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Integrated identification, functional characterization, and clinical significance of key genes during cervical cancer progression

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Abstract

Introduction: Cervical cancer evolves from normal cervical epithelium through a multifaceted pathway, first to high-grade squamous intraepithelial lesions (HSILs) and subsequently culminating in invasive cancer, yet the specific molecular mechanisms driving this progression remain poorly understood.

Objective: To identify genes persistently dysregulated during cervical lesion progression and to evaluate their clinical relevance with respect to prognosis, immune infiltration, and therapeutic response.

Methods: Two independent gene expression datasets (GSE7803 and GSE64217) were analyzed to screen persistently differentially expressed genes (DEGs) across normal cervix, HSIL, and cervical cancer. Public databases and clinical samples were used to validate the hub genes initially identified through functional enrichment and protein–protein interaction analyses. Associations with patient survival, immune infiltration, and drug sensitivity were further analyzed using integrated bioinformatics platforms.

Results: Eighteen common persistent DEGs were identified, primarily enriched in cell cycle regulation and epithelial differentiation. Four hub genes (*AURKA*, *ECT2*, *RFC4*, and *PCNA*) showed progressive upregulation as cervical lesions progressed and were associated with clinicopathological features. Elevated RFC4 and PCNA levels were significantly associated with improved survival. Immune analysis revealed distinct correlation patterns for *RFC4* and *PCNA* expression: positive with B cells and effector memory immune cells, and negative with regulatory T cells. Drug sensitivity analysis showed that elevated *RFC4* and *PCNA* expression were associated with lower IC_{50} values, whereas *AURKA* and *ECT2* were linked to potential drug resistance.

Conclusion: *AURKA*, *ECT2*, *RFC4*, and *PCNA* are key regulators of cervical lesion progression, among which RFC4 and PCNA are promising prognostic biomarkers and potential therapeutic targets.

Keywords: Cervical cancer; High-grade squamous intraepithelial lesions; Bioinformatics analysis; Hub genes; Immune infiltration

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1. Introduction

Cervical carcinoma represents a major factor in cancer-related morbidity and

mortality for females globally; consequently, numerous areas lacking structured diagnostic initiatives carry a substantial medical burden.[1] The development of this disease comprises three stages: initially healthy cervical tissue, followed by high-grade squamous intraepithelial lesions (HSIL), and ultimately cervical cancer.[2] Persistent infection with high-risk human papillomavirus (HPV) is necessary for carcinogenesis; nevertheless, it alone is insufficient to trigger aggressive cellular changes. Most HPV infections are temporary and are naturally eradicated by the human immune response within a brief period without intervention.[3] Only a small proportion of HPV-infected individuals eventually develop high-grade lesions or invasive cancer, highlighting the critical role of host genetic susceptibility and tumor-intrinsic molecular changes in disease progression.[4,5] These additional factors include alterations in cell cycle regulators, DNA damage repair pathways, epigenetic modifications, and the local immune microenvironment. Collectively, they determine whether an HPV infection will regress, remain dormant, or evolve into malignancy, underscoring the need to identify key molecular drivers that are consistently dysregulated across the entire progression spectrum from normal cervix to invasive cancer.[6]

Growing evidence indicates that malfunctions in cell cycle regulation, DNA replication, and epithelial differentiation play pivotal roles in the progression from premalignant states to infiltrative malignancy. Uncontrolled activation of mitogenic signaling cascades and impaired genomic integrity are regarded as fundamental milestones fueling cervical cancer genesis and development.[7,8] These abnormalities often arise from the combined effects of high-risk HPV oncoproteins and host genetic alterations, which collectively override normal cell cycle checkpoints, inactivate tumor suppressors, and induce persistent replication stress. The resulting genomic instability promotes the acquisition of additional somatic mutations, thereby further accelerating malignant transformation.[9] In particular, sustained activation of cell cycle-related genes and DNA replication machinery may confer a proliferative advantage to dysplastic epithelial cells, facilitating clonal expansion and accumulation of oncogenic alterations.[10] However, most transcriptomic studies have focused on comparisons between normal cervical tissues and cervical cancer, whereas molecular changes that persist across the entire progression spectrum remain poorly characterized.[11] This gap is clinically relevant because precancerous lesions represent a window of opportunity for early intervention; understanding the continuous molecular alterations that drive the transition from HSIL to carcinoma could inform risk stratification and guide preventive strategies. Identifying genes that are consistently dysregulated from the normal cervix to HSIL and cervical cancer may therefore provide more robust biomarkers for disease monitoring and therapeutic stratification.[12] Such persistent molecular alterations are more likely to represent core drivers of cervical carcinogenesis rather than stage-specific or context-dependent changes, making them attractive candidates for targeted intervention. Moreover, genes exhibiting gradual, continuous changes in expression across disease stages may better reflect the biological continuum of cervical lesion evolution and offer greater translational value for early intervention.[13]

In addition, the tumor immune microenvironment and therapeutic response have emerged as key determinants of clinical outcome in cervical cancer.[14] Increasing evidence indicates that tumor-intrinsic proliferative programs can shape immune cell infiltration patterns and influence sensitivity to anticancer therapies, including chemotherapy and immunotherapy. For instance, tumors with high proliferative activity often exhibit distinct immune landscapes, characterized by altered abundance of regulatory T cells and effector lymphocyte subsets, which collectively determine the balance between immune evasion and antitumor immunity.[15] Alterations in genes regulating cell proliferation and DNA repair have been shown to modulate antitumor immune surveillance by affecting antigen presentation, cytokine signaling, and immune checkpoint regulation. Notably, the DNA damage response pathway intersects with innate immune signaling through the cGAS–STING axis, where replication stress or genomic instability can trigger type I interferon production and recruit cytotoxic immune cells.[16] Nevertheless, integrative analyses that integrate transcriptomic alterations with immune infiltration characteristics and pharmacogenomic features remain limited.[17] Most existing studies have focused either on immune profiles or on drug sensitivity separately, lacking a unified framework that links persistent molecular dysregulation to both immune contexture and therapeutic response. A comprehensive understanding of how persistent molecular dysregulation interacts with immune contexture and drug response may help identify prognostic and therapeutic biomarkers.

Furthermore, advances in bioinformatics and public cancer databases have enabled systematic integration of multidimensional data, including gene expression, clinical outcomes, immune infiltration profiles, and drug sensitivity information.[18] Such integrative approaches provide a powerful framework to uncover key molecular drivers underlying cervical carcinogenesis and to explore their potential clinical implications more holistically. In this study, we systematically integrated two independent transcriptomic datasets (GSE7803 and GSE64217) to

identify genes persistently dysregulated during cervical lesion progression. By combining clinical validation, immune infiltration profiling, and drug sensitivity assessment, we aimed to delineate key molecular drivers of cervical carcinogenesis and to clarify their potential prognostic and therapeutic relevance.

2. Materials and methods

2.1. Data acquisition and processing

The Gene Expression Omnibus (GEO) database included gene expression profiling datasets associated with cervical lesions. Two separate datasets were used: GSE7803 and GSE64217. The raw expression data were downloaded and prepared in R software. The data were normalized, and the background was corrected. The batch effects between datasets were adjusted using the sva package.

After preprocessing, the public-dataset cohort comprised 47 samples: 12 normal cervical samples, 9 HSIL samples, and 26 cervical cancer samples.

2.2. Identification of differentially expressed genes

Differential gene expression analysis was completed using R's limma package. Pairwise comparisons were performed between healthy cervical tissue and cervical cancer, between healthy cervical tissue and HSIL, and between HSIL and cervical cancer across all datasets. Genes with an adjusted $p < 0.05$ and a log fold change ≥ 2 were considered significantly differentially expressed.

Genes exhibiting persistent differential expression across all contrast trios were identified as persistent differentially expressed genes (DEGs), indicating sustained aberrant regulation throughout the progression of cervical cancer. Subsequently, the overlap of persistent DEGs detected within both datasets was identified to identify common DEGs for subsequent investigation.

2.3. Functional enrichment analysis and construction of protein–protein interaction network

Functional characterization via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted using the Metascape web tool. Enriched GO terms and KEGG pathways with $p < 0.05$ were considered statistically significant. For protein–protein associations, the detected DEGs were uploaded to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, and a network map was generated utilizing the default confidence score. Cytoscape facilitated the visualization of the protein–protein interaction (PPI) network, while the CytoHubba plugin enabled the identification of hub genes.

2.4. Expression validation and survival analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) platform, which combines RNA-seq datasets from The Cancer Genome Atlas and the Genotype–Tissue Expression projects, was used to assess the expression profiles and prognostic importance of hub genes. Differentially expressed mRNA levels were analyzed across cervical cancer samples and normal cervical epithelial cells. For survival analyses, we divided cervical carcinoma patients into high- and low-expression cohorts using the median expression levels of the selected hub genes as the cutoff. The prognostic role of hub gene expression was assessed using overall survival (OS) as the endpoint.

Immunohistochemistry data from the Human Protein Atlas (HPA) database were employed to assess protein expression of the hub genes.

2.5. Immune infiltration and drug sensitivity analysis

A correlation between hub genes and cancer immune infiltration was studied using the Gene Set Cancer Analysis tool. Spearman's correlation analysis was performed to assess the associations between hub gene expression patterns and the density of 24 distinct immune cell types.

The Genomics of Drug Sensitivity in Cancer (GDSC) and Cancer Therapeutics Response Portal (CTRP) databases were used to assess pharmacodynamic sensitivity. To assess the predictive utility of hub genes for treatment outcomes, the relationship between hub gene expression patterns and the half-maximal inhibitory concentration (IC_{50}) values of anticancer medications was examined.

2.6. Human samples

Tissue specimens included 20 normal cervical samples, 16 HSIL, and 40 cervical cancer samples, all confirmed histologically. Eligible patients had a histologically confirmed diagnosis of normal cervix, HSIL, or cervical cancer, had not received any chemotherapy, radiotherapy, or immunotherapy before sample collection, and had complete clinical and pathological records. Patients with concurrent other malignancies, pregnancy, autoimmune diseases requiring immunosuppressive therapy, or active infection were excluded. Biopsies were rapidly cooled with liquid nitrogen after surgical extraction and preserved at −80 °C prior to subsequent evaluations. All individuals participating in this study provided formal written consent before enrollment, and the initiative obtained formal authorization from the institutional ethics committee. Medical records and pathology reports provided the clinicopathological and histological data.

2.7. Quantitative real-time polymerase chain reaction

Following the manufacturer's instructions, total RNA was isolated from tissue samples via TRIzol reagent. Reverse transcription was performed using the PrimeScriptTM RT Reagent Kit (Takara, Japan). A real-time polymerase chain reaction (PCR) machine (QuantStudio™ 5, Applied Biosystems, United States of America [USA]) was utilized for quantitative real-time PCR analysis with TB Green Premix ExTaq II (Takara, Japan). The *GAPDH* gene was used as the endogenous control. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ procedure. Primer sequences are outlined in Table A1.

2.8. Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (IBM, USA) and GraphPad Prism version 9.1.0 (GraphPad Software, USA). Quantitative data were presented as mean ± standard deviation. To examine differences between two groups, the Student's *t*-test was employed. A two-sided *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Identification of differentially expressed genes

Gene expression patterns were retrieved from the GEO database, including the GSE7803 and GSE64217 cohorts, which contained 12 normal cervical tissues, 26 cervical cancer tissues, and 9 HSIL tissues. Differential gene expression analysis was conducted to contrast transcriptional profiles across normal cervical, HSIL, and invasive carcinoma samples. Within the GSE7803 dataset, 4,775, 1,446, and 1,365 DEGs were detected in the contrasts of healthy versus cancer, normal versus HSIL, and HSIL versus cancer, respectively. Among them, 53 genes showed sustained differential expression in the transition from normal to cervical cancer (Figure 1A). Similarly, in the GSE64217 dataset, 3,155, 3,000, and 3,145 DEGs were identified in the corresponding comparisons, with 2,734 genes showing continuous differential expression across disease progression (Figure 1B). Furthermore, Venn diagram analysis revealed 18 common DEGs shared between the two datasets, which were consistently dysregulated during cervical cancer progression (Figure 1C). Heatmaps were generated to visualize the expression patterns of representative DEGs in both datasets (Figure 1D and 1E).

3.2. Functional enrichment analysis and protein–protein interaction network construction

Functional enrichment analysis was conducted for 18 common DEGs. GO analysis showed that the genes were enriched in the biological processes of sensory organ morphogenesis, positive regulation of catalytic activity, nuclear receptor-mediated signaling pathway, positive regulation of cell cycle, and epithelial cell differentiation (Figure 2A). No KEGG pathways were significantly enriched, likely due to the limited number of persistent DEGs (n = 18) and the fact that these genes may function through tightly interconnected regulatory networks rather than through a single dominant canonical pathway.

After constructing a 17-node, 8-edge PPI network from the STRING database, we visualized it in Cytoscape and used the CytoHubba plugin with the maximal clique centrality algorithm to detect hub genes. As a result, four hub genes—*AURKA*, *ECT2*, *RFC4*, and *PCNA*—were identified (Figure 2B).

3.3. Expression validation and survival analysis of hub genes

Expression patterns of these four hub genes were verified using the GEPIA platform, which includes 306 cervical cancer samples alongside 13 healthy cervical specimens. Findings indicated that the abundance of *AURKA*, *ECT2*, *RFC4*, and *PCNA* mRNA was notably increased in cervical cancer tissues relative to their counterparts (Figure 3A). Additionally, immunohistochemical profiles retrieved from the HPA database supported the upregulated protein concentrations of these identified genes in cervical cancer samples (Figure 3B).

To assess the predictive value of the hub genes, Kaplan–Meier survival curves were used to examine OS in individuals with cervical cancer. The findings suggested that *RFC4* and *PCNA* expression exhibited a substantial correlation with OS in cervical cancer patients; however, no meaningful association was detected regarding *AURKA* or *ECT2* levels and clinical survival outcomes (Figure 3C).

3.4. Immune infiltration analysis

Gene set variation analysis (GSVA) scores were originally used to assess the associations between hub gene expression and immune cell infiltration. The infiltration levels of various immune cell types were substantially associated with hub gene expression (Figure 4A). Specifically, *PCNA* expression was positively correlated with the infiltration of CD8 naive T cells, B cells, effector memory T cells, dendritic cells, Th1 cells, $CD8^+$ T cells, and T follicular helper (Tfh) cells, whereas it exhibited a negative association with neutrophils, Th17 cells, monocytes, and natural killer T cells. *RFC4* expression showed positive correlations with effector memory T cells and B cells, along with inverse correlations with central memory induced regulatory T cells and T cells. *AURKA* levels were positively associated

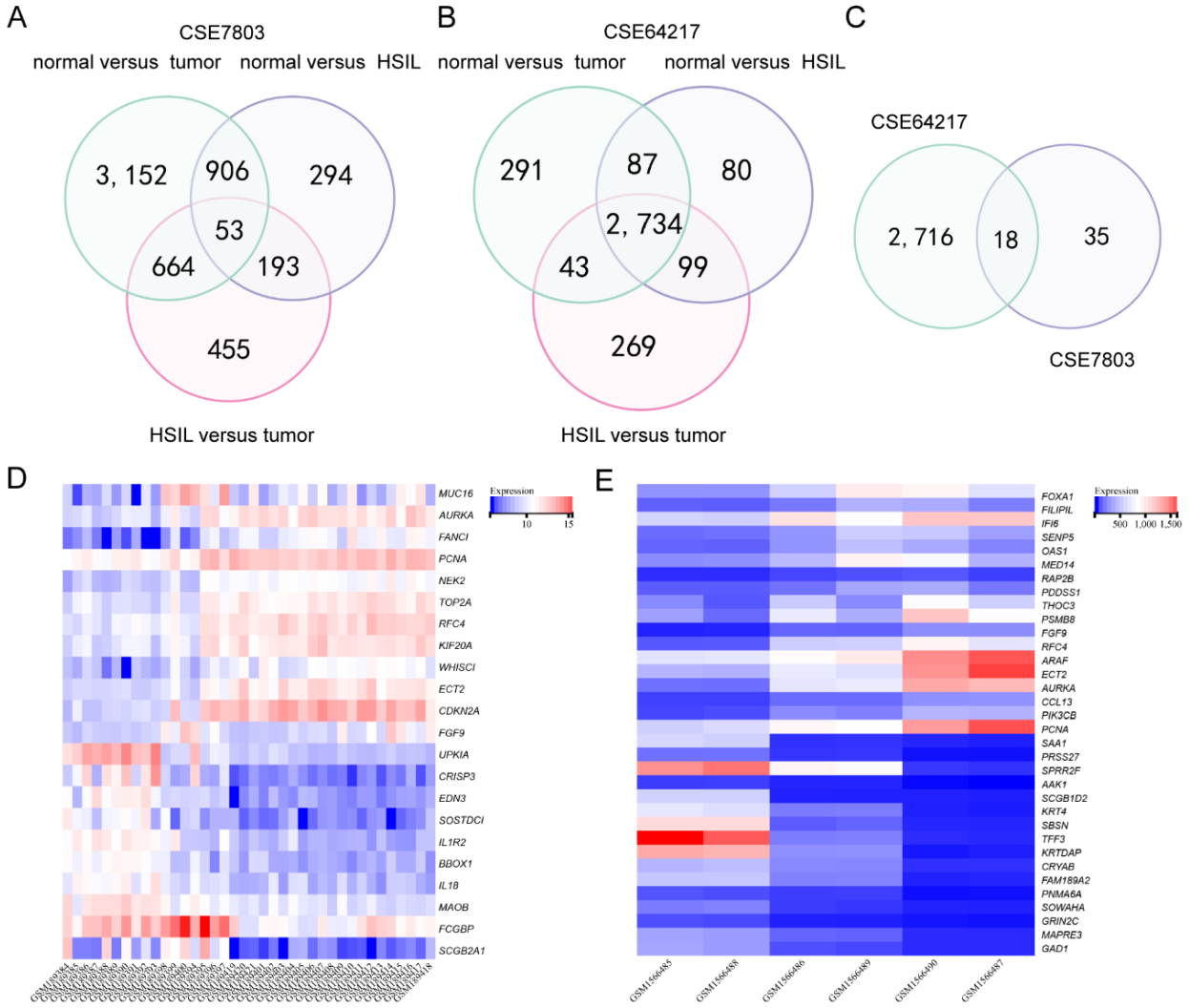


Figure 1. Identification of DEGs during cervical lesion progression. (A) DEGs identified in the GSE7803 dataset among normal cervix, HSIL, and cervical cancer tissues. (B) DEGs identified in the GSE64217 dataset. (C) Venn diagram showing the overlap of persistently dysregulated DEGs shared by the two datasets during cervical carcinogenesis. (D) Heatmap depicting typical sustained DEGs from the GSE7803 dataset. (E) Heatmap illustrating typical sustained DEGs within the GSE64217 dataset. Red and blue hues represent relatively elevated and diminished gene expression intensities, respectively. Abbreviations: DEGs: Differentially expressed genes; HSIL: High-grade squamous intraepithelial lesions.

with neutrophils and Th1 cells, yet negatively linked with $CD4^+$ T cells. Levels of *ECT2* showed positive associations with $CD8^+$ naive T cells, neutrophils, natural regulatory T cells (nTregs), and Th17 cells, and negative associations with $CD8^+$ T cells, cytotoxic T cells, exhausted T cells, macrophages, natural killer cells, Tfh cells, and Th2 cells (Figure 4B).

Furthermore, the correlations between copy number alterations (CNVs) and the DNA methylation status of hub genes, as well as immune infiltration, were thoroughly investigated. CNV levels of *PCNA* and *AURKA* exhibited a pronounced positive relationship with B cell infiltration, while CNV levels of *RFC4* and *ECT2* showed a substantial negative association with nTreg cells (Figure 5A). Additionally, DNA methylation of *ECT2* was positively associated with the recruitment of central memory cells and Type 1 regulatory T cells (Figure 5B).

3.5. Drug sensitivity analysis

The association between hub gene activity and sensitivity to anticancer medications was examined using data from the CTRP and GDSC databases. In the CTRP database, the IC_{50} values of all 30 examined drugs were positively correlated

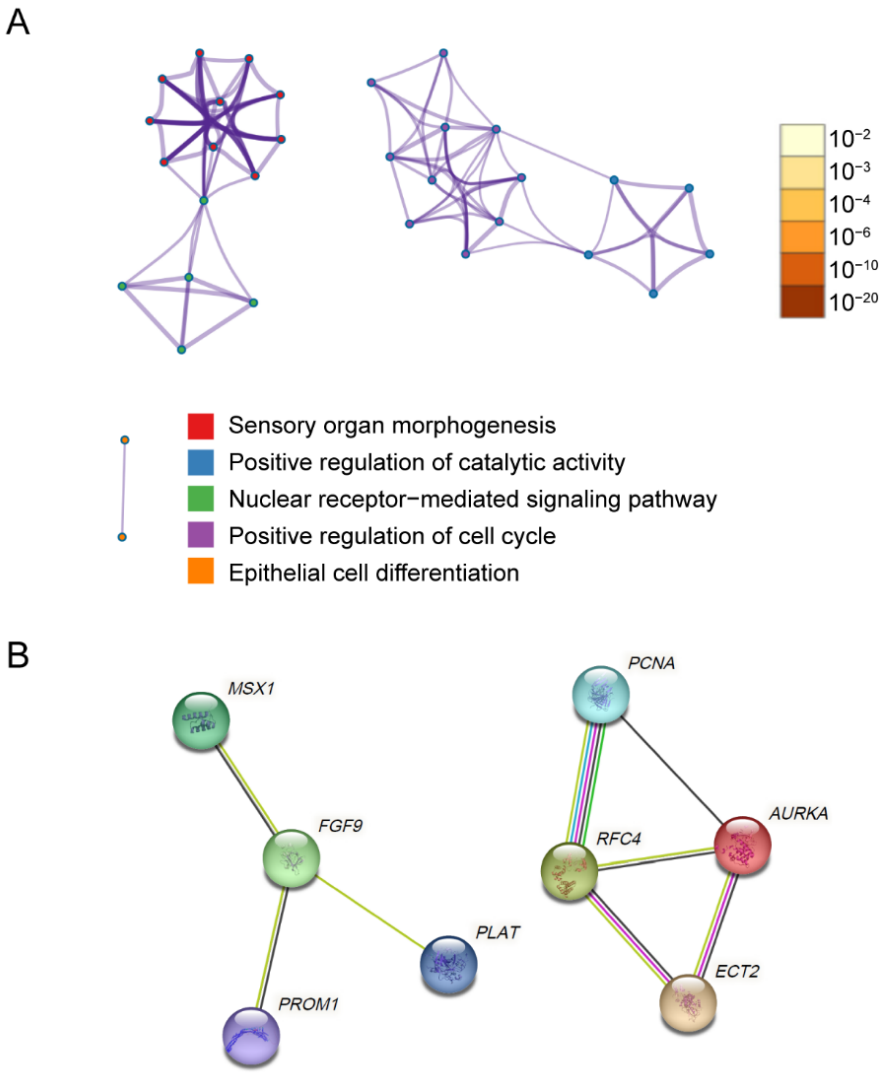


Figure 2. Analysis of persistent differentially expressed genes (DEGs). (A) GO enrichment analysis performed on persistent DEGs. (B) Protein–protein interaction network of persistent DEGs built with the Search Tool for the Retrieval of Interacting Genes/Proteins database. In this network, each node corresponds to a protein, and each edge represents a predicted or known interaction.

with the expression patterns of *ECT2* and *AURKA*, implying a possible link with therapeutic resistance. Conversely, *RFC4* and *PCNA* expression concentrations exhibited a negative correlation with the IC_{50} values of these drugs, suggesting increased drug sensitivity (Figure 6A).

Consistent results were observed in the GDSC database. The levels of *ECT2* and *AURKA* exhibited a positive relationship with IC_{50} values of 24 drugs, apart from elesclomol. Conversely, *RFC4* and *PCNA* levels demonstrated a negative association with IC_{50} values of 21 drugs, excluding Nutlin-3a(-), BAY 61-3606, VX-11e, and elesclomol. Furthermore, *AURKA* levels showed a negative correlation with the IC_{50} of elesclomol, whereas *RFC4* expression showed a positive correlation with the IC_{50} of Nutlin-3a(-) (Figure 6B).

3.6. Experimental validation and clinical significance of hub genes

To verify the bioinformatic findings, quantitative real-time PCR was used to quantify the abundance of *AURKA*, *ECT2*, *RFC4*, and *PCNA* mRNAs within normal cervical tissue, HSIL tissue, and cervical cancer tissue. The results revealed a stepwise elevation in the levels of these four hub genes from normal cervix through HSIL and finally to cervical cancer specimens (Figure 7A–D).

To uncover the clinicopathological significance of these

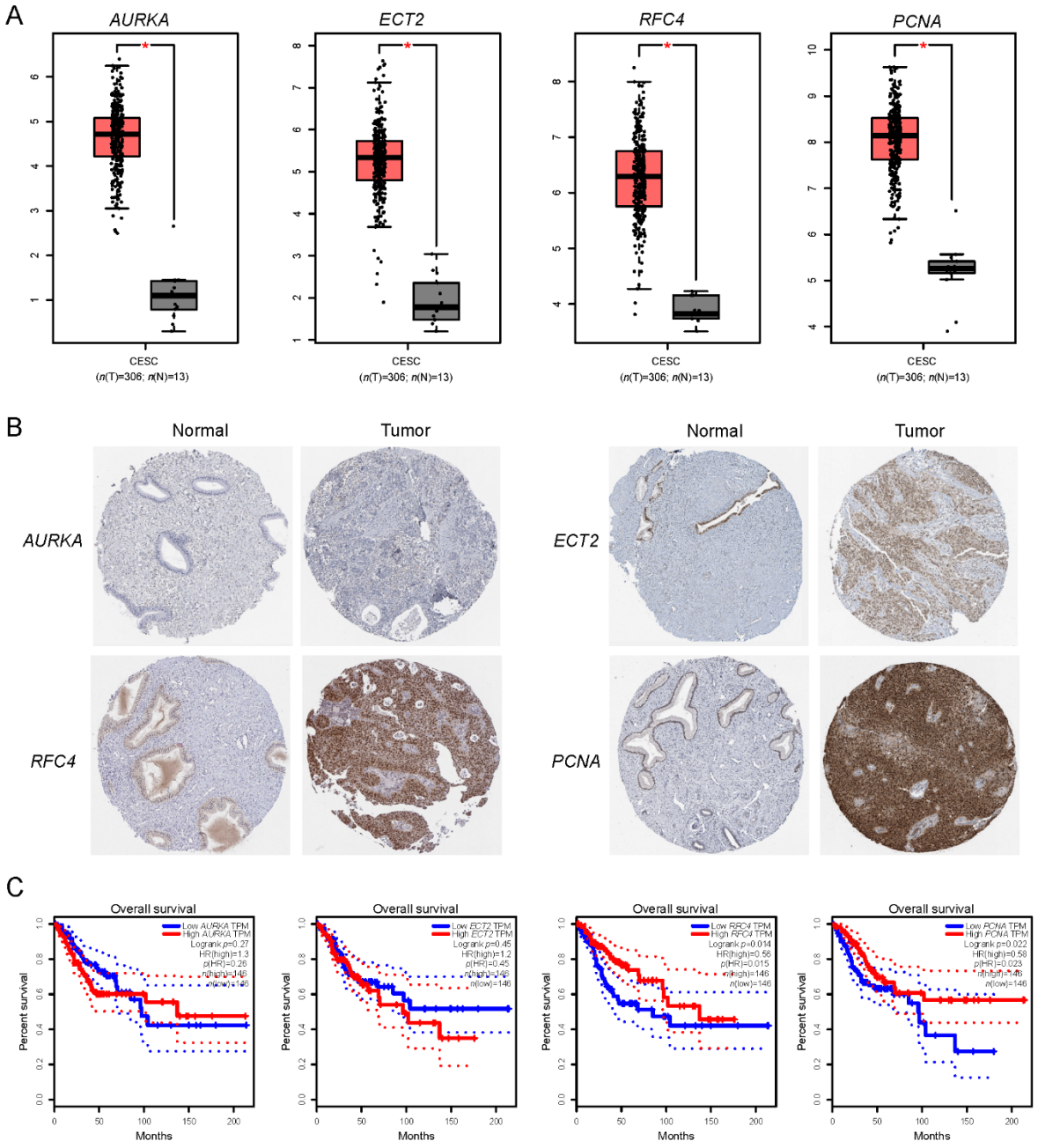
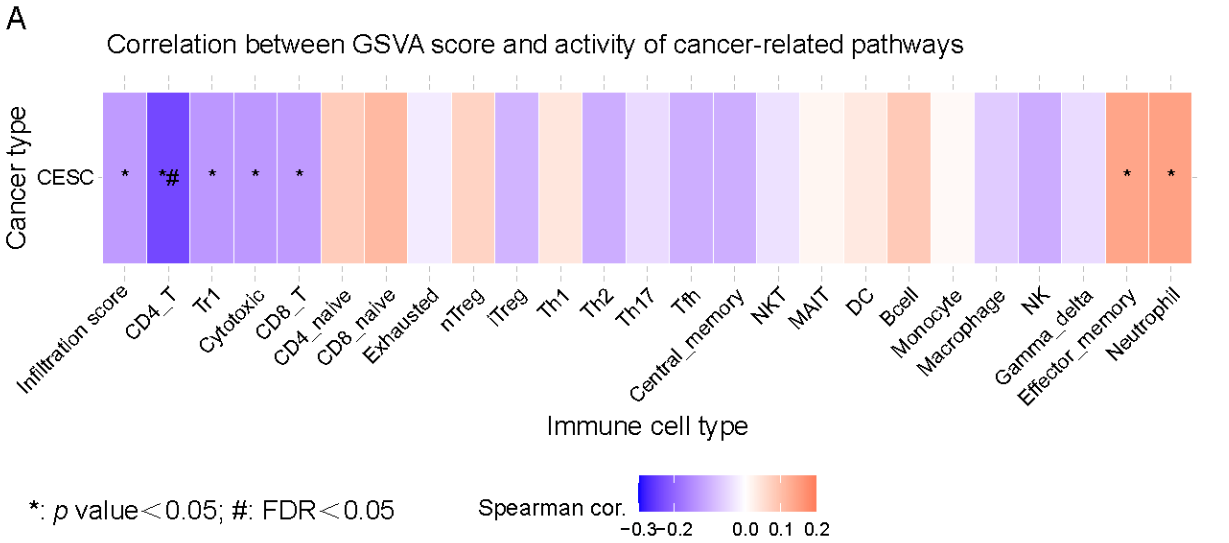


Figure 3. Validation of hub gene expression and survival prognosis. (A) Evaluation of mRNA expression levels for hub genes by the GEPIA website. (B) Immunohistochemical analysis of protein expression patterns for hub genes between cervical cancer and healthy tissues derived from the HPA database. (C) Kaplan–Meier survival plots demonstrating the association between hub gene expression intensity and overall survival in patients with cervical cancer. Note: $^{*}p < 0.05$.

Abbreviations: CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; GEPIA: Gene Expression Profiling Interactive Analysis; HPA: Human Protein Atlas; HR: Hazard ratio.

hub genes, we further assessed the relevance between their expression and clinical parameters. The expression intensities of the four hub genes were significantly associated with tumor sizes. In addition, *AURKA*, *RFC4*, and *PCNA* expression levels were significantly associated with tumor stage. Notably, *RFC4* expression was also significantly correlated with vascular invasion (Tables 1 and 2).



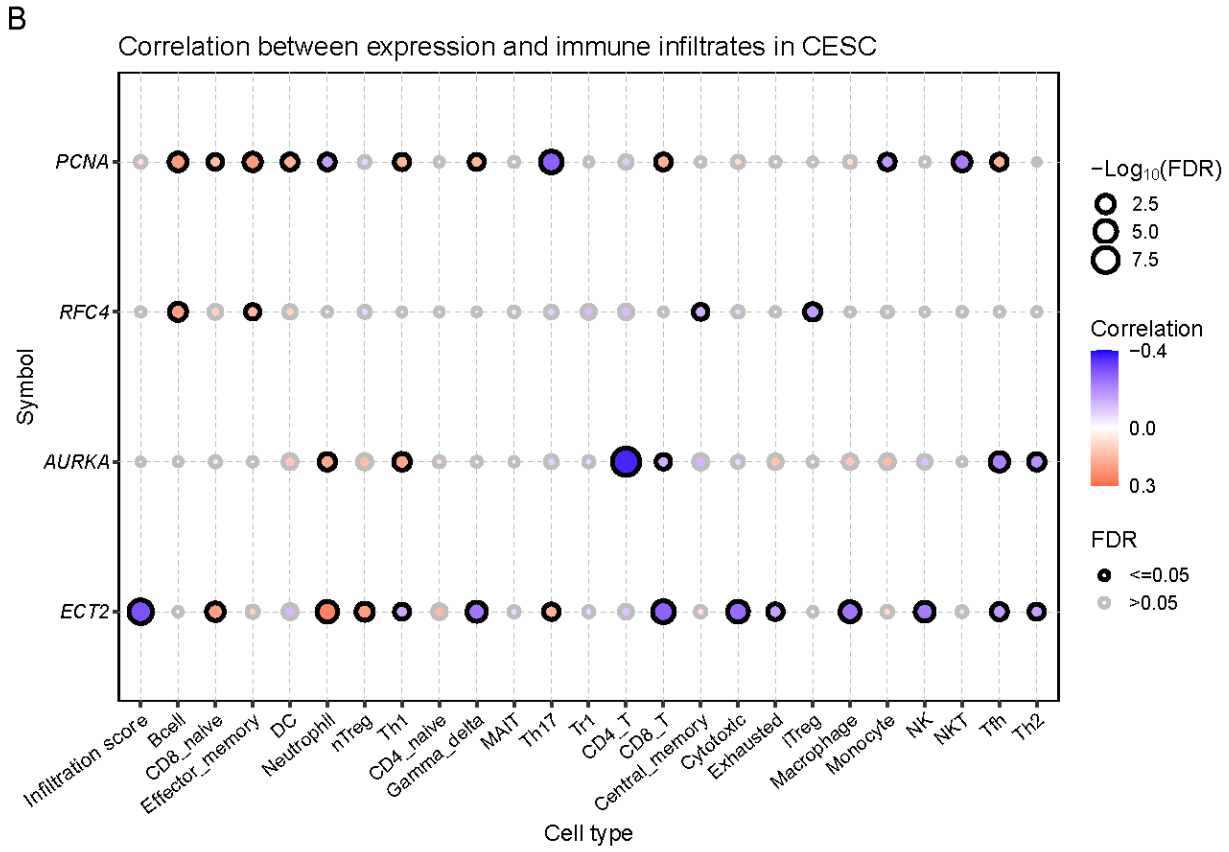


Figure 4. Association between hub gene expression and immune infiltration in cervical cancer. (A) GSVA scores of hub genes: correlation with the activity of immune pathways in cervical cancer. (B) Association between hub gene expression and infiltration of multiple immune cell subsets in cervical cancer. Abbreviations: CD: Cluster of differentiation; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; DC: Dendritic cell; FDR: False discovery rate; GSVA: Gene set variation analysis; iTreg: Induced regulatory T cells; MAIT: Mucosal-associated invariant T cells; NK: Natural killer; NKT: Natural killer T cell; nTreg: Natural regulatory T cells; Tfh: T follicular helper; Tr1: Type 1 regulatory T cells.

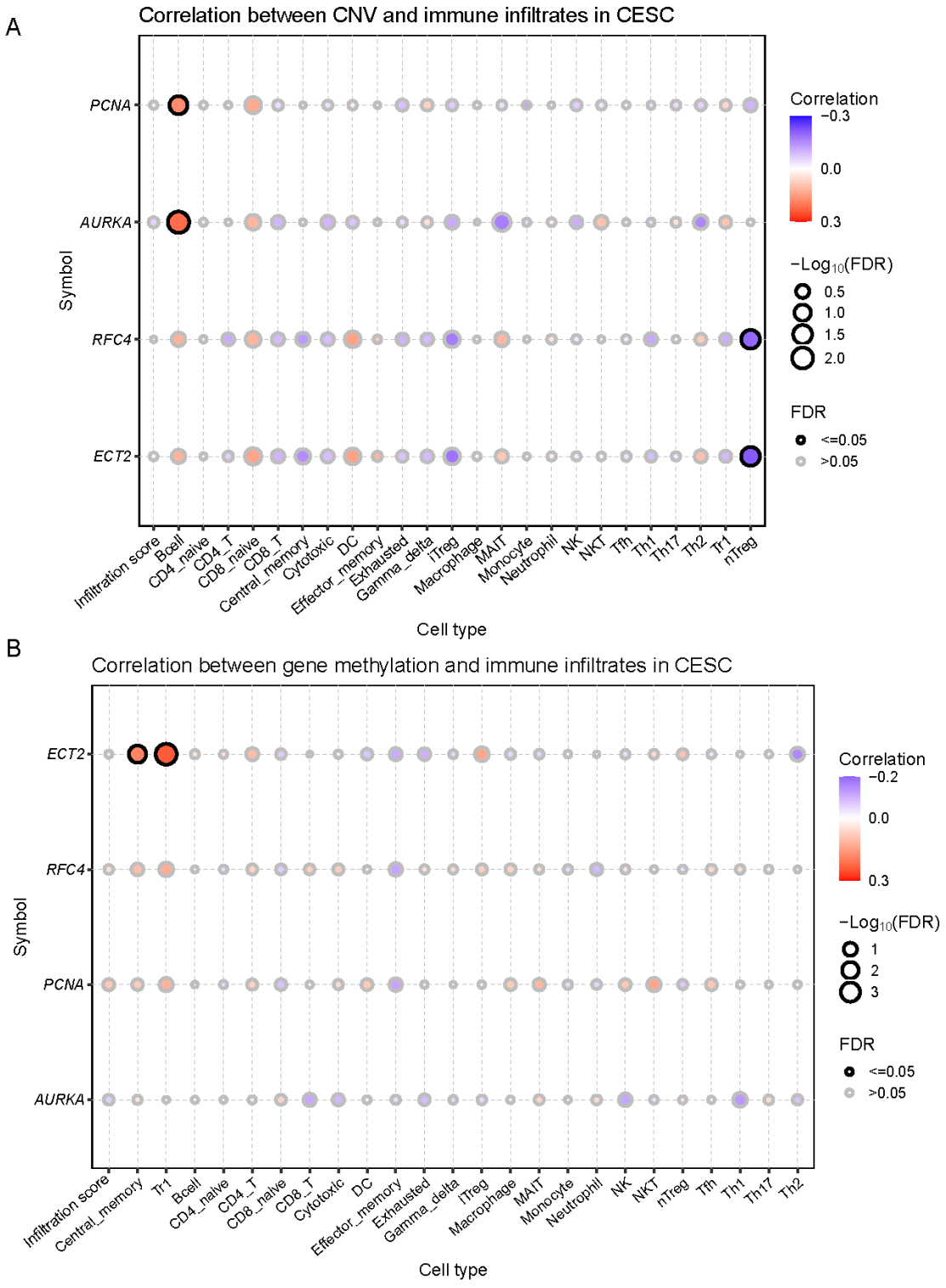


Figure 5. Relationship between hub gene CNVs and DNA methylation in relation to immune infiltration in cervical cancer. (A) Association involving CNV of hub genes and immune cell infiltration degrees. (B) Association between DNA methylation concentrations of hub genes and immune cell infiltration. Abbreviations: CD: Cluster of differentiation; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CNV: Copy number alterations; DC: Dendritic cell; FDR: False discovery rate; iTreg: Induced regulatory T cells; MAIT: Mucosal-associated invariant T cells; NK: Natural killer; NKT: Natural killer T cell; nTreg: Natural regulatory T cells; Tfh: T follicular helper; Tr1: Type 1 regulatory T cells.

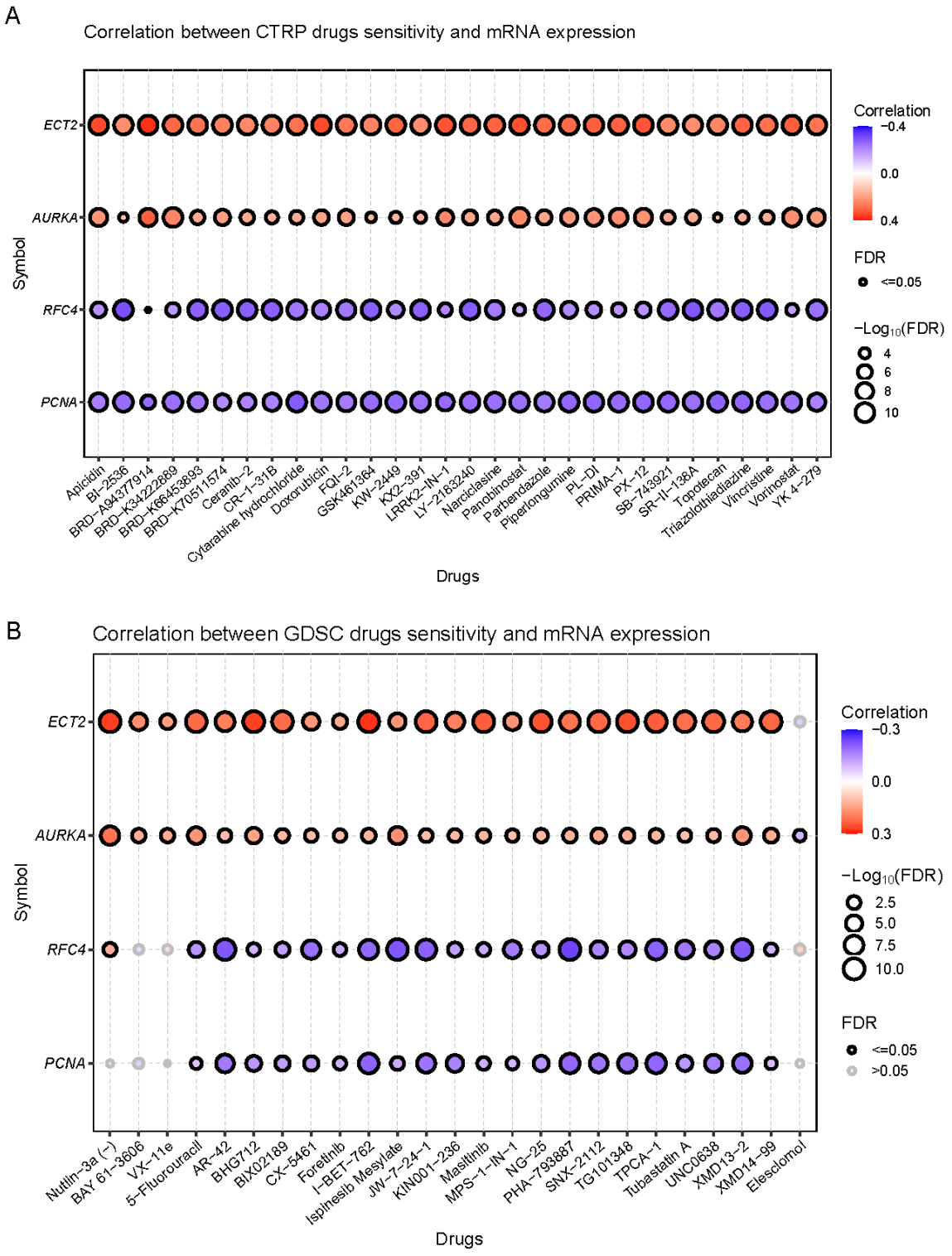


Figure 6. Association between hub gene expression and drug sensitivity in cervical cancer. Drug sensitivity (IC_{50} values) relative to hub gene expression, based on CTRP (A) and GDSC (B) data.

Abbreviations: CTRP: Cancer Therapeutics Response Portal; FDR: False discovery rate; GDSC: Genomics of Drug Sensitivity in Cancer; IC_{50}: Half-maximal inhibitory concentration.

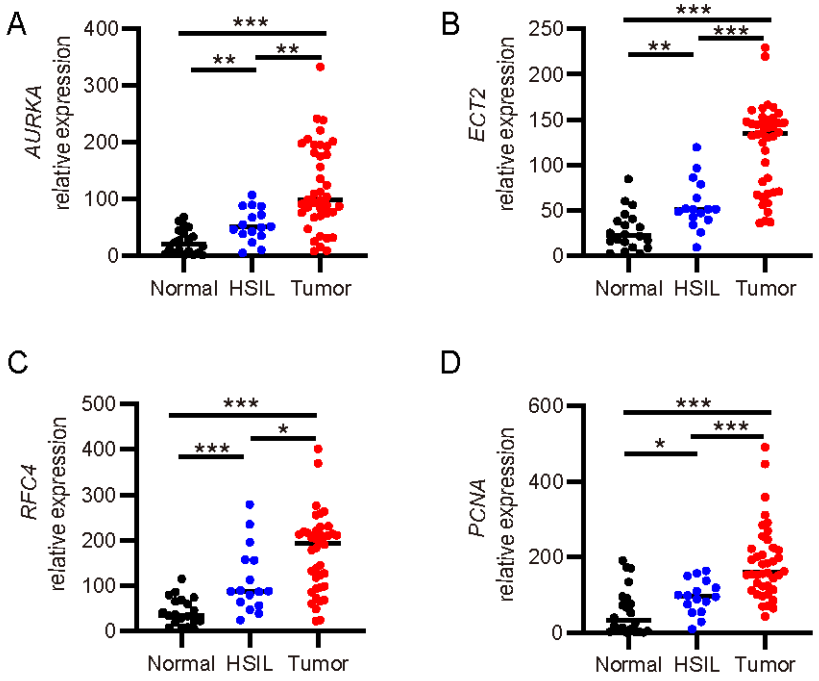


Figure 7. Quantitative real-time polymerase chain reaction (qRT-PCR) validation of hub gene expression in clinical samples. qRT-PCR analysis showing the mRNA expression levels of *AURKA* (A), *ECT2* (B), *RFC4* (C), and *PCNA* (D) in normal cervical tissues, HSIL samples, and cervical cancer tissues. Note: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
Abbreviation: HSIL: High-grade squamous intraepithelial lesions.

Table 1. Associations between *ECT2* and *AURKA* expression levels with clinicopathological features of cervical cancer patients

Characteristics	Cases	*ECT2* expression			*AURKA* expression		
		Low	High	*p*	Low	High	*p*
Age (years)				0.614			0.595
<45	16	10	6		9	7	
≥45	24	10	14		11	13	
International Federation of Gynecology and Obstetrics staging				0.130			0.025*
Ib	29	18	11		17	12	
IIa–IIb	11	2	9		3	8	
Tumor size (cm)				0.019*			0.035*
<4	25	17	8		16	9	
≥4	15	3	12		4	11	
Lymph node invasion				0.128			0.232
Absent	27	18	9		15	12	
Present	13	2	11		5	8	
Vascular invasion				0.119			0.571
Absent	30	17	13		16	14	
Present	10	3	7		4	6	

Note: $^{*}p < 0.05$.

Table 2. Associations among *RFC4* and *PCNA* expression levels alongside clinicopathological features of cervical cancer individuals

Characteristics	Cases	*RFC4* expression			*PCNA* expression		
		Low	High	*p*	Low	High	*p*
Age (years)				0.712			0.567
<45	16	8	8		9	7	
≥45	24	12	12		11	13	
International Federation of Gynecology and Obstetrics staging				0.012*			0.006*
Ib	29	19	10		18	11	
IIa–IIb	11	1	10		2	9	
Tumor size (cm)				0.048*			0.013*
<4	25	17	8		18	7	
≥4	15	3	12		2	13	
Lymph node invasion				0.118			0.190
Absent	27	18	9		16	11	
Present	13	2	11		4	9	
Vascular invasion				0.002*			0.242
Absent	30	19	11		16	14	
Present	10	1	9		4	6	

Note: * $p < 0.05$.

4. Discussion

Cervical malignancy remains a significant factor in cancer-induced fatalities for females globally and evolves via a clearly established progression pathway from healthy cervical tissue to HSIL and finally invasive cancer.[19] Elucidating the key molecular events that drive this progression can improve early diagnosis, prognostic stratification, and therapeutic decision-making.[20] In this work, we integrated bioinformatics analyses with experimental validation to systematically identify and characterize core genes persistently dysregulated during cervical carcinogenesis. Our approach combined multiple public transcriptomic datasets, functional enrichment analysis, and validation in clinical tissue samples. By focusing on persistently altered genes rather than transient changes, we aimed to uncover stable molecular signatures associated with disease progression.

By analyzing two independent GEO datasets, we identified 18 persistent DEGs that exhibited consistent expression alterations across normal cervix, HSIL, and cervical cancer. This stringent screening strategy minimized dataset-specific bias and enhanced the robustness of our findings.[21,22] Functional enrichment studies revealed that these genes are primarily involved in cell cycle regulation, nuclear receptor-mediated signaling, and epithelial differentiation, emphasizing the central role of cell cycle disruption.[23] The absence of statistical significance in KEGG pathway analyses—likely due to the limited number of persistent DEGs—suggests that these genes may act through tightly interconnected regulatory networks rather than through a single dominant signaling pathway.[24] PPI network analysis further identified four hub genes—*AURKA*, *ECT2*, *RFC4*, and *PCNA*—each serving as a critical modulator of cell-cycle progression and DNA replication.

Aurora kinase A functions as a serine/threonine kinase necessary for assembling the mitotic spindle and segregating chromosomes. In multiple malignant tumors, this protein contributes significantly to genomic instability.[25] Beyond its canonical role in mitosis, *AURKA* also participates in centrosome duplication, spindle checkpoint regulation, and the DNA damage response, thereby influencing both cell cycle progression and genome integrity.[26] Enhanced *AURKA* levels have been detected within multiple human malignancies, such as breast,

ovarian, and cervical cancer, and are commonly linked to inferior clinical outcomes and treatment resistance.[27] This regulatory function is particularly relevant in HPV-driven tumors, given that the viral proteins E6 and E7 promote immune evasion through reduced antigen presentation and creation of an immunosuppressive environment. Studies have shown that combining aurora A inhibitors with immune checkpoint blockade may synergistically restore antitumor immunity, suggesting a novel treatment option for cervical cancer patients who experience relapse or develop resistance to standard therapies.[28] These results indicate that *AURKA* might function not only as a mitotic controller but additionally as a prospective immunoregulatory target within HPV-induced malignancies.[29] *ECT2* serves as a guanine nucleotide exchange factor essential for cytokinesis, and it has been observed to facilitate oncogenic proliferation, invasion, and metastatic spread.[30,31] Concretely, *ECT2* stimulates the minor GTPase RhoA, which subsequently controls actin filament rearrangement, cellular polarity, and contractile ring development throughout the cytokinesis process.[32] Aberrant *ECT2* overexpression can lead to failure in cytokinesis, multinucleation, and aneuploidy, thereby fueling genomic instability and malignant progression. Beyond its function during cytokinesis, *ECT2* has been tied to epithelial–mesenchymal transition, a crucial mechanism that enhances cancer cells' migration and invasion.[33] *ECT2* dysregulation has been linked to aberrant activation of the RhoA–ROCK signaling axis and metabolic reprogramming during HPV-associated carcinogenesis, highlighting its multifaceted oncogenic role.[34] Notably, alterations in *ECT2* CNV and methylation status in cervical cancer remain largely unexplored,[35,36] and the results provide new insights into tumor–immune interactions.

Replication factor C subunit 4 is a structure-specific DNA-binding protein that is a vital component of the replication factor C complex, primarily involved in DNA replication, damage checkpoint regulation, and the preservation of genomic integrity.[37] Its aberrant expression has attracted considerable research interest due to its high prevalence across diverse human malignancies. Evidence indicates that *RFC4* is markedly overexpressed in numerous malignancies, including hepatocellular carcinoma, colorectal cancer, neuroblastoma, glioblastoma, and leukemia, suggesting that *RFC4* may serve as a general oncogenic driver and a promising therapeutic target.[38] However, the expression dynamics of *RFC4* during cervical precancerous progression have remained largely unexplored. In our current study, we noticed the upregulation of *RFC4* from normal cervical epithelium to HSIL and further to invasive cervical cancer. This stepwise upregulation strongly implied that *RFC4* played an active role in driving the malignant transformation from precancerous lesions to invasive cervical carcinoma. Notably, a previous study reported that *RFC4* expression was 3.4fold higher in HPVpositive head and neck tumors than in normal counterparts.[39] Although this finding originates from head and neck tumors, it carries important implications for cervical cancer, as persistent infection with highrisk HPV is the predominant etiological driver of both malignancies. The consistent upregulation of *RFC4* in HPVpositive lesions raises the intriguing possibility that HPV oncoproteins may directly or indirectly enhance *RFC4* transcription, thereby facilitating viral replication and host cell proliferation. Although the mechanisms by which HPV infection induces *RFC4* upregulation remain unclear, our findings, together with existing evidence, support a model in which HPVmediated dysregulation of *RFC4* contributes to the acquisition of a hyperproliferative phenotype during cervical carcinogenesis. Additional clinical analyses showed that higher *RFC4* expression was positively related to advanced International Federation of Gynecology and Obstetrics stage, increased tumor dimensions, and vascular invasion among cervical cancer patients. These clinicopathological correlations suggest that *RFC4* not only participates in disease progression but may also serve as a surrogate indicator of aggressive tumor behavior.

Proliferating cell nuclear antigen forms a homotrimeric sliding clamp that wraps around DNA, serving as a core scaffold for recruiting proteins involved in DNA replication, repair, and damage tolerance.[40] Within this current investigation, we noted a gradual increase in *PCNA* expression from healthy cervix through HSIL to cervical cancer, aligning with its crucial function in maintaining proliferative potential throughout oncogenic progression. Beyond its role in regular DNA replication, *PCNA* is vital to the DNA damage tolerance pathway.[41] Upon replication stress, *PCNA* undergoes monoubiquitination at the Lys164 residue, a modification catalyzed by the Rad6–Rad18 complex, which recruits Y-family translesion synthesis polymerases to bypass replication-blocking lesions. This process prevents replication fork collapse and enables cells to complete DNA synthesis despite DNA damage, thereby promoting cell survival.[42] In the context of cervical carcinogenesis, persistent HPV infection induces replication stress and genomic instability, conditions that may heavily rely on *PCNA*-mediated damage tolerance. Targeting the ubiquitination status or interaction partners of *PCNA*, therefore, represents a promising strategy to interfere with the proliferative and stress-adaptive capacity of cervical cancer cells.

An investigation into the oncogenic immunological

landscape revealed that the migration of diverse immune cell populations was regulated by the key genes. Importantly, high *RFC4* and *PCNA* expression were positively associated with infiltration by B cells and effector memory cell subsets, in which they exhibited antagonistic relationships with immunosuppressive entities such as regulatory T cells. These correlation patterns suggest that tumors with high *RFC4*/*PCNA* expression may harbor a more immunologically "hot" microenvironment, characterized by enhanced adaptive immunity and reduced suppressive signals. In contrast, *AURKA* and *ECT2* exhibited distinct immune correlation profiles: *AURKA* was positively associated with neutrophils and Th1 cells, while *ECT2* showed complex immune associations, including positive correlations with nTregs and Th17 cells and negative correlations with $CD8^+$ T cells and natural killer cells. The favorable immune microenvironment associated with high *RFC4* and *PCNA* expression may be partly attributable to their roles in DNA replication and genome maintenance. Enhanced replication stress may activate the cGAS–STING pathway, resulting in increased type I interferon secretion and subsequent recruitment of effector lymphocytes, thereby promoting antitumor immune responses.[43] These immune-related patterns provide a plausible biological explanation for the observed association between high *RFC4* and *PCNA* expression and more favorable clinical outcomes, suggesting that enhanced proliferative activity may influence immune surveillance and antitumor immunity in cervical cancer.[44,45] Furthermore, the negative correlation between *RFC4* and *PCNA* and T regulatory cells, which are known to suppress effector Tcell function, may relieve local immunosuppression and facilitate more effective antitumor responses. Collectively, these findings highlight that the prognostic value of *RFC4* and *PCNA* is determined not only by their cellintrinsic proliferative functions but also by their capacity to shape the tumor immune microenvironment toward a more active state.

In addition, drug sensitivity analyses using CTRP and GDSC datasets revealed that high *RFC4* and *PCNA* expression levels were associated with lower IC_{50} values for multiple anticancer agents, indicating increased drug sensitivity. In contrast, *AURKA* and *ECT2* expression was positively correlated with IC_{50} values, suggesting a potential role in drug resistance.[46] Although the involvement of these genes in cervical cancer drug resistance has not been extensively reported, these findings carry practical implications for personalized chemotherapy. For patients whose tumors exhibit high *RFC4* or *PCNA* expression, the lower IC_{50} values suggest increased sensitivity to multiple anticancer agents. Assessment of *RFC4* and *PCNA* expression in biopsy specimens may help identify patients who are more likely to benefit from these agents, thereby avoiding ineffective regimens and reducing unnecessary toxicity. Conversely, tumors with low *RFC4*/*PCNA* expression or high *AURKA*/*ECT2* expression may display relative resistance, suggesting that alternative or combination strategies—such as incorporating poly(ADP-ribose) polymerase inhibitors or ataxia telangiectasia and Rad3-related kinase inhibitors to synthetically increase replication stress—could be considered to overcome primary chemoresistance.[47,48] Moreover, the favorable drug-sensitivity profile associated with high *RFC4* and *PCNA* expression suggests that these tumors might also respond to emerging targeted therapies directed against the DNA replication and repair machinery, including the inhibitors of checkpoint kinase 1 or WEE1 tyrosine kinase. Therefore, *RFC4* and *PCNA* hold promise as predictive biomarkers to guide first-line chemotherapy selection and to inform the addition of targeted agents in cervical cancer. Prospective clinical studies are warranted to establish validated expression cutoffs and to further evaluate the effects of these markers on treatment decision-making.

In summary, the present study systematically identified *AURKA*, *ECT2*, *RFC4*, and *PCNA* as persistently dysregulated hub genes during the progression from normal cervical epithelium to HSIL and invasive cervical cancer. The overexpression of *RFC4* and *PCNA* was correlated with aggressive clinicopathological features and associated with patient survival, as well as with enhanced sensitivity to multiple anticancer agents. These seemingly paradoxical findings may be explained by their dual roles in promoting DNA replication while simultaneously facilitating error-free damage tolerance and modulating the tumor immune microenvironment. Several limitations should be acknowledged. The bioinformatics predictions are primarily derived from public datasets, and the survival analyses are based on retrospective data. In addition, although protein expression was confirmed in the HPA database, immunohistochemical validation using our own clinical cohort was not performed due to the limited availability of paraffin-embedded tissues; this will be addressed in future studies. The underlying molecular mechanisms by which *RFC4* and *PCNA* influence chemosensitivity and immune infiltration require further experimental validation. Furthermore, the potential confounding effects of HPV genotypes and patient treatment histories were not fully accounted for in the current analysis. Consequently, future prospective studies with larger independent cohorts are warranted to validate the predictive value of *RFC4* and *PCNA* expression for guiding chemotherapy selection, as well as to explore the therapeutic efficacy of targeting these proteins in combination with DNA-damaging agents or replication stress-inducing drugs in cervical cancer.

5. Conclusion

Aurora kinase A, *ECT2*, *RFC4*, and *PCNA* were identified as key hub genes associated with cervical cancer progression. Among them, *RFC4* and *PCNA* exhibit particularly strong prognostic and therapeutic relevance, potentially mediated through immune modulation and enhanced drug sensitivity. These findings provide new insights into the cellular pathways driving cervical oncogenesis and present potential markers for early detection and personalized therapy. Specifically, higher expression of *RFC4* and *PCNA* correlated with favorable survival outcomes and distinct immune infiltration patterns, including positive associations with B cells and effector memory immune cells. Moreover, their expression levels were associated with sensitivity to multiple chemotherapeutic agents, suggesting that targeting these genes could improve treatment efficacy in patients with cervical cancer. Collectively, this integrated bioinformatics and experimental approach highlights *RFC4* and *PCNA* as dual-function regulators—both as prognostic indicators and as potential therapeutic targets—warranting further preclinical and clinical investigation.

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Conflict of interest

The authors have no conflicts of interest.

Author contributions

Conceptualization: Meiqin Yang
Formal analysis: Shengyi Gu
Investigation: Shengyi Gu
Methodology: Meiqin Yang
Writing–original draft: Shengyi Gu
Writing–review & editing: Meiqin Yang

Ethics approval and consent to participate

All the clinical experiments in this study were approved by the Ethics Committee of the Shanghai First Maternity and Infant Hospital (approval no: KS24427). Written informed consent was obtained from all participating patients.

Consent for publication

Written informed consent for publication was obtained from all participating patients.

Availability of data

Data are available from the corresponding author upon reasonable request.

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Appendix

Table A1. Primers used in this study

Gene name	Primer sequences (5'–3')
AURKA	F: TGGGTGGTCAGTACATGCTC
	R: TGCATCCGACCTTCAATCATTTC
ECT2	F: TGTAGTCACGGACTTTCAGGA
	R: GTACAATACAACGGGCGACAT
RFC4	F: GCCGTGTCCGCCTTTTAAGAT
	R: AGATAAGACAGAATCGGGTGGT
PCNA	F: GCGTGAACCTCACCAGTATGT
	R: TCTTCGGCCCTTAGTGTAATGAT
GAPDH	F: CTGACTTCAACAGCGACACC
	R: TGCTGTAGCCAAATTCGTTGT