in HPV + CEAD, with Epi1 showing hypoxic/glycolytic (IL2/STAT5) features and Epi4 exhibiting proliferative (G2M/E2F/DNA repair) signatures, highlighting the necessity for subtype-specific therapies [80]. Additionally, PCLAF + tumor-associated epithelial cells (TAEpis) in CSCC correlate with poor anti-PD-1 response and CD8 + T-cell dysfunction, promoting tumor-immune evasion [82]. Post-RCT single-cell analysis identified dynamic shifts in epithelial subsets, with Epi2 expansion and Epi3 reduction. Notably, MHC-II-mediated antigen presentation was upregulated in Epi1/4/5, while MHC-I expression declined in Epi2, suggesting differential immunomodulatory effects of RCT [55]. Sialylation-related genes are highly enriched in CC epithelial cells. GALNT3-negative epithelial cells are protective, negatively correlating with immunosuppressive macrophages and MDSCs [83]. GALNT12, GCNT4, and NPL serve as prognostic biomarkers [84]. SALL4, an oncogenic driver, promotes tumor progression and represents a promising therapeutic target [85]. Single-cell analyses reveal subtype-specific epithelial heterogeneity in CC, with distinct metabolic/immune signatures (hypoxic/GALNT3-/PCLAF + subsets) driving therapy resistance and immune evasion, while identifying potential targets such as SALL4 and Sialylation-related genes.

Cancer stem cells

Cancer stem cells (CSCs) exhibit significant heterogeneity in CC, with single-cell analyses revealing distinct subpopulations characterized by varying expression of stemness markers (e.g., OCT4, SOX2, and NANOG), metabolic profiles (glycolytic vs. OXPHOS-dominant), and tumorigenic potentials. Chumduri et al. conducted a comprehensive investigation of cervical regions, identifying distinct lineage-specific stem cell populations regulated by opposing Wnt signals from the stromal microenvironment [86]. Their work revealed that endocervical stroma undergoes remodeling with upregulation of DKK2, which promotes ectocervical stem cell outgrowth, highlighting niche-dependent regulation [56]. Liu et al. found the CCII group had substantially more CSCs (24.75%) than CCI (15.63%), along with increased mesenchymal stem cells, marked by elevated CFTR, PROM1, CD55, and RHEX expression [64]. Li et al. identified a specific epithelial cluster (Cluster 8) with high ALDH1A1 and SOX2 expression, containing six functional states [59]. Pseudotime analysis showed their progression from differentiation to proliferation phases, with activated TGF-β, Hedgehog, and Notch pathways. Age-related CSC differences emerged, with Cluster 5 (elderly patients) showing CSC-like traits while Cluster 9 (younger patients) exhibited oncogenic and immunosuppressive features[87]. Molecular subtyping identified key transcription factors (ASCL1, NEUROD1, POU2F3, and YAP1) defining cellular states[51]. Malignant transformation studies documented CSCs shifting from proliferative capacity to tumorigenic EMT/invasive potential. Tissue microarray analysis confirmed stem-like clusters associated with recurrence[88]. Concurrent TME analysis revealed progressing interferon responses, suggesting CSC-immune crosstalk.

Cancer-associated fibroblasts

Most fibroblasts originate from normal tissues, while cancer-associated fibroblasts are functionally enriched in IL-17 and IFN signaling and antigen presentation pathways, indicating their interaction with immune cells to promote tumor progression [41]. CAFs are highly heterogeneous. Qiu et al. identified two major subtypes: myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs). iCAFs are enriched in inflammatory pathways (NF-κB, complement, and coagulation), while myCAFs are involved in ECM remodeling and antigen presentation [44]. Liu et al. further classified fibroblasts into six subclusters across disease stages, including two normal matrix fibroblast subsets (Fib1 and Fib2), a myCAF subset enriched in CIN and CESC samples, and three vascular CAFs (vCAF1- 3). myCAFs showed high expression of cancer-associated genes and regulons and were linked to poor survival via NRG1-ERBB3 and WNT5A-FZD6 signaling [42]. During RCT, the proportion of iCAFs and a subtype of activated CAFs (apCAF2) decreased, while vCAF1 increased, accompanied by upregulated TNF and JAK/STAT signaling and downregulated angiogenesis pathways [55]. Single-cell and spatial transcriptomics uncovered spatially regulated CAF subtypes. A specific CAF population located outside high metabolism regions expressed ACTA2, POSTN, ITGB4, and FAP. Multimodal intersection analysis (MIA) classified tumors into myCAF + and myCAF − subtypes. myCAF + tumors exhibited enhanced energy metabolism, proliferation, reduced apoptosis, and immune responses. They also showed significantly decreased infiltration of DCs, B cells, neutrophils, T cells, NK cells, and Th1 cells, suggesting that myCAFs suppress immune surveillance and facilitate tumor progression [45]. CAF spatial heterogeneity was further supported by MYH11 + CAFs being enriched in normal regions. The C0 MYH11 + CAF subset promoted tumor cell proliferation and migration and inhibited apoptosis via soluble SDC1[89]. Importantly, MYH9 was identified as a key regulator of myofibroblast development. Spatial transcriptomics revealed

distinct profiles between MYH9 − and MYH9 + myofibroblasts. High infiltration of MYH9 + myofibroblasts was correlated with poor prognosis and reduced immunotherapeutic efficacy in CC, potentially via Hedgehog pathway activation(Fig. 5)[90]. Single-cell and spatial analyses reveal functionally diverse CAF subtypes (myCAFs/iCAFs/vCAFs) in CC that spatially regulate tumor metabolism, immune suppression (via NRG1/WNT5A/MYH9 pathways), and therapy resistance, with MYH9 + myofibroblasts emerging as key prognostic determinants.

Endothelial cell

Endothelial cell (EC) heterogeneity in CC manifests through distinct angiogenic subtypes, with tip cells (ESM1 + / APLN +) emerging as key regulators of vascular remodeling and immune suppression. Qiu et al. identified four EC types, including tip cells characterized by ESM1, COL4A1, ADAMTSL2, ANGPTL2, and APLN expression, which are involved in angiogenesis and tumor progression. These tip cells represent potential therapeutic targets in CC [91] [44]. Liu et al. further classified ECs into two major subclusters: C0 tumor endothelial cells, marked by HPSG2 and PLVAP, and C1 blood ECs expressing FLT[18]. Further subclustering revealed arteriolar and venular ECs with distinct markers. Arterial ECs were enriched in migration and angiogenesis-related metabolic pathways and potentially interacted with immune cells via CD74-COPA, CD74-MIF, and CD74-APP ligand–receptor pairs [59]. RCT significantly alters the EC landscape. Among the five EC subclusters, EC2 was notably reduced post-RCT, although EC2-4 still accounted for over 50% of ECs. Post-treatment samples also showed increased expression of genes linked to epithelial proliferation [55]. MHC-high ECs indicated reduced antitumor effects, whereas PODXL + ECs enhanced the pro-tumor effects in advanced CESC cases (Fig. 5) [92]. PODXL was predominantly expressed in TECs of CC, as confirmed by tissue staining. High PODXL expression was strongly

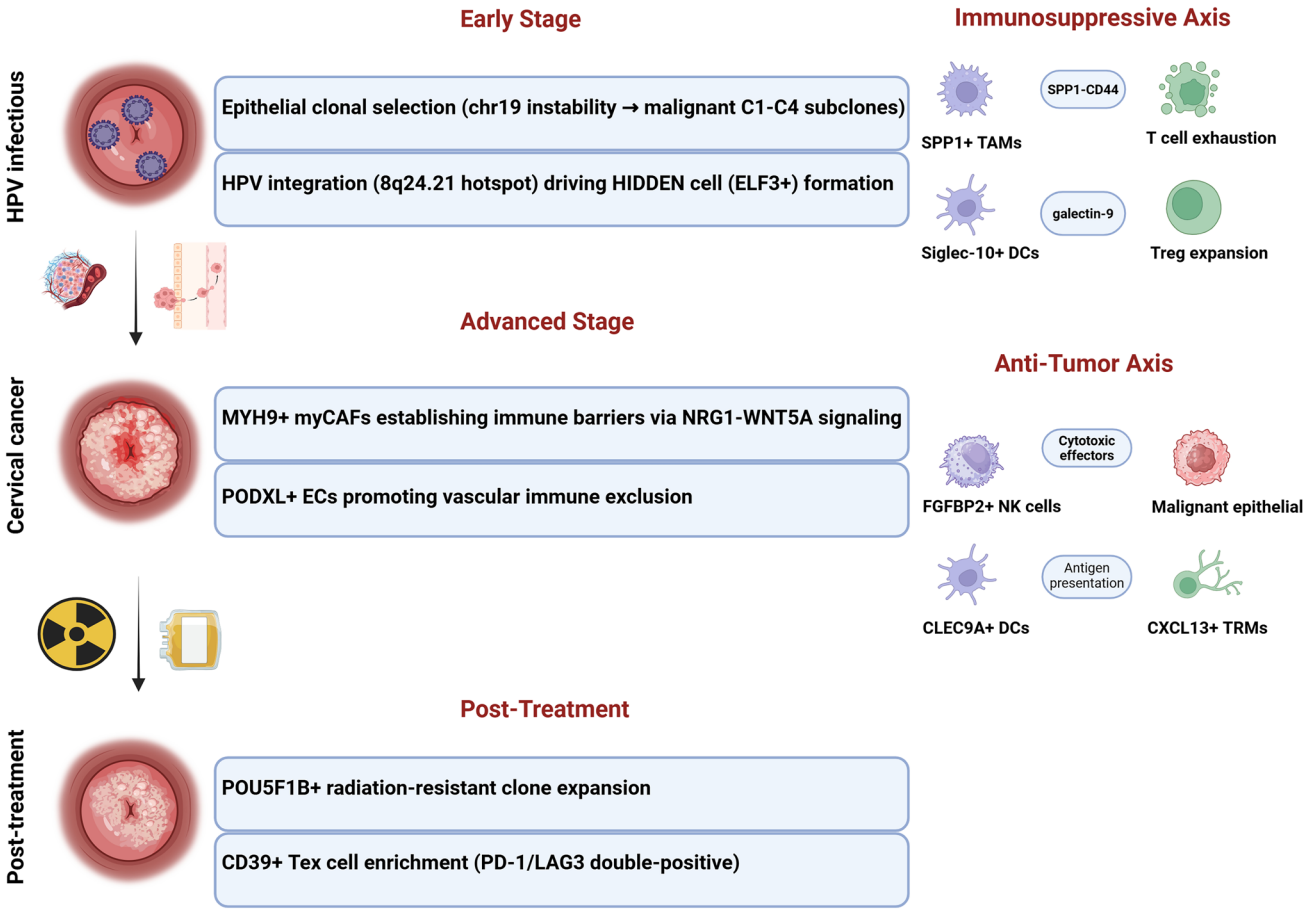


Fig. 5 Spatiotemporal evolution of tumor-immune crosstalk features: (1) Stage-specific progression from HPV-induced ELF3 + HIDDEN cells through myCAF-mediated immune exclusion (NRG1-WNT5A) to PD-1/LAG3 + Tex cell-dominated resistance; (2) dual network regulation balancing immunosuppressive axes (SPP1 + TAM-CD44-CD8 + Tex, Siglec-10 + DC-galectin-9-Treg) against antitumor effectors (FGFBP2 + NK cytotoxicity, CLEC9A + DC-TRM priming)

associated with reduced progression-free and overall survival (both $p < 0.001$) in 180 CC patients receiving radiotherapy or chemoradiotherapy. Compared to PODXL + low TECs, PODXL + high TECs exhibited impaired antitumor immunity and enhanced tumor-promoting features. Consistently, bulk RNA-seq data further demonstrated that PODXL overexpression was negatively correlated with immune response and prognosis in treated CC, supporting its role as a potential therapeutic target and prognostic marker [93]. These findings position EC subpopulations—particularly PODXL + tumor ECs—as dual diagnostic biomarkers and therapeutic targets, offering new avenues to disrupt tumor vasculature while reversing immune evasion in CC treatment.

T cells

T-cell subtypes and distribution in cervical cancer

Single-cell sequencing has revolutionized our understanding of T-cell heterogeneity in tumors, revealing critical subsets with distinct functional states, exhaustion profiles, and tumor-reactive potentials that shape immunotherapy responses and patient outcomes. Liu et al. identified three T-cell subtypes in CESC: $CD8^+$ T cells, γδT cells, and Tregs. $CD8^+$ T cells were more abundant in CCII, while γδT cells and Tregs were more prevalent in CCI. Interestingly, despite higher proportions of $CD8^+$ T cells in CCII, these cells—along with Tregs—showed elevated expression of complement and inflammatory genes, suggesting greater immunosuppressive potential [64]. γδT cells are positively correlated with response to immunotherapy and may serve a protective antitumor role in CC [94]. Treg cells in CESC showed high expression of Th17 and TNFRSF9, linked to tumor progression [42]. TCR analysis revealed pronounced clonal expansion of $CD4^+$ Tregs within the TME, where their spatial enrichment in hypometabolic niches correlated with potent immunosuppressive activity [45]. Moreover, the upregulation of BCL10 in Tregs correlated with increased Treg/$CD4^+$Th2 infiltration and reduced $CD8^+$/Th1/NK infiltration. BCL10 induced PD-1 expression via NF-κB activation in $CD8^+$ T cells, promoting their apoptosis[95].

$CD8^+$ T cells: functional states and exhaustion

scRNA-seq and pseudotemporal analysis delineated distinct $CD8^+$ T-cell populations in CESC, including early-effector ($CXCR4^+$), exhausted ($PDCD1^+$ $LAG3^+TIM3^+$), and proliferative ($MKI67^+$) subsets. While exhausted cells showed downregulated effector markers (CXCR4/CX3CR1), some retained proliferative capacity, nominating LAG3/TIM3 as therapeutic targets [41]. Notably, $GZMK^+$ cells differentiated into functionally impaired $GZMB^+PDCD1^+TIGIT^+CTLA\text{-}4^+$ effectors [81], and $CD39^+$ exhausted T cells correlated with poor prognosis. CD39 blockade enhanced cytotoxicity and synergized with anti-PD-1 therapy via CXCL13-mediated B-cell recruitment, with liposomal POM-1 showing promise [96]. Conversely, $FGFBP2^+$ cytotoxic $CD8^+$ T/NK cells and rare but highly cytotoxic $PLAC8^+$ TRMs are associated with favorable outcomes [60, 97]. $CXCL13^+$ Tissue Resident Memory cells (TRM), exhibiting dual cytotoxic/inhibitory features, predicted radiotherapy survival [97], highlighting TRM heterogeneity as both a prognostic marker and therapeutic determinant. These findings map the functional continuum of $CD8^+$ T cells in CESC, from cytotoxic ($FGFBP2^+$/$PLAC8^+$) to exhausted ($PDCD1^+$/$CD39^+$) states, informing checkpoint blockade and radiotherapy strategies (Fig. 5).

TCR clonality and HPV status

TCR clonality analysis reveals HPV-type-specific immune landscapes in CC, where HPV16 + tumors demonstrate greater $CD4^+$/$CD8^+$ T-cell clonal overlap compared to HPV18 + and non-HPV SCC cases. This possibly explains higher recurrence and metastasis in HPV-positive tumors [44]. $CD8^+$ Tcm and Tex cells exhibited more clonal expansion than Temra/Teff subsets, suggesting immune exhaustion dominance. $CD4^+$ Tregs showed the highest clonal expansion among $CD4^+$ subsets [98]. The above research highlights how HPV status shapes distinct T-cell exhaustion patterns, with clonally expanded Tregs and exhausted $CD8^+$ subsets driving immune evasion, providing mechanistic insights for recurrence risk and immunotherapy stratification in HPV-associated tumors.

Prognostic and predictive T-cell markers

Emerging T-cell biomarkers, particularly $CXCL13^+$ populations, are transforming prognostic prediction and therapy in CC. $CXCL13^+$ T cells correlate with non-recurrence post-CCRT and predict immunotherapy response. A five-gene random forest model using this biomarker accurately predicts recurrence and treatment response [54]. Notably, sorafenib enhances $CD8^+$ T-cell cytotoxicity (↑ IFN-γ, TNF-α), positioning it as a promising immunotherapy sensitizer [99], while iPSC-derived E6-specific CTLs effectively target $HPV16^+$ tumors[100]. These advances—from predictive algorithms to novel cell therapies—underscore the pivotal role of T-cell modulation in CC precision oncology.

B cells

While immunotherapeutic strategies in CC have traditionally focused on T cells, recent studies underscore the critical involvement of B cells. TCGA analyses of HPV-associated head and neck squamous cell carcinoma and CC

revealed that higher expression of B-cell-related genes, such as CD19 and IGJ, correlated with improved 3-year overall survival in HPV-positive patients [101]. In the AT-84-E7 murine model, B-cell depletion accelerated tumor progression, whereas combination therapies increased MHC class II expression in mature and T2 B cells. Notably, RT induced immunosuppressive CD1d high $CD5^+$ regulatory B cells, which were mitigated by PD-L1 blockade. Moreover, PD-L1 checkpoint blockade combined with RT enhanced B-cell responses, as evidenced by an increase in somatic hypermutations (SHM) in productive BCR sequences [102]. Single-cell RNA sequencing identified two functionally distinct B-cell subsets-germinal center (GC) and follicular B cells enriched upon RT and PD-1 inhibition, promoting antigen presentation and tumor cytotoxicity. In the CCII subgroup, elevated B-cell abundance and antigen presentation-related gene expression were observed, alongside high expression of COL3A1, COL1A1, and COL1A2 in native B cells. Additionally, this group showed upregulation of IL-18 and chemokine signaling, as well as enrichment of TSLP and B-cell receptor pathways. In contrast, the CCI group exhibited enhanced interferon signaling [64]. These findings collectively demonstrate that B cells play multifaceted roles in CC immunity, influencing prognosis through antigen presentation, regulatory functions, and synergistic interactions with immunotherapy and radiotherapy.

Macrophage

Recent studies have revealed the functional heterogeneity of tumor-associated macrophages (TAMs) and their critical role in CC progression and immune modulation. Qiu et al. reclassified macrophages into three subsets—monocytes, $C1QC^+$ TAMs, and $SPP1^+$ TAMs—highlighting a prognostic dichotomy between C1QC and SPP1 expression profiles [103]. Gene signatures of $C1QC^+$ and $SPP1^+$ TAMs outperformed conventional M1/M2 classifications in predicting clinical outcomes. Locally advanced CC exhibited reduced $C1QC^+$ TAMs and increased $SPP1^+$ TAMs, whereas patients with high $C1QC^+$ and low $SPP1^+$ TAM signatures had the most favorable overall and disease-specific survival[104]. $C1QC^+$ TAMs were associated with elevated immune checkpoint molecule expression and enrichment of TCR and IFN-γ signaling pathways, suggesting an antitumor phenotype. In contrast, $SPP1^+$ TAMs were enriched in TGF-β signaling, extracellular matrix remodeling, and keratinization pathways, indicating a pro-tumor role. The SPP1–CD44 axis (Fig. 5), in particular, was found to facilitate immunosuppressive communication between $SPP1^+$ macrophages and immune cells, promoting tumor cell survival and correlating with advanced tumor stage and poor prognosis[105].

Comparative analysis of CCI and CCII subtypes revealed distinct macrophage profiles, with CCII exhibiting elevated immune-regulatory gene expression (5.65% vs 5.30% abundance) and activation of EMT, p53, TNF-α/NF-κB, and apoptosis pathways [64]. Metabolic reprogramming was evident through increased oxidative phosphorylation gene expression in CCII-stage macrophages, contrasting with early-stage immune-dominant profiles. HK3-expressing TAMs were found to compromise $CD8^+$ T-cell responses by impairing antigen presentation [105, 106]. Post-NACT studies showed enhanced macrophage-tumor cell interactions, particularly $CD74^+$ macrophages, which promoted M2 polarization; CD74 blockade restored phagocytic function and inhibited tumor growth [62]. Notably, lipid-associated macrophages (LAMs) emerged as predictive biomarkers for LSIL-to-HSIL progression, engaging in pro-tumor SPP1-CD44 signaling with epithelial cells [61]. These findings collectively position TAM-targeted strategies, including SPP1-CD44 axis inhibition and macrophage reprogramming, as promising therapeutic avenues in CC.

NK cells

NK cells, although comprising a relatively small proportion of tumor-infiltrating lymphocytes (2.52% in CCI and 0.85% in CCII), demonstrate distinct functional characteristics across CC subtypes. In the CCI group, NK cells predominantly expressed activation and cytotoxicity-associated genes, including KLRD1, KLRC1, GZMA, GZMB, and NKG7, while NK cells in the CCII group upregulated genes enriched in IL-17 and TNF signaling pathways, such as TRAF4, FOSB, CXCL1, and FOS. Gene set enrichment analysis further revealed enhanced IFN-γ response and allograft rejection pathways in CCI, whereas EMT and coagulation pathways were prominent in CCII [64]. Following RCT, infiltration of $CD16^+$ NK cells increased within the cervical tumor microenvironment, accompanied by elevated expression of cytotoxic effector genes, which correlated with improved patient survival [55]. Subclustering of NK cells identified two major subsets: $CD16^+$ and $CD56^+$ NK cells. Notably, a higher frequency of $CD16^+$ NK cells was associated with better prognosis in CESC patients. These $CD16^+$ NK cells displayed elevated expression of FGFBP2, NKG7, and GZMH, suggesting enhanced cytolytic function compared to the $CD56^+$ subset [42]. Further analysis identified $FGFBP2^+$ NK cells as a potent cytotoxic subset characterized by upregulation of effector molecules, including GZMA, GZMB, GNLY, and PRF1, alongside decreased expression of inhibitory checkpoint genes such as PDCD1, TIGIT, and CTLA4 (Fig. 5). The high cytotoxicity signature score associated with $FGFBP2^+$ NK cells underscores their significant antitumor potential and highlights their relevance as prognostic biomarkers and therapeutic targets in CC [60].

Dendritic cells

Liu et al. characterized seven distinct myeloid cell subpopulations in CC, including three TAMs and two M-MDSCs, with DCs emerging as particularly crucial players that orchestrate the delicate equilibrium between pro- and antitumor immunity [107]. Post-RCT analysis revealed a myeloid compartment shift marked by increased $FCN1^+$ M-MDSCs and decreased $CD1C^+$ DCs, with both populations showing enhanced antigen processing signatures. Extended profiling identified eight myeloid subsets, including prognostically significant $CCL20^+/APOE^+$ macrophages (associated with poor survival) and tumor-suppressive $CLEC9A^+$ DCs (depleted in advanced disease) [42]. Subtype analysis showed CCI tumors harbored more plasmacytoid DCs and $CD141^+CLEC9A^+$ DCs with upregulated antigen presentation genes (Fig. 5), while CCII tumors exhibited broader DC activation of antigen presentation pathways[55]. Notably, immunosuppressive DC subsets were identified: $Siglec\text{-}10^+$ DCs impaired T-cell responses via galectin-9 (Fig. 5), and their blockade restored PD-1 efficacy[108], while $LAMP3^+$ mature DCs formed immunosuppressive niches through IDO1-mediated tryptophan metabolism and spatial co-localization with exhausted T cells. Therapeutic targeting proved synergistic—combined IDO1/PD-1 inhibition achieved superior tumor control versus monotherapy in vivo [109]. These findings collectively highlight the pivotal yet dual role of DCs in both promoting and suppressing antitumor immunity, offering new targets for combination immunotherapy in CC.

The role of single-cell sequencing in the treatment and prognosis of cervical cancer

Single-cell sequencing studies on treatment resistance in cervical cancer

While RCT remains fundamental for CC treatment, recurrence persists due to complex TME alterations involving immune and stromal components [102, 110]. Key resistance mechanisms include: (1) Genetic evolution post-RT, with NFKB1 (G430E) mutant allele frequency increasing from 9.8% to 31.9%, converting this tumor suppressor into a resistance driver dysfunctional through acquired mutations during RT, contributing to therapy failure [111]; (2) immune dysfunction featuring PDCD1 + LAG3 + TIM3 + exhausted T-cell clusters (C1) and ABC transporter-upregulated chemoresistant ECs [58, 59, 112]; and (3) therapy-induced immunosuppression through ACKR2 + tumor cells that secrete TGF-β to drive CD8 + T-cell senescence [49], and mast cell-mediated ECM remodeling [52]. Notably, treatment also activates potential therapeutic vulnerabilities: PD-1 blockade synergizes with RT by enhancing B-cell memory responses [102]. While residual epithelial cells upregulate MHC-II antigen presentation [55], multi-omics analysis revealed IL1R1 as an actionable target, with anakinra combination therapy mitigating relapse [49, 113]. These findings reveal the Janus-faced nature of TME remodeling post-therapy, where survival adaptations simultaneously create therapeutic resistance while exposing new targetable weaknesses.

Prospects of targeted therapy and prognostic biomarkers in single-cell sequencing datasets

Several reviews have summarized the current landscape of targeted therapies in [44, 114]. Recent advances in CC research have identified multiple promising biomarkers and therapeutic targets through comprehensive multi-omics analyses. Key findings include CD3G's progressive upregulation from CIN to invasive tumors, particularly in HPV + patients and CD4 + T cells, where it enhances TCR-CD3 complex formation and correlates with improved prognosis [40]. Conversely, EFNA1 overexpression in tumor cells associates with poorer survival, especially in younger patients with advanced disease [102]. Angiogenesis profiling revealed distinct immune microenvironments, with high-AGS tumors showing neutrophil/DC enrichment and upregulated checkpoint molecules (IL10RB and TGFB1), while low-AGS tumors exhibited M2 macrophage and Treg infiltration [115]. A CESC-specific hypoxia-related score (CSHRS) was established using LASSO and Cox regression, revealing prognostic associations with immune infiltration, genomic mutations, and drug resistance[116]. Promising targeted therapeutic strategies are emerging, particularly TGX-221 and CAL-101 for high-risk CC patients [117], while the immunomodulatory SPP1-CD44 axis that mediates critical epithelial–macrophage crosstalk represents a novel therapeutic avenue for modulating tumor progression and treatment response [118]. The TME's complexity is further highlighted by MMP expression patterns predicting treatment response [119], DSG2-mediated immune exclusion[120], TNFRSF12A's role in mast cell signaling [121], and PGK1's regulation of macrophage polarization and necroptosis [122]. Metastatic progression appears driven by TGF-β-secreting neutrophils/CAFs and responsive epithelial cells [123], with TMED2 emerging as a pan-cancer promoter of tumor growth via ER stress pathways [124]. These discoveries collectively advance our understanding of CC pathogenesis while revealing multiple actionable targets for precision therapy.

Bridging single-cell biology and clinical therapy: advances and hurdles

Single-cell RNA sequencing has revolutionized our understanding of TME, revealing cell-type-specific vulnerabilities with therapeutic potential. Epithelial cell targeting remains challenging, as PCLAF + TAEpis drive PD-1 resistance without available inhibitors, whereas SALL4 is druggable via epigenetic modulators in clinical development [85, 125, 126]. Sialylation enzymes (GALNT3/12) represent another actionable axis, with inhibitors showing cross-cancer applicability [127, 128]. CSC interception currently focuses on Wnt/β-catenin (LGK974) [129]and ALDH1A1/SOX2 inhibition [130], though DKK2's role as a Wnt antagonist remains unexplored therapeutically. Stromal reprogramming efforts are hampered by the lack of inhibitors for protumorigenic CAF subsets (MYH11 + /MYH9 + ; NRG1-ERBB3 + myCAFs). Immunomodulation strategies are most advanced for checkpoint blockade (PD-1/CTLA-4/TIGIT) [131, 132] and CD39 inhibition[133], while TRM subsets ($CXCL13^+$/$PLAC8^+$) and BCL10 + T cells lack targeted agents. Myeloid cell targeting shows promise with SPP1 + [134]and HK3 + subsets (2-deoxyglucose) [106], but $CD74^+$ macrophage depletion remains unfeasible. NK cell therapeutics lag despite prognostic associations of $CD16^+$/$FGFBP2^+$ subsets. DC-focused approaches—notably galectin-9 blockade for $Siglec\text{-}10^+$ DCs [135] and IDO1 inhibition for $LAMP3^+$ DCs [136]—warrant clinical testing in CC. Critical barriers include target validation in CC models, combinatorial toxicity management, and patient stratification. Future work should prioritize functional genomics to credential novel targets (e.g., DKK2), optimize multi-compartment targeting (e.g., PD-1 + $LAMP3^+$ DC co-inhibition), and launch biomarker-driven trials anchored to single-cell classifications. In summary, while single-cell technologies have unveiled novel therapeutic vulnerabilities across the TME, translating these discoveries into clinical applications still faces key challenges, including target validation, combinatorial optimization, and patient stratification. Future efforts should integrate functional genomics with multi-omics-guided precision medicine to advance biomarker-driven therapies anchored to single-cell classifications.

Conclusion and prospect

Single-cell sequencing has revolutionized our understanding of CC biology by decoding its cellular architecture with unprecedented resolution. These technologies have unveiled the disease's remarkable heterogeneity, identifying molecular subtypes (hypoxic/proliferative/immunoreactive) and distinct epithelial states (cytokeratin$^+$/immune-interacting/senescent) that underlie clinical variability. Particularly transformative has been the elucidation of HPV-driven oncogenesis, where viral integration hotspots (8q24.21), radiation-induced resistant clones ($POU5F1B^+$), and sophisticated immune evasion mechanisms ($SPP1^+$ TAMs, GALNT3-mediated immunosuppression) have been mapped at single-cell resolution. Equally groundbreaking are the metabolic insights revealing spatially organized Warburg effect and OXPHOS-dominant regions with unique immune correlates, and the characterization of the immune ecosystem's dual nature—from exhausted $PD\text{-}1^+LAG3^+TIM3^+$ T-cell subsets and immunosuppressive $MYH9^+$ CAFs/$PODXL^+$ ECs to rare but therapeutically promising $FGFBP2^+$ NK cells and $CXCL13^+$ TRMs. While these discoveries have opened new therapeutic avenues, significant translational challenges persist, including NFKB1 mutation-driven radioresistance, $ACKR2^+$ tumor cell-induced T-cell senescence, and stromal NRG1-ERBB3 signaling networks that sustain therapeutic resistance. The field now stands at a critical juncture where promising targets such as SALL4, the SPP1-CD44 axis, and GALNT3/12 must be balanced against the urgent need for clinical-grade inhibitors against $PCLAF^+$ TAEpis, $MYH9^+$ CAFs, and $CD74^+$ macrophages. Moving forward, the research community must prioritize three key areas: (1) mechanistic studies to validate novel targets (DKK2, ELF3/ESE-1) and develop HPV integration monitoring technologies; (2) therapeutic innovation through modular clinical trials incorporating single-cell classifiers and stromal-immune co-targeting strategies; and (3) building translational infrastructure with CC-specific functional genomics platforms and TME-preserving preclinical models. Realizing the full potential of single-cell guided precision oncology will require unprecedented collaboration across computational biology, immunology, and clinical oncology, coupled with investments in adaptive trial frameworks capable of incorporating dynamic cellular insights. As we transition from empirical approaches to mechanism-driven combinations, the coming decade promises to redefine CC management through spatial target engagement and true precision medicine anchored in single-cell ecosystem understanding. The remaining challenge is not technological, but rather our collective capacity to creatively harness these cellular insights for transformative patient benefit.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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