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Aberrant PAX6 Expression in Cervical Cancer: Integrated Molecular Profiling and Clinical Cohort Evaluation

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Abstract

Background: Paired box 6 (PAX6) is a developmental transcription factor implicated in multiple malignancies, but its molecular and clinical associations in cervical cancer remain incompletely characterized. This study investigated PAX6 expression, HPV16-associated alterations, developmental gene networks, and clinical outcomes in cervical cancer.

Materials & Methods: Integrated analyses of TCGA-CESC and public molecular databases evaluated PAX6 transcript expression, HPV16 status, genomic alterations, protein-expression patterns, survival outcomes, and developmental gene associations. Peripheral-blood PAX6 expression was independently assessed using quantitative real-time PCR in 103 cervical cancer patients receiving cisplatin-based chemoradiotherapy and 108 healthy controls.

Results: PAX6 expression was elevated in cervical cancer tissues (GEPIA3, $p = 0.000542$) and HPV16-positive tumours ($n = 175$) compared with HPV16-negative tumours ($n = 14$; $p = 2.2 \times 10^{-7}$). Exploratory survival analyses showed divergent associations with overall survival (HR = 0.62, 95% CI 0.39–0.99) and relapse-free survival (HR = 2.32, 95% CI 1.06–5.06). In 304 primary tumours, PAX6 correlated weakly with SOX2 ($\rho = 0.296$) and SIX3 ($\rho = 0.274$), both significant after false-discovery-rate correction. Peripheral-blood PAX6 was elevated in 84/103 patients (81.6%; $p = 0.001$), without significant associations with chemoradiotherapy response ($p = 0.804$) or overall survival.

Conclusions: PAX6 exhibits altered expression in cervical cancer, with associations involving HPV16 status, developmental transcriptional networks, and selected survival outcomes. Peripheral-blood findings provide complementary clinical evidence, but independent validation is required to establish prognostic utility.

1. Introduction

Cervical cancer is the fifth most common cancer among women with 604,196 new cases and 279,583 deaths in 2024 globally[1]. Persistent infection with carcinogenic high-risk HPV types is the necessary cause of cervical cancer, with HPV16 and HPV18 being among the most carcinogenic types and accounting for a large proportion of cervical cancers[2]. However, only a fraction of HPV-infected individuals ultimately develops invasive disease, indicating that additional molecular alterations are required for malignant transformation, tumor progression, and clinical heterogeneity[3].

Concurrent chemoradiotherapy remains the standard treatment for locally advanced cervical cancer, yet considerable variability exists in treatment response, recurrence, and subsequent recurrence and survival outcomes[4,5]. Current prognostic assessment relies predominantly on clinicopathological factors, including tumor stage, histological subtype, lymph node status, and treatment response[6,7]. Although these factors provide essential clinical information for clinical decision-making and risk assessment, they incompletely capture the underlying biological diversity of cervical cancer[8]. Consequently, there

remains a need for molecular biomarkers capable of improving risk stratification predict treatment response and providing biological insight into disease progression and recurrence[7].

Developmental transcription factors are increasingly recognized as important regulators of cancer cell state and tumor behavior[9,10]. During tumorigenesis, transcriptional programs that normally govern embryonic development, lineage specification, and tissue differentiation can be reactivated or co-opted, enabling cancer cells to undergo phenotypic plasticity, dedifferentiation, and adaptation to the tumor microenvironment and therapeutic stress[9,11]. Among these developmental regulators, the paired box (PAX) family of transcription factors has attracted considerable attention because of its central roles in organogenesis, lineage specification, cell-fate determination, proliferation, and differentiation, as well as its context-dependent involvement in tumor initiation and progression[12].

PAX6 is a highly conserved paired box transcription factor that functions as a key regulator of ocular, neural, endocrine, and epithelial development[12,13]. Beyond its established developmental functions, accumulating evidence suggests that PAX6 contributes to tumor biology in a context-dependent manner[13]. Aberrant PAX6 expression has been reported in several malignancies, including non-small cell lung cancer, pancreatic cancer, colorectal cancer, and glioma, where it has been associated with altered cellular differentiation, proliferation, invasion, tumor progression, and clinical outcome[14–17]. However, the biological significance of PAX6 appears highly tissue-specific, and its role in cervical cancer remains poorly defined.

Notably, preliminary observations from our clinical cohort demonstrated frequent PAX6 overexpression in cervical cancer patients and suggested a potential association with HPV-positive disease. These findings, together with emerging evidence linking PAX6 to cancer progression in other tumor types, prompted a systematic investigation of its role in cervical carcinogenesis. Despite this biological rationale, a comprehensive evaluation integrating transcriptomic expression, HPV association, protein expression, survival outcomes, molecular interaction networks, and independent clinical validation have not previously been performed in cervical cancer.

Recent advances in cancer genomics and publicly accessible multi-omics resources provide unprecedented opportunities to evaluate candidate biomarkers across multiple biological layers[18–21]. Integration of transcriptomic, proteomic, genomic, functional, and clinical datasets enables more robust biomarker discovery than single-cohort studies and facilitates identification of biologically meaningful molecular signatures with potential translational relevance[19,21]. Such integrative approaches are particularly valuable for developmental transcription factors whose biological effects extend beyond differential gene expression and involve broader regulatory networks.

Based on these observations, we hypothesized that PAX6 is recurrently activated in cervical cancer, associated with HPV-positive disease, and linked to biological programs that contribute to disease progression and recurrence. To test this hypothesis, we employed an integrative multi-omics and clinical validation strategy to characterize PAX6 expression patterns, HPV association, prognostic significance,

molecular interaction networks, and functional pathways across independent datasets. Findings from in-silico analyses were subsequently validated in an independent cervical cancer cohort. Through this approach, we sought to determine whether PAX6 represents a clinically relevant biomarker associated with HPV-linked cervical carcinogenesis and relapse-prone disease behavior.

2. MATERIALS AND METHODS

2.1 Study Design

This study employed an integrative multi-omics and clinical validation framework to investigate the molecular and clinical significance of PAX6 in cervical cancer. Publicly available transcriptomic, proteomic, genomic, survival, and functional datasets were interrogated to characterize PAX6 expression patterns, HPV association, prognostic relevance, molecular interaction networks, and biological pathways. Complementary PAX6 expression analysis was performed using quantitative real-time PCR (qPCR) in peripheral-blood samples from a clinically characterized cervical cancer cohort.

2.2 In-Silico Multi-Omics Analysis

A systematic bioinformatics workflow was implemented to evaluate PAX6 across multiple molecular layers. Gene expression analyses were performed using GEPIA3[22], UALCAN[23], and OncoDB databases[24]. Pan-cancer expression profiling was conducted using GEPIA3 and UALCAN, while cervical cancer-specific analyses focused on the Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC) cohort from The Cancer Genome Atlas (TCGA). Differential expression was evaluated using GEPIA3 (306 tumors and 13 normal tissues), UALCAN (305 tumors and 3 normal tissues), and OncoDB (304 tumors and 22 normal tissues) **(**Table 1**).**

Table 1
Molecular, clinical, and bioinformatic resources used for the integrated evaluation of PAX6 in cervical cancer.

Data Layer	Database/Resource	Dataset/Source	Purpose	Key Output
Transcriptomics	GEPIA3	TCGA + GTEx	Pan-cancer and CESC expression analysis	Differential expression of PAX6
Transcriptomics	UALCAN	TCGA-CESC	Tumor–normal expression comparison	Concordant expression evidence
Transcriptomics	OncoDB	TCGA-CESC	Expression and HPV association	HPV16-related expression analysis
Proteomics	Human Protein Atlas (HPA)	IHC tissue atlas	Qualitative protein-expression assessment	PAX6 protein abundance in CESC
Survival Analysis	KM-Plotter	GEO + TCGA + EGA	Prognostic significance	OS and RFS analysis
Cancer Genomics	cBioPortal	TCGA-CESC	Mutation and copy-number alterations	Genomic alteration profile
Functional Genomics	Enrichr	PAX6-associated gene set	GO and KEGG enrichment	Biological processes and pathways
Network Biology	STRING	PAX6-associated proteins	Protein interaction analysis	Molecular interaction network
Clinical Validation	Independent CaCx cohort	103 CaCx + 108 controls	Peripheral-blood qPCR evaluation	Peripheral-blood PAX6 expression

CaCx, cervical cancer; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; GEPIA3, Gene Expression Profiling Interactive Analysis 3; UALCAN, University of Alabama at Birmingham Cancer Data Analysis Portal; HPA, Human Protein Atlas; IHC, immunohistochemistry; GEO, Gene Expression Omnibus; EGA, European Genome-phenome Archive; KM-Plotter, Kaplan–Meier Plotter; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; qPCR, quantitative polymerase chain reaction; OS, overall survival; RFS, relapse-free survival; HPV16, human papillomavirus type 16.

Initially, investigated *PAX6* gene modulation in HPV-mediated cervical cancer, where we analyse its expression 175 HPV16-positive and 14 HPV16-negative tumors. The HPV16-negative category was interpreted as negative for HPV16 according to the dataset annotation and was not assumed to represent samples negative for all HPV types. The available data and metadata were reviewed to

establish whether HPV typing, histological subtypes and FIGO stage were available for these groups. Protein expression patterns were assessed using immunohistochemistry images obtained from The Human Protein Atlas (HPA)[25].

The prognostic significance of PAX6 was evaluated using KM-Plotter[26] and cBioPortal[27]. Relapse-free survival (RFS), overall survival (OS), and disease-specific survival (DSS) were analyzed according to database-specific analytical pipelines. Genomic alterations, including mutations and copy-number changes, were examined using cBioPortal.

For the survival analyses, patients were stratified into high- and low-PAX6-expression groups according to the cutoff specified by the respective analysis platform. Kaplan–Meier curves and log-rank tests were used to compare survival outcomes, and HRs with 95% CIs were reported where available. The direction of each HR was interpreted according to the reference group specified in the corresponding analysis. In the clinical cohort, overall survival was compared between PAX6 expression groups using Kaplan-Meier analysis and the log-rank test.

To explore the biological context of PAX6 activation, protein–protein interaction networks were generated using STRING[28], and functional enrichment analyses were performed using Enrichr. Gene Ontology (GO) Biological Process, GO Molecular Function, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted. Enrichment was considered statistically significant when the multiple-testing-adjusted p-value < 0.05.

2.3 Clinical Cohort

The clinical cohort comprised 103 histopathologically confirmed cervical cancer patients and 108 age-matched healthy women. Patients were recruited from the Department of Radiotherapy, King George's Medical University, Lucknow, India, over a 60-month period. Peripheral blood samples were collected at the initial clinical visit. Cervical cancer diagnosis and staging were established according to the International Federation of Gynecology and Obstetrics (FIGO) and World Health Organization (WHO) guidelines. The clinicopathological characteristics of the study population are summarized in Table 2.

Table 2
Clinicopathological characteristics of patients with cervical cancer (n = 103).

Variable	Category	Number of Patients (%)
Age (years)	≤ 40	16 (15.5)
	> 40	87 (84.5)
Histopathological Type	Squamous Cell Carcinoma	100 (97)
	Adenocarcinoma	03 (3)
	Other	00 (0)
FIGO Stage	I	00 (0)
	II	54 (52)
	III	44 (43)
	IV	05 (5)
HPV Status	HPV Positive	86 (83.5)
	HPV Negative	17 (16.5)
Clinical Response to CRT	Complete Response (CR)	31 (30.1)
	Partial Response (PR)	26 (25.2)
	Stable Disease (SD)	22 (21.4)
	Progressive Disease (PD)	24 (23.3)
Response Category	Responders (CR + PR)	57 (55.3)
	Non-Responders (SD + PD)	46 (44.7)
Vital Status	Alive	73 (70.9)
	Deceased	30 (29.1)
PAX6 Expression Status	Upregulated	84 (81.6)
	Downregulated	19 (18.4)
Follow-up	Months	60
Median Overall Survival in months	Up-regulation	28
	Downregulation	23

Eligible patients were aged 30–70 years and had histologically confirmed cervical squamous cell carcinoma or adenocarcinoma. Patients with secondary malignancies, major systemic comorbidities, or incomplete clinical information were excluded. Healthy controls had no previous history of malignancy

or cervical neoplastic disease. The cohort was previously investigated for DNA repair gene expression and chemoradiotherapy outcomes (Kushwah et al., 2023) [29]. The present investigation extends its molecular characterization through PAX6-specific expression analysis.

The clinical investigation was conducted within a larger cervical cancer research programme approved by the Institutional Ethics Committee of King George's Medical University, Lucknow, Uttar Pradesh, India (Approval No. 256/Ethics/R.Cell-18; dated 24 September 2018). Written informed consent was obtained from all participants before enrolment and biological sample collection, in accordance with the Declaration of Helsinki.

2.4 RNA Isolation and Quantitative Real-Time PCR

Blood samples (2ml) were collected for RNA extraction from all study participants in vials with TRIzol reagent (Life Technologies, USA) and all samples were stored at -20 ∘C until analysis. Total RNA was isolated according to the manufacturer's instructions and quantified using a BioPhotometer (Eppendorf, Germany). cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) and quantified. Expression of PAX6 gene with *GAPDH* as the endogenous reference gene was performed by real-time PCR (qPCR) using Maxima SYBR Green/ROX Master Mix (Thermo Scientific, USA) on a LightCycler 480 platform (Roche Diagnostics, Germany). The reaction mixture (10 µl) contained 1x Maxima SYBR Green/ROX qPCR master mix (Thermo Scientific USA), 25 ng of cDNA, 2.5 pmol specific primers and 1 µl of milliQ water. The PAX6 gene expression primers were design by using Primer 3.0(online version) and cross validated by NCBI Primer BLAST (online version) forward primer 5'-AGCGGTGCATTTGCATGTT-3' and the reverse 5'-GATTGGTGATGGCTCAAGTGT-3'. The annealing temperature was 57°C. The reaction condition was set as one cycle at 95°C for 5 min; followed by 35 cycles at 95°C for 10s, 57°C for 20s, 72°C for 10s, and a final hold at 10°C for 1 min. The relative PAX6 expression was calculated using the 2^-ΔΔCt method[29].

For clinical analyses, samples exhibiting fold-change values > 1 were classified as upregulated, whereas samples with fold-change values < 1 were classified as downregulated. A relative expression value of 1 indicated expression equivalent to the designated calibrator. Classification of individual samples as upregulated or downregulated was descriptive and did not independently establish statistically significant differential expression. Group-level differences were evaluated separately using the specified statistical tests[29].

2.5 Treatment Response and Survival Assessment

All patients received cisplatin-based concurrent chemoradiotherapy according to institutional treatment protocols. Clinical response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0. Patients achieving complete response (CR) or partial response (PR) were categorized as responders, whereas patients with stable disease (SD) or progressive disease (PD) were categorized as non-responders.

Patients were followed after treatment. Overall survival (OS) was defined as the interval between diagnosis and death from any cause or last follow-up. Relapse-free survival (RFS) was defined as the interval between diagnosis and documented disease recurrence or last follow-up.

2.6 Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 5.01[30], SPSS version 21.0[31], and database-specific analytical pipelines. Continuous variables are presented as geometric mean ± standard error (SE). Differences between groups were assessed using Mann–Whitney U or Wilcoxon rank-sum tests as appropriate.

Pairwise associations were assessed using Spearman's rank correlation coefficient (ρ). Raw *p*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure. Associations were considered statistically significant at an FDR-adjusted *p*-value < 0.05. For Fig. 6, cells were left blank when the corresponding associations did not meet this significance threshold after FDR correction.

Associations between PAX6 expression status and clinicopathological variables were evaluated using Chi-square or Fisher's exact tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated where appropriate. Survival analyses were performed using Kaplan–Meier curves and Cox proportional hazards regression models. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

2.7 Transcriptomic Correlation Analysis

To evaluate gene co-expression, Level 3 RNA-sequencing data (raw counts processed via the STAR pipeline to transcripts per million [TPM]) for the TCGA-CESC cohort[7] were acquired using the TCGAbiolinks R package[32]. Analysis was strictly restricted to primary tumor samples (N = 304). Gene expression values were log2-transformed (log2(TPM + 1)). The associations between PAX6 and candidate developmental regulators (SOX1, SOX2, NEUROG2, SIX3) were evaluated using both Pearson and Spearman rank correlation coefficients. To account for multiple testing, p-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR). Statistical analyses and visualizations were performed in R version 4.6.1[33] using the ggplot2[34], ggcorrplot, and patchwork packages. An FDR-adjusted p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 PAX6 overexpression shows cross-platform concordance in cervical cancer

To determine whether PAX6 is aberrantly expressed in cervical cancer, we first evaluated its expression pattern across multiple publicly available transcriptomic datasets. Pan-cancer analysis using the GEPIA3

platform revealed significant dysregulation of PAX6 across several malignancies. Among these, Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC) exhibited significant PAX6 overexpression compared with normal tissues, together with cholangiocarcinoma, oesophageal carcinoma, lung squamous cell carcinoma, and sarcoma.

To specifically investigate cervical cancer, differential expression analyses were performed using GEPIA3, UALCAN, and OncoDB. Consistent across all platforms, PAX6 expression was significantly elevated in cervical tumor tissues relative to normal cervical tissues. In the GEPIA3 dataset, PAX6 expression was significantly increased in 306 cervical cancer samples compared with 13 normal cervical tissues ($p = 0.000542$). Similar overexpression was observed in the UALCAN TCGA-CESC cohort comprising 305 primary tumors and 3 normal cervical samples. Analysis using OncoDB showed a concordant pattern of increased PAX6 transcript abundance in cervical carcinoma (Fig. 1A–E).

The analyses using GEPIA3, UALCAN and OncoDB showed concordant patterns of increased PAX6 transcript abundance in cervical cancer. These findings provide cross-platform support for the observed expression pattern; however, they do not independently establish increased PAX6 protein expression.

Collectively, these findings demonstrate concordant PAX6 transcript overexpression across cervical cancer datasets, with supportive qualitative immunohistochemical evidence of protein expression, suggesting a potential association between PAX6 dysregulation and cervical carcinogenesis.

Expression values are presented as $\log_2(\text{TPM} + 1)$. Statistical significance was determined according to the respective platform-specific analytical pipelines.

3.2 PAX6 expression is associated with HPV16 status in cervical cancer

Persistent infection with high-risk human papillomavirus (HPV), particularly HPV16, represents the principal etiological driver of cervical carcinogenesis. To investigate whether PAX6 activation is associated with HPV-mediated tumor biology, PAX6 expression was compared between HPV16-positive and HPV16-negative cervical cancer samples using the OncoDB platform.

A total of 175 HPV16-positive and 14 HPV16-negative cervical cancer samples were included in the analysis. PAX6 expression was significantly higher in HPV16-positive tumors than in HPV16-negative tumors ($p = 2.2 \times 10^{-7}$). The average expression level of PAX6 was 4.4 in HPV16-positive tumors compared with 1.0 in HPV16-negative tumors, corresponding to a log2 fold change of 1.32.

To further evaluate the relationship between HPV infection and PAX6 activation in our clinical cohort, differential expression patterns were examined according to HPV status. PAX6 expression was significantly elevated in both HPV-positive and HPV-negative cervical cancer patients compared with healthy controls. However, relative expression levels were consistently higher among HPV-positive patients, supporting observations from public datasets **[**Figure 2 **(A-B)]**. These findings demonstrate an association between higher PAX6 expression and HPV16-positive tumour status. Whether this

relationship reflects HPV16-specific regulation or broader differences in HPV-associated tumour biology requires mechanistic investigation.

3.3 PAX6 expression is associated with relapse-free survival in cervical cancer

To evaluate the clinical significance of PAX6 expression, survival analyses were performed using independent public datasets and subsequently compared with findings from the clinical validation cohort (Table 3).

Table 3
Association of peripheral-blood PAX6 expression status with chemoradiotherapy response and vital status in patients with cervical cancer (N = 103).

Clinical Response				
Differential Expression	**Responders (CR + PR) (n = 57) (%)**	**Non-responders (SD + PD) (n = 46) (%)**	**P-value**	**OR (95% CI)**
Down regulated	10(17.5)	09(19.6)	0.804	1.14(0.42–3.10)
Up regulated	47(82.5)	37(80.4)		
Vital Status				
Differential Expression	**Alive (n = 73) (%)**	**Deceased (n = 30) (%)**	**P-value**	**OR (95% CI)**
Down regulated	09(12.3)	10(33.3)	0.023	3.56(1.27–9.98)
Up regulated	64(87.7)	20(66.7)		

Kaplan–Meier analysis revealed a significant association between PAX6 expression and overall survival (OS) in the public dataset. Patients with high PAX6 expression exhibited longer OS than those with low expression (HR = 0.62, 95% CI: 0.39 to 0.99; log-rank $p = 0.045$). This result indicates an association between higher PAX6 expression and a lower observed hazard of death in the analyzed dataset (Fig. 3A).

In contrast, relapse-free survival (RFS) analysis showed that patients with high PAX6 expression had shorter RFS than those with low expression (HR = 2.32, 95% CI: 1.06 to 5.06; log-rank $p = 0.03$; Fig. 3B). This finding indicates an association between elevated PAX6 expression and an increased hazard of relapse in the analyzed dataset. The contrasting associations observed for OS and RFS highlight the importance of interpreting each endpoint separately (Fig. 3B).

In the clinical cohort, PAX6 expression was not significantly associated with immediate response to cisplatin-based chemoradiotherapy. Similarly, the clinical-cohort OS analysis did not demonstrate a statistically significant difference between patients with high and low PAX6 expression (Fig. 3C).

Overall, PAX6 expression showed different associations with OS and RFS in the public dataset, whereas no significant association was observed with immediate treatment response or OS in the clinical cohort. These findings warrant further investigation in independent cohorts, with clearly defined expression groups, survival endpoints and adjustment for relevant clinical factors.

3.4 PAX6-associated networks show enrichment of developmental and transcriptional programs

To investigate the biological context of PAX6 dysregulation, functional enrichment and molecular association analyses were performed using Enrichr and STRING. Gene Ontology (GO) Biological Process analysis demonstrated enrichment of processes related to cellular differentiation and developmental regulation. Among the significantly enriched terms were enteroendocrine cell differentiation, pancreatic beta-cell differentiation, neuron fate commitment, negative regulation of nervous system development, epithelial cell differentiation, visual system development, and regulation of microRNA transcription. These findings indicate that genes associated with PAX6 are preferentially represented in biological programs involved in lineage specification and developmental control.

GO Molecular Function analysis further identified enrichment of transcriptional and regulatory functions, including histone acetyltransferase binding, R-SMAD binding, transcription coregulator binding, transcription factor activity, kinase binding, and sequence-specific DNA binding. These findings are consistent with the established role of PAX6 as a developmental transcription factor involved in regulation of cell identity and differentiation.

KEGG pathway analysis identified enrichment of pathways related to signaling mechanisms regulating pluripotency of stem cells and developmental transcriptional programs. Such pathways are relevant to cellular plasticity and transcriptional reprogramming in cancer, although the present enrichment analysis does not establish a direct functional role for PAX6 in maintaining a stem-like phenotype.

A STRING-derived molecular association network was subsequently examined to identify developmental regulators associated with PAX6. The network included SOX2, SOX1, NEUROG2, SIX3, TBR1, MITF, MAF, INS, BANF1, and DCAF7. Several of these proteins, particularly SOX-family and SIX3 developmental regulators, participate in transcriptional programs governing lineage specification and cell-state regulation **[**Figure 4 **(A-D)]**.

Together, these analyses place PAX6 within a developmental regulatory context characterized by differentiation-associated, transcriptional, and pluripotency-related molecular programs. To determine whether selected regulators identified in the STRING network also exhibited coordinated transcriptional behavior with PAX6 in primary cervical tumors, their expression relationships were subsequently examined in the TCGA-CESC cohort.

3.4.1 Transcriptomic co-expression of PAX6 with selected developmental regulators in primary cervical tumours

To determine whether selected developmental regulators identified in the STRING-derived PAX6 association network also exhibited coordinated transcriptional patterns with PAX6 in primary cervical tumours, we examined SOX1, SOX2, NEUROG2, and SIX3 expression in the TCGA-CESC cohort. Analysis was restricted to primary tumour specimens, yielding 304 evaluable cases (Table 4).

Table 4
Transcriptomic co-expression of PAX6 with selected developmental regulators in the TCGA-CESC primary tumour cohort (N = 304).

Gene pair	N	Spearman ρ	Raw *p*-value	FDR-adjusted *p*-value
PAX6–SOX1	304	0.011	0.845	0.845
PAX6–SOX2	304	0.296	1.41×10^{-7}	5.65×10^{-7}
PAX6–NEUROG2	304	0.055	0.343	0.457
PAX6–SIX3	304	0.274	1.28×10^{-6}	2.57×10^{-6}

Note: Spearman's rank correlation coefficients (ρ) are reported. Raw *p*-values were adjusted using the Benjamini–Hochberg FDR procedure. Associations with FDR-adjusted $p < 0.05$ were considered statistically significant.

Spearman rank correlation analysis demonstrated a weak positive correlation between PAX6 and SOX2 expression ($\rho = 0.296$, FDR-adjusted $p = 5.65 \times 10^{-7}$) (Fig. 5). PAX6 expression was similarly correlated with SIX3 ($\rho = 0.274$, FDR-adjusted $p = 2.57 \times 10^{-6}$). In contrast, SOX1 and NEUROG2 showed very weak and non-significant correlations with PAX6 ($\rho = 0.011$, FDR-adjusted $p = 0.845$; and $\rho = 0.055$, FDR-adjusted $p = 0.457$, respectively).

A Spearman correlation matrix was additionally generated to visualize pairwise co-expression relationships among the evaluated developmental regulators (Fig. 6). Collectively, these findings provide transcriptomic support for selected components of the PAX6-associated developmental network, particularly SOX2 and SIX3, in primary cervical tumours. The modest correlation coefficients indicate coordinated expression rather than direct transcriptional regulation and therefore warrant functional validation.

3.5 Peripheral-blood PAX6 expression and clinical associations

PAX6 transcript levels were assessed in peripheral-blood samples from 103 patients with cervical cancer and compared with those from 108 healthy controls. Consistent with the observed expression patterns in the public transcriptomic datasets, PAX6 expression was significantly elevated in patients with cervical cancer compared with healthy controls. Based on the study-defined expression classification, PAX6 was upregulated in 84 patients (81.6%), whereas 19 patients (18.4%) showed downregulation. These findings support the frequent alteration of peripheral-blood PAX6 expression in cervical cancer and provide clinical evidence complementary to the tumour-tissue transcriptomic analyses. These

findings demonstrate frequent peripheral-blood PAX6 elevation relative to the specified control calibrator; they do not independently validate tumour-tissue expression.

To investigate whether HPV infection influenced PAX6 expression, subgroup analyses were performed according to HPV status. Elevated PAX6 expression was observed in both HPV-positive and HPV-negative cervical cancer patients relative to healthy controls; however, expression levels were higher among HPV-positive patients, consistent with observations from public datasets.

The relationship between PAX6 expression and patient outcome was subsequently examined. Comparison according to vital status revealed a significant association between differential PAX6 expression and survival outcome. PAX6 downregulation was more frequently observed among deceased patients than among surviving patients (33.3% versus 12.3%; OR = 3.56, 95% CI = 1.27–9.98, p = 0.023). Conversely, PAX6 upregulation was more frequently detected among surviving patients.

To determine whether PAX6 expression influenced treatment response, expression patterns were analyzed according to clinical response following cisplatin-based chemoradiotherapy. No significant association was observed between PAX6 expression status and treatment response (p = 0.804). Similarly, Kaplan–Meier analysis of overall survival did not demonstrate statistically significant differences according to PAX6 expression status **(**Table 5**)**.

Table 5
Summary of molecular and clinical evidence for PAX6 dysregulation in cervical cancer.

Evidence Layer	Dataset	Finding	Significance	Biological/Clinical Implication
Pan-cancer Transcriptomics	GEPIA3	PAX6 significantly upregulated in CESC	p = < 0.000542	Tumor-associated activation
Tumour–normal expression comparison	UALCAN	Higher expression in CESC tumors than normal tissues	Significant	Concordant evidence of increased PAX6 expression
Tumour–normal expression comparison	OncoDB	Elevated expression in CESC	Significant	Consistent across public analysis platforms
Protein Expression	Human Protein Atlas	Stronger PAX6 staining in cervical tumors	Significant	Protein-level support (Qualitative IHC evidence)
HPV Association	OncoDB	Higher expression in HPV16-positive tumors	$p = 2.2\times10^{-7}$	Association with HPV16-positive tumour status
Relapse-Free Survival	KM-Plotter	Additional reported survival association; cohort independence unverified	HR = 2.32, p = 0.03	Reported association; verify source and HR direction
Disease-Specific Survival	cBioPortal	Altered PAX6 associated with poorer DSS	p = 0.0338	Clinical outcome association
Genomic Alterations	cBioPortal	Very low mutation frequency (~ 0.3–1%)	Rare event	Genomic alteration is unlikely to be the predominant explanation for PAX6 dysregulation
Functional Enrichment	Enrichr	Developmental differentiation pathways enriched	p < 0.001	Association with developmental regulatory programs

Statistical estimates were obtained from the respective public databases or clinical cohort analyses. GEPIA3, UALCAN, and OncoDB may include overlapping TCGA-CESC samples; therefore, concordant transcriptomic findings do not constitute independent biological validation. Human Protein Atlas immunohistochemical findings represent qualitative protein-expression evidence. Survival associations should be interpreted according to their respective endpoints, expression-defined or alteration-defined groups, and reference categories. Peripheral-blood PAX6 expression was evaluated in a separate clinical cohort and does not directly validate tumour-tissue expression.
Abbreviations:PAX6, paired box 6; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; GEPIA3, Gene Expression Profiling Interactive Analysis 3; TCGA, The Cancer Genome Atlas; HPA, Human Protein Atlas; IHC, immunohistochemistry; HPV16, human papillomavirus type 16; DSS, disease-specific survival; RFS, relapse-free survival; HR, hazard ratio; OR, odds ratio; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; CRT, chemoradiotherapy.

Evidence Layer	Dataset	Finding	Significance	Biological/Clinical Implication
Functional Enrichment	Enrichr	Stem-cell pluripotency pathways enriched	$p = 7.05\times10^{-3}$	Enrichment of pluripotency-related pathways
Network Analysis	STRING	Interaction with SOX2, SOX1, NEUROG2, SIX3	Significant	Developmental regulatory context
Clinical Validation	Patient cohort (n = 103)	PAX6 upregulated in 81.6% of patients	p = 0.001	Peripheral-blood clinical corroboration (different tissue compartment)
Vital Status Association	Patient cohort	Reduced peripheral-blood PAX6 expression was more frequent among deceased patients	OR = 3.56, p = 0.023	Outcome-associated peripheral-blood expression pattern
CRT Response	Patient cohort	No association with treatment response	p = 0.804	Not predictive of CRT response

Statistical estimates were obtained from the respective public databases or clinical cohort analyses. GEPIA3, UALCAN, and OncoDB may include overlapping TCGA-CESC samples; therefore, concordant transcriptomic findings do not constitute independent biological validation. Human Protein Atlas immunohistochemical findings represent qualitative protein-expression evidence. Survival associations should be interpreted according to their respective endpoints, expression-defined or alteration-defined groups, and reference categories. Peripheral-blood PAX6 expression was evaluated in a separate clinical cohort and does not directly validate tumour-tissue expression.
Abbreviations:PAX6, paired box 6; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; GEPIA3, Gene Expression Profiling Interactive Analysis 3; TCGA, The Cancer Genome Atlas; HPA, Human Protein Atlas; IHC, immunohistochemistry; HPV16, human papillomavirus type 16; DSS, disease-specific survival; RFS, relapse-free survival; HR, hazard ratio; OR, odds ratio; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; CRT, chemoradiotherapy.

Collectively, these findings confirm frequent peripheral-blood PAX6 upregulation in cervical cancer but do not establish PAX6 as a predictor of chemoradiotherapy response or overall survival. The association between reduced peripheral-blood PAX6 expression and adverse vital status warrants further investigation, particularly given the distinct biological compartments represented by circulating blood and tumour-tissue transcriptomic datasets.

4. DISCUSSION

The present study provides the first comprehensive integration of transcriptomic, clinical, and systems-level analyses examining the significance of PAX6 in cervical cancer. By integrating TCGA-based molecular analyses with peripheral-blood expression measurements in a clinically characterized cohort, we identified altered PAX6 expression associated with cervical cancer, HPV16-positive tumour status, selected survival endpoints, and developmental transcriptional programmes.

A major finding of this study is the remarkable consistency of PAX6 overexpression across independent datasets. Transcriptomic analyses performed using GEPIA3, UALCAN, and OncoDB uniformly demonstrated significantly elevated PAX6 expression in cervical carcinoma compared with normal cervical tissues. These findings were further supported by Human Protein Atlas data showing increased PAX6 protein expression in cervical cancer tissues. Importantly, clinical validation confirmed PAX6 upregulation in more than 80% of cervical cancer patients, indicating that PAX6 activation is a reproducible molecular feature of cervical cancer rather than a dataset-specific observation.

The concordant PAX6 expression patterns observed across GEPIA3, UALCAN, and OncoDB provide cross-platform support for the observed association between PAX6 expression and cervical cancer. However, these resources may analyze overlapping TCGA-CESC samples, and their agreement should not be interpreted as independent cohort validation with separately collected samples is required to establish the reproducibility and generalisability of these findings.

PAX6 is a highly conserved developmental transcription factor that plays essential roles in cellular differentiation, lineage commitment, and organogenesis. While traditionally studied in ocular and neural development, increasing evidence suggests that aberrant reactivation of developmental transcription factors contributes to malignant transformation by promoting cellular plasticity and adaptation[12,13]. In several malignancies, including non-small cell lung cancer and pancreatic cancer, elevated PAX6 expression has been associated with aggressive disease phenotypes, enhanced invasion, epithelial–mesenchymal transition, and unfavorable clinical outcomes[15]. Our findings suggest that cervical cancer may represent another malignancy in which developmental reprogramming involving PAX6 contributes to tumor progression.

One of the most biologically relevant observations in this study was the strong association between PAX6 expression and HPV16 infection. HPV16-positive tumors exhibited substantially higher PAX6 expression than HPV16-negative tumors, and this relationship was independently supported by the clinical cohort. Persistent HPV infection induces extensive transcriptional and epigenetic remodelling through the actions of E6 and E7 oncoproteins[35–37]. Although the precise mechanisms regulating PAX6 activation in cervical cancer remain unknown, our findings suggest that PAX6 may represent part of the broader transcriptional reprogramming landscape associated with HPV-driven carcinogenesis. Future mechanistic studies should investigate whether HPV oncogenes directly or indirectly regulate PAX6 expression and whether PAX6 contributes to HPV-mediated cellular transformation.

Another important observation was the association between elevated PAX6 expression and relapse-related outcomes. Kaplan–Meier analyses demonstrated that increased PAX6 expression was associated with significantly shorter relapse-free survival in cervical cancer patients. This finding was consistently observed across independent survival datasets and suggests that PAX6 may identify tumors with a greater propensity for recurrence[38,39]. Interestingly, although relapse-related endpoints demonstrated significant associations, overall survival in our clinical cohort did not differ significantly according to PAX6 expression status. Several factors may explain this discrepancy. Relapse-free survival

and overall survival capture distinct clinical events, and recurrence often precedes mortality by several years[40]. Furthermore, differences in treatment regimens, sample size, follow-up duration, and patient heterogeneity may influence overall survival analyses[6,38]. Collectively, these observations suggest that PAX6 may be more closely linked to biological processes governing recurrence and disease progression than to short-term survival outcomes. However, these association between higher PAX6 expression and shorter RFS (HR = 2.32) was not directionally consistent with the association between higher expression and longer OS (HR = 0.62). These exploratory, unadjusted associations must be interpreted separately and do not establish independent prognostic value.

Functional enrichment analyses provide additional insight into the biological context of PAX6 activation. Gene Ontology and KEGG analyses consistently highlighted developmental differentiation pathways, transcriptional regulatory programs, and signaling pathways associated with stem cell pluripotency. Moreover, protein interaction network analyses identified strong associations between PAX6 and developmental regulators such as SOX2, SOX1, NEUROG2, and SIX3[12,41–43]. These findings support a model in which PAX6 participates in developmental transcriptional networks that may facilitate cellular plasticity and tumor adaptation[12]. Such biological programs have been increasingly recognized as important drivers of tumor heterogeneity, treatment resistance, and disease recurrence across multiple cancer types[44,45]. Transcriptomic analysis of primary cervical tumours identified modest but statistically significant positive correlations between PAX6 and SOX2 and between PAX6 and SIX3. These associations provide additional support for a shared developmental transcriptional context but do not demonstrate direct regulatory interactions. As SOX2 is a well-established master regulator of cellular plasticity and stemness[39], provides transcriptomic evidence that PAX6 expression is associated with selected developmental reprogramming programs in primary cervical tumours.

The peripheral-blood findings require separate interpretation from the tumour-tissue analyses. Although higher tumour PAX6 expression was associated with shorter relapse-free survival (RFS) in the analyzed public dataset, peripheral-blood PAX6 downregulation was more frequent among deceased patients in the clinical cohort. These observations are not directly contradictory because they represent different biological compartments and clinical endpoints. Blood-derived gene-expression profiles reflect both cellular transcriptional states and the relative composition of circulating leukocyte populations; therefore, peripheral-blood expression cannot be assumed to represent tumour-tissue expression directly[46,47]. Pre-analytical factors, including blood collection, processing and RNA preparation, may also influence blood-derived RNA measurements[48]. As matched tumour and blood expression data were not available to establish a direct relationship between the two compartments, these associations should not be combined into a single mechanistic or prognostic conclusion. Future studies should assess paired tumour and blood samples and evaluate these markers using appropriately adjusted clinical models.

From a translational perspective, our findings suggest that PAX6 may have utility as a biomarker for cervical cancer progression[49]. The consistent overexpression of PAX6 across independent cohorts,

together with its association with HPV infection and relapse-related outcomes, supports its potential value for risk stratification[7]. However, the absence of a significant association with immediate chemoradiotherapy response indicates that PAX6 should not currently be considered a predictive biomarker of treatment response. Instead, PAX6 may be more appropriately viewed as a biomarker reflecting disease biology and progression risk[49].

The identification of clinically relevant biomarkers remains a major challenge in cervical cancer management, particularly for distinguishing biologically aggressive tumors from those with more favorable outcomes. The present study demonstrates that PAX6 is consistently activated across independent cervical cancer cohorts and is associated with HPV16-positive disease and relapse-related clinical outcomes. These observations suggest that PAX6 may serve as a molecular indicator of tumor progression and disease aggressiveness.

The peripheral-blood findings provide complementary evidence of altered PAX6 expression in cervical cancer but do not establish concordance with tumour-tissue expression. Furthermore, the endpoint-specific survival associations and absence of a significant clinical-cohort association with overall survival limit conclusions regarding prognostic utility. Larger, independently recruited cohorts with paired tumour and blood samples are required to determine whether PAX6 provides clinically meaningful information beyond established clinicopathological variables.

Beyond its biomarker potential, the enrichment of developmental and stemness-associated pathways among PAX6-related networks suggests that PAX6 may represent a biologically informative node within cervical cancer progression programs. Further mechanistic studies are warranted to determine whether PAX6-associated pathways could be exploited for therapeutic intervention or incorporated into future molecular classification frameworks for cervical cancer.

The present findings warrant further investigation to define the functional significance of PAX6 in cervical carcinogenesis. Partial overlap among public transcriptomic datasets, differences between tumour-tissue and peripheral-blood expression profiles, and heterogeneity in clinical outcomes may influence the observed molecular associations. Functional characterization of PAX6-associated regulatory networks and prospective validation in independent clinical cohorts are needed to establish its biological significance and prognostic relevance in cervical cancer.

5. CONCLUSION

The present study identifies aberrant PAX6 expression in cervical cancer through integrated molecular profiling and clinical cohort evaluation. Increased PAX6 transcript expression was observed across TCGA-based analytical platforms, with supportive immunohistochemical evidence and elevated peripheral-blood expression in an independent clinical cohort. PAX6 expression was significantly associated with HPV16-positive tumour status and showed positive correlations with developmental regulators SOX2 and SIX3, suggesting its involvement in altered transcriptional programmes associated

with cervical carcinogenesis. Although survival analyses revealed endpoint-specific associations, PAX6 expression was not significantly associated with chemoradiotherapy response or overall survival in the clinical cohort. Collectively, these findings identify PAX6 as a candidate molecular marker in cervical cancer and provide a basis for further functional characterization and independent clinical validation.

Abbreviations

CaCx
Cervical cancer
CESC
Cervical squamous cell carcinoma and endocervical adenocarcinoma
CI
Confidence interval
CRT
Chemoradiotherapy
FDR
False discovery rate
GEPIA3
Gene Expression Profiling Interactive Analysis 3
GO
Gene Ontology
GTEx
Genotype-Tissue Expression
HPA
Human Protein Atlas
HPV
Human papillomavirus
HPV16
Human papillomavirus type 16
HR
Hazard ratio
IHC
Immunohistochemistry
KEGG
Kyoto Encyclopedia of Genes and Genomes
OR
Odds ratio
OS
Overall survival
qPCR

Quantitative polymerase chain reaction

RFS

Relapse-free survival

STRING

Search Tool for the Retrieval of Interacting Genes/Proteins

TCGA

The Cancer Genome Atlas

TCGA-CESC

The Cancer Genome Atlas cervical squamous cell carcinoma and endocervical adenocarcinoma cohort

UALCAN

University of Alabama at Birmingham Cancer Data Analysis Portal

Declarations

Ethics approval

The clinical component of this study was conducted within a larger cervical cancer research programme approved by the Institutional Ethics Committee of King George's Medical University, Lucknow, Uttar Pradesh, India (Approval No. 256/Ethics/R.Cell-18; dated 24 September 2018). The present investigation included 103 cervical cancer patients and 108 healthy controls selected from the parent study population according to predefined inclusion and exclusion criteria.

Consent to participate

Written informed consent was obtained from all participants enrolled in the parent research programme before biological sample collection and clinical data acquisition. The present analysis included 103 cervical cancer patients and 108 healthy controls who fulfilled the study eligibility criteria.

Consent for publication

Not applicable. No identifiable individual participant information is presented in this manuscript.

Clinical trial registration

Clinical trial number: not applicable.

Author contributions

MS: Data curation; Formal analysis; Visualization; Writing – original draft; Methodology. **TSN**: Formal analysis; Visualization; Writing – original draft. **VT**: Formal Analysis, Validation, Visualization. **PV**: Formal analysis; Validation; Visualization. **KS**: Investigation; Resources; Writing – review & editing. **RM**: Writing – review & editing; Investigation; Validation. **MB**: Conceptualization; Supervision; Writing – review & editing; Validation; Resources **VP**: Validation; Writing – review & editing. **ASK**: Conceptualization; Funding

acquisition; Methodology; Resources; Software; Supervision; Writing – original draft; Writing – review & editing; Project administration.

Data and Code Availability Statement

The publicly available molecular and clinical outcome datasets analysed in this study were accessed through TCGA-CESC and the GEPIA3, UALCAN, OncoDB, cBioPortal, KM-Plotter, STRING, Enrichr, and Human Protein Atlas platforms. TCGA-CESC RNA-sequencing data are accessible through the National Cancer Institute Genomic Data Commons (https://portal.gdc.cancer.gov/). Individual-level quantitative real-time PCR and clinical data from the King George's Medical University cohort are not publicly available due to institutional ethical and patient-confidentiality considerations but may be obtained from the corresponding author upon reasonable request, subject to applicable institutional approvals. The R scripts used for TCGA-CESC transcriptomic correlation analysis, multiple-testing correction, and figure generation are available at https://github.com/atarskushwah/PAX6-CervicalCancer-MultiOmics.

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References

1. Global burden of cancer attributable to infections in 2024: One in eight cancers worldwide linked to infections. Accessed October 3, 2026. https://www.iarc.who.int/cancer-type/cervical-cancer
2. Cervical cancer – IARC. Accessed October 3, 2026. https://www.iarc.who.int/cancer-type/cervical-cancer/
3. Vallejo-Ruiz V, Gutiérrez-Xicotencatl L, Medina-Contreras O, Lizano M. Molecular aspects of cervical cancer: a pathogenesis update. *Front Oncol*. 2024;14. doi:10.3389/fonc.2024.1356581
4. Massobrio R, Bianco L, Campigotto B, et al. New Frontiers in Locally Advanced Cervical Cancer Treatment. *JCM*. 2024;13(15):4458. doi:10.3390/jcm13154458
5. Kushwah A, Gupta M, Singh R, Banerjee M. Cytokine gene variants and treatment outcome of cisplatin-based concomitant chemoradiotherapy in cervical cancer. *British Journal of Biomedical*

Science. 2020;77(2):81-86. doi:10.1080/09674845.2020.1714164

6. Cibula D, Raspollini MR, Planchamp F, et al. ESGO/ESTRO/ESP Guidelines for the management of patients with cervical cancer – Update 2023*. *Int J Gynecol Cancer*. 2023;33(5):649-666. doi:10.1136/ijgc-2023-004429
7. Lizano M, Carrillo-García A, De La Cruz-Hernández E, Castro-Muñoz LJ, Contreras-Paredes A. Promising predictive molecular biomarkers for cervical cancer (Review). *Int J Mol Med*. 2024;53(6):50. doi:10.3892/ijmm.2024.5374
8. The Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer. *Nature*. 2017;543(7645):378-384. doi:10.1038/nature21386
9. Fazilaty H, Basler K. Reactivation of embryonic genetic programs in tissue regeneration and disease. *Nat Genet*. 2023;55(11):1792-1806. doi:10.1038/s41588-023-01526-4
10. Mehta A, Stanger BZ. Lineage Plasticity: The New Cancer Hallmark on the Block. *Cancer Res*. 2024;84(2):184-191. doi:10.1158/0008-5472.CAN-23-1067
11. Zhang Z, Wu Y, Fu J, et al. Proteostatic reactivation of the developmental transcription factor TBX3 drives BRAF/MAPK-mediated tumorigenesis. *Nat Commun*. 2024;15(1):4108. doi:10.1038/s41467-024-48173-9
12. Shaw T, Barr FG, Üren A. The PAX Genes: Roles in Development, Cancer, and Other Diseases. *Cancers (Basel)*. 2024;16(5):1022. doi:10.3390/cancers16051022
13. Giacobbi E, Scioli MP, Servadei F, et al. PAX Family, Master Regulator in Cancer. *Diagnostics*. 2025;15(11):1420. doi:10.3390/diagnostics15111420
14. Wu D ming, Zhang T, Liu Y bin, et al. The PAX6-ZEB2 axis promotes metastasis and cisplatin resistance in non-small cell lung cancer through PI3K/AKT signaling. *Cell Death Dis*. 2019;10(5):349. doi:10.1038/s41419-019-1591-4
15. Mascarenhas JB, Young KP, Littlejohn EL, Yoo BK, Salgia R, Lang D. PAX6 Is Expressed in Pancreatic Cancer and Actively Participates in Cancer Progression through Activation of the MET Tyrosine Kinase Receptor Gene. *J Biol Chem*. 2009;284(40):27524-27532. doi:10.1074/jbc.M109.047209
16. Li Y, Li Y, Liu Y, Xie P, Li F, Li G. PAX6, a novel target of microRNA-7, promotes cellular proliferation and invasion in human colorectal cancer cells. *Dig Dis Sci*. 2014;59(3):598-606. doi:10.1007/s10620-013-2929-x
17. Mayes DA, Hu Y, Teng Y, et al. PAX6 Suppresses the Invasiveness of Glioblastoma Cells and the Expression of the Matrix Metalloproteinase-2 Gene. *Cancer Res*. 2006;66(20):9809-9817. doi:10.1158/0008-5472.CAN-05-3877
18. Boehm KM, Khosravi P, Vanguri R, Gao J, Shah SP. Harnessing multimodal data integration to advance precision oncology. *Nat Rev Cancer*. 2022;22(2):114-126. doi:10.1038/s41568-021-00408-3
19. Acharya D, Mukhopadhyay A. A comprehensive review of machine learning techniques for multi-omics data integration: challenges and applications in precision oncology. *Brief Funct Genomics*. 2024;23(5):549-560. doi:10.1093/bfgp/elae013

20. Hernández-Lemus E, Ochoa S. Methods for multi-omic data integration in cancer research. *Front Genet*. 2024;15. doi:10.3389/fgene.2024.1425456
21. Liu F, Beck S, Yang L, Luo H, Zhang K. Advancing AI for multi-omics and clinical data integration in basic and translational cancer research. *Nat Rev Cancer*. 2026;26(7):497-512. doi:10.1038/s41568-026-00922-2
22. GEPIA3. February 22, 2026. Accessed October 16, 2026. https://bio.tools/gepia3
23. UALCAN. Accessed October 16, 2026. https://ualcan.path.uab.edu/
24. Cho M, Tang G, Rogers CS, et al. OncoDB 2.0: a comprehensive platform for integrated pan-cancer omics analysis. *Nucleic Acids Research*. 2026;54(D1):D1537-D1544. doi:10.1093/nar/gkaf952
25. The Human Protein Atlas. Accessed October 16, 2026. https://www.proteinatlas.org/
26. Balázs G. KM-plot. Kaplan-Meier plotter. Accessed October 16, 2026. http://www.kmplot.com/analysis
27. cBioPortal for Cancer Genomics. Accessed October 16, 2026. https://www.cbioportal.org/
28. Getting started - STRING Help. Accessed October 16, 2026. https://string-db.org/help/getting_started/
29. Kushwah AS, Srivastava K, Banerjee M. Differential expression of DNA repair genes and treatment outcome of chemoradiotherapy (CRT) in cervical cancer. *Gene*. 2023;868:147389. doi:10.1016/j.gene.2023.147389
30. What's new in Prism 5.01? - FAQ 1242 - GraphPad. Accessed October 17, 2026. https://www.graphpad.com/support/faq/whats-new-in-prism-501/
31. IBM SPSS Statistics 21 Documentation. August 14, 2012. Accessed October 17, 2026. https://www.ibm.com/support/pages/ibm-spss-statistics-21-documentation
32. Colaprico A, Silva TC, Olsen C, et al. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res*. 2016;44(8):e71. doi:10.1093/nar/gkv1507
33. R: The R Project for Statistical Computing. Accessed October 17, 2026. https://www.r-project.org/
34. Wickham H. Getting Started with ggplot2. In: Wickham H, ed. *Ggplot2: Elegant Graphics for Data Analysis*. Springer International Publishing; 2016:11-31. doi:10.1007/978-3-319-24277-4_2
35. Liu H, Ma H, Li Y, Zhao H. Advances in epigenetic modifications and cervical cancer research. *Biochim Biophys Acta Rev Cancer*. 2023;1878(3):188894. doi:10.1016/j.bbcan.2023.188894
36. Sisodiya S, Singh P, Joshi T, et al. Human papillomavirus-mediated cervical cancer: epigenetic interplay and clinical implications. *Front Microbiol*. 2025;16. doi:10.3389/fmicb.2025.1633283
37. Kushwah AS, Masood S, Mishra R, Banerjee M. Genetic and epigenetic alterations in DNA repair genes and treatment outcome of chemoradiotherapy in cervical cancer. *Crit Rev Oncol Hematol*. 2024;194:104240. doi:10.1016/j.critrevonc.2023.104240
38. Gao C, Zhou Q, Liu L, et al. Stratification by Mutational Landscape Reveals Differential Immune Infiltration and Predicts the Recurrence and Clinical Outcome of Cervical Cancer. *Phenomics*. 2025;5(4):384-403. doi:10.1007/s43657-024-00158-w

39. Zhang Y, Zou J, Li L, Han M, Dong J, Wang X. Comprehensive assessment of postoperative recurrence and survival in patients with cervical cancer. *Eur J Surg Oncol*. 2024;50(10):108583. doi:10.1016/j.ejso.2024.108583
40. Mayadev J, Limón JCV, Godinez FJR, et al. Ultrasensitive detection and tracking of circulating tumor DNA to predict relapse and survival in patients with locally advanced cervical cancer: phase III CALLA trial analyses. *Annals of Oncology*. 2025;36(9):1047-1057. doi:10.1016/j.annonc.2025.05.533
41. Kamachi Y, Uchikawa M, Tanouchi A, Sekido R, Kondoh H. Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev*. 2001;15(10):1272-1286. doi:10.1101/gad.887101
42. Sansom SN, Griffiths DS, Faedo A, et al. The Level of the Transcription Factor Pax6 Is Essential for Controlling the Balance between Neural Stem Cell Self-Renewal and Neurogenesis. *PLoS Genet*. 2009;5(6):e1000511. doi:10.1371/journal.pgen.1000511
43. Xu Y, Xi J, Wang G, et al. PAUPAR and PAX6 sequentially regulate human embryonic stem cell cortical differentiation. *Nucleic Acids Res*. 2021;49(4):1935-1950. doi:10.1093/nar/gkab030
44. Bhat GR, Sethi I, Sadida HQ, et al. Cancer cell plasticity: from cellular, molecular, and genetic mechanisms to tumor heterogeneity and drug resistance. *Cancer Metastasis Rev*. 2024;43(1):197-228. doi:10.1007/s10555-024-10172-z
45. Srivastava M, Singh S, Kumar P, et al. From Epigenetic Dysregulation to Therapeutic Reprogramming in Endometrial Cancer: A State–Response Framework for Treatment Resistance. *Int J Mol Sci*. 2026;27(10):4371. doi:10.3390/ijms27104371
46. Chaussabel D, Pascual V, Banchereau J. Assessing the human immune system through blood transcriptomics. *BMC Biol*. 2010;8:84. doi:10.1186/1741-7007-8-84
47. O'Connell GC, Wang J, Smothers C. Donor white blood cell differential is the single largest determinant of whole blood gene expression patterns. *Genomics*. 2023;115(6):110708. doi:10.1016/j.ygeno.2023.110708
48. Van Der Schueren C, Decruyenaere P, Avila Cobos F, et al. Subpar reporting of pre-analytical variables in RNA-focused blood plasma studies. *Mol Oncol*. 2024;19(7):1968-1978. doi:10.1002/1878-0261.13647
49. Kushwah AS, Gupta MK, Srivastava K, Banerjee M. Cytokine Gene Variants as Risk Predictors and Prognostic Biomarkers for Cervix Cancer: A Revisit. *Current Cancer Therapy Reviews*. 2025;22(3):234-244. doi:10.2174/0115733947351277250217065714

Figures

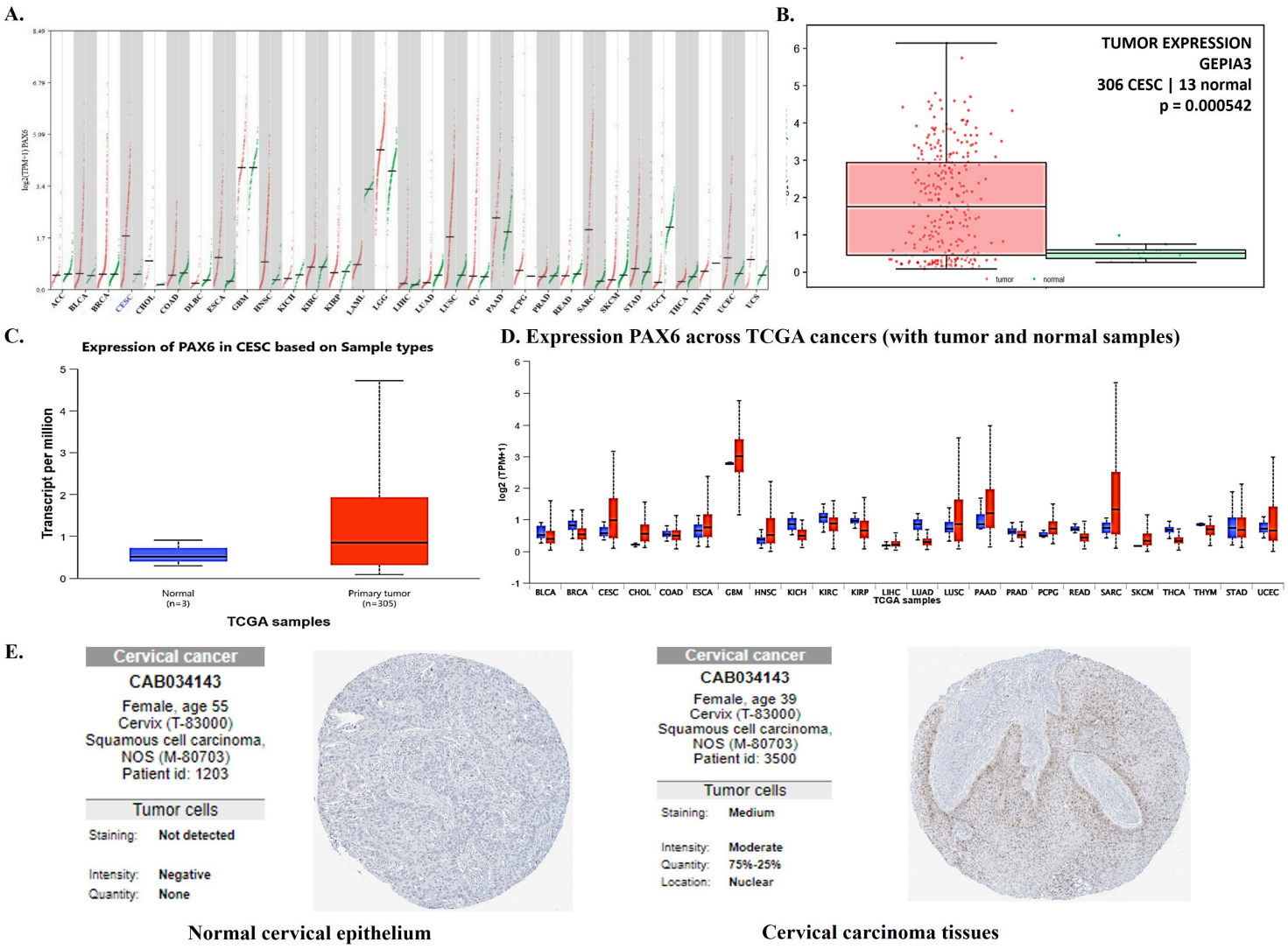


Figure 1

PAX6 is consistently overexpressed in cervical cancer across independent transcriptomic and proteomic datasets. (A) Pan-cancer differential expression analysis of PAX6 using the GEPIA3 platform. PAX6 expression levels were compared between tumor and corresponding normal tissues across TCGA and GTEx datasets. Significant overexpression was observed in multiple cancer types, including Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (CESC). (B) Differential expression of PAX6 in CESC determined using GEPIA3. PAX6 expression was significantly elevated in cervical cancer tissues (n = 306) compared with normal cervical tissues (n = 13). (C) Independent validation of PAX6 overexpression using the UALCAN TCGA-CESC cohort (primary tumors, n = 305; normal tissues, n = 3). (D) Confirmation of elevated PAX6 expression using the OncoDB platform (CESC, n = 304; normal tissues, n = 22). (E) Representative immunohistochemistry images from The Human Protein Atlas showing increased PAX6 protein expression in cervical carcinoma tissues compared with normal cervical epithelium.

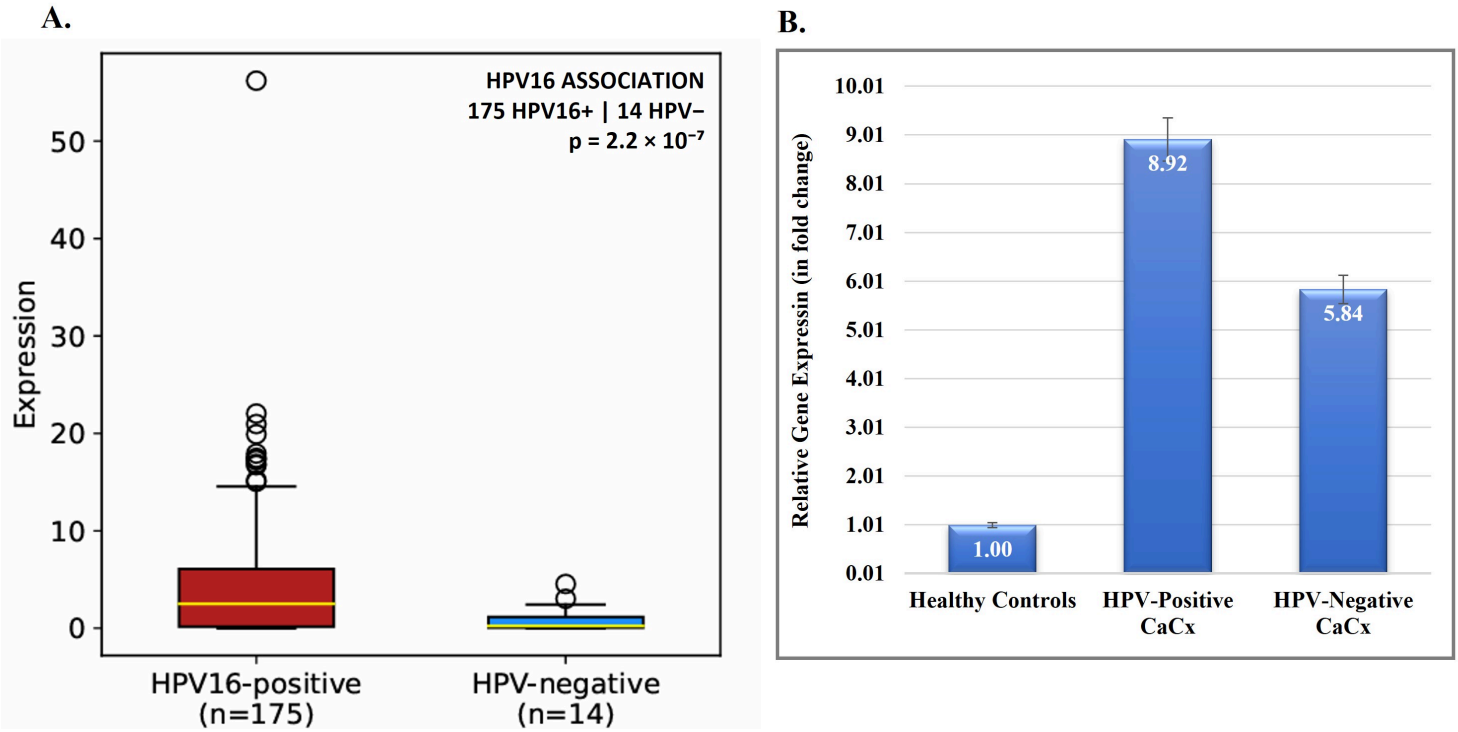


Figure 2

Association between PAX6 expression and HPV16 infection in cervical cancer.

(A) Differential expression of PAX6 between HPV16-positive (n = 175) and HPV16-negative (n = 14) cervical cancer samples analyzed using the OncoDB platform. PAX6 expression was significantly higher in HPV16-positive tumors ($p = 2.2 \times 10^{-7}$). (B) Relative PAX6 expression in healthy controls, HPV-negative cervical cancer patients, and HPV-positive cervical cancer patients from the clinical validation cohort. PAX6 expression was elevated in both cervical cancer groups compared with controls, with the highest expression observed in HPV-positive tumors. Data are presented as $\log_2(TPM+1)$ for public datasets and relative fold change for the clinical cohort. Statistical significance was determined using platform-specific analyses or appropriate comparative statistical tests.

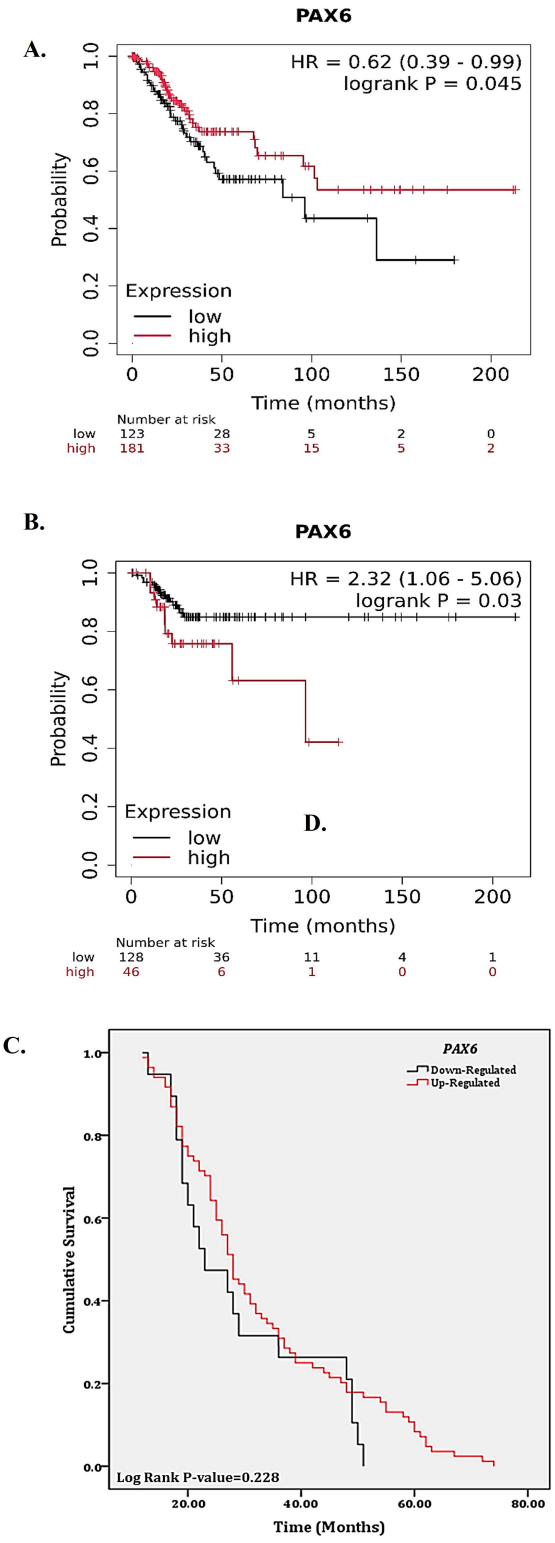


Figure 3

Association of PAX6 expression with survival and treatment outcomes in cervical cancer.

(A) Kaplan–Meier overall survival (OS) analysis according to PAX6 expression in the public dataset. (B) Kaplan–Meier relapse-free survival (RFS) analysis according to PAX6 expression in the public dataset. (C) Kaplan–Meier OS analysis according to PAX6 expression in the clinical cohort. Higher PAX6

expression was associated with longer OS but shorter RFS in the public dataset, whereas the clinical-cohort OS analysis did not show a statistically significant difference between expression groups.

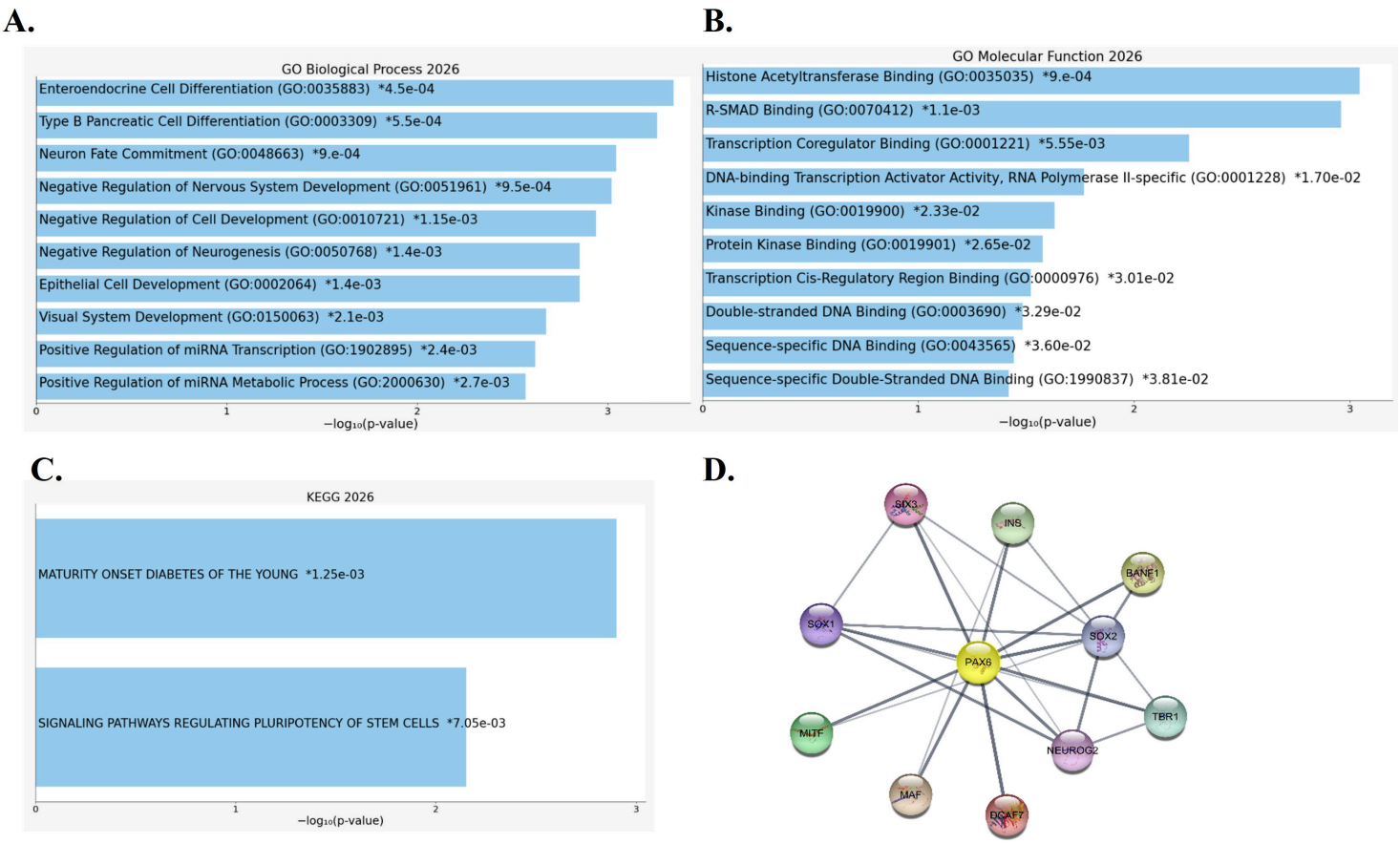


Figure 4

Functional enrichment and molecular association analyses of PAX6-associated genes. (A) Gene Ontology Biological Process enrichment showing significant representation of developmental and differentiation-related biological processes. (B) Gene Ontology Molecular Function enrichment showing transcriptional regulatory, protein-binding, and DNA-binding functions. (C) KEGG pathway enrichment highlighting developmental and stem-cell pluripotency-related signaling programs. (D) STRING-derived molecular association network linking PAX6 with developmental regulators including SOX2, SOX1, NEUROG2, SIX3, TBR1, MITF, MAF, INS, BANF1, and DCAF7. These analyses identify predicted associations between PAX6 and developmental regulatory programs. Enrichment and network results are hypothesis-generating and do not independently establish direct PAX6 regulation or functional stemness activation.

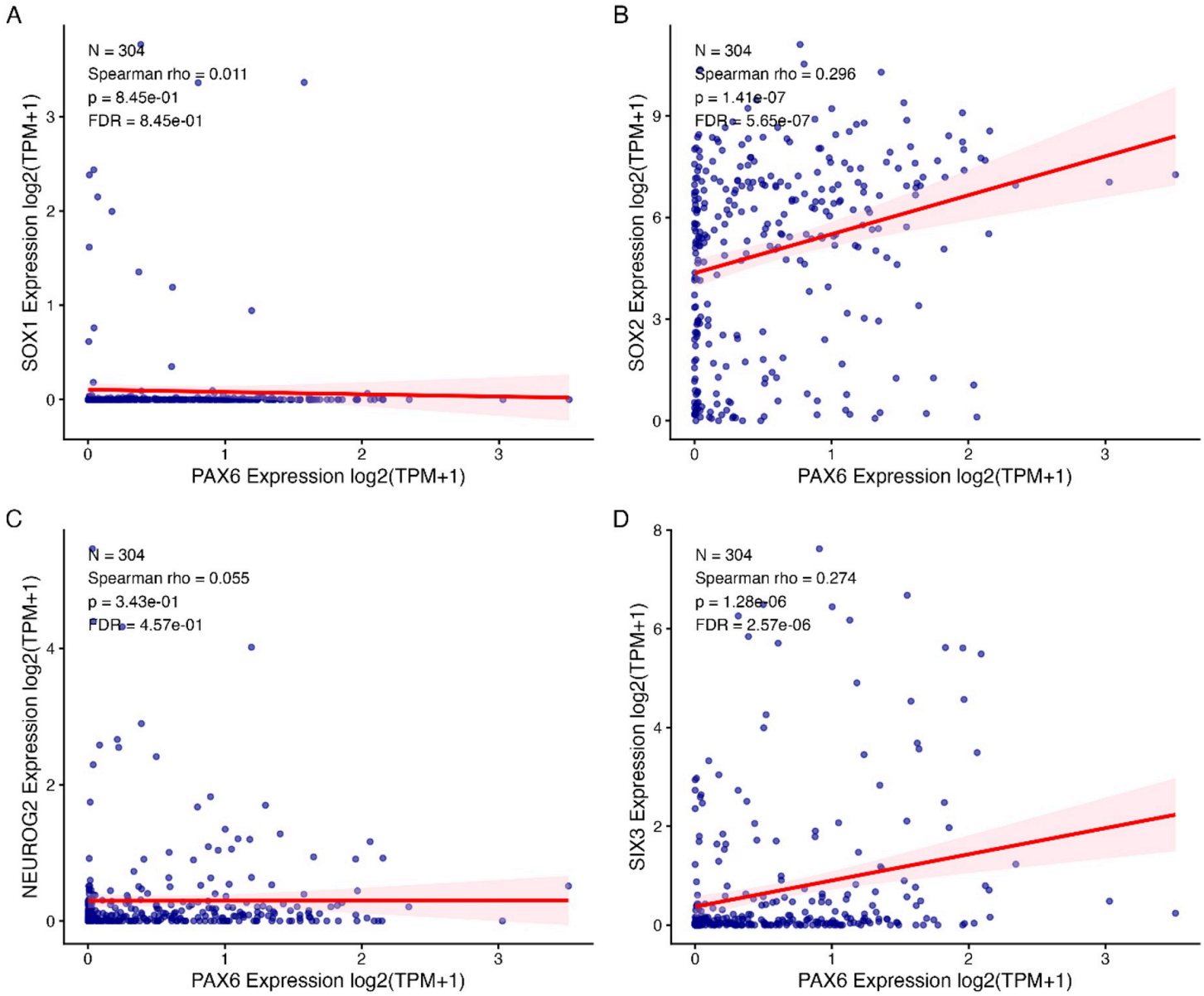


Figure 5

Transcriptomic co-expression of PAX6 with selected developmental regulators in primary cervical tumours. Scatter plots showing expression relationships between PAX6 and (A) SOX1, (B) SOX2, (C) NEUROG2, and (D) SIX3 in 304 primary tumour samples from the TCGA-CESC cohort. Expression values are shown as $\log_2(\text{TPM} + 1)$. The fitted line and shaded region represent the linear regression fit and 95% confidence interval, respectively. Spearman correlation coefficients, raw *p*-values, and Benjamini–Hochberg FDR-adjusted *p*-values are shown for each comparison. Significant positive correlations were observed for PAX6–SOX2 and PAX6–SIX3, whereas PAX6–SOX1 and PAX6–NEUROG2 were not significantly correlated.

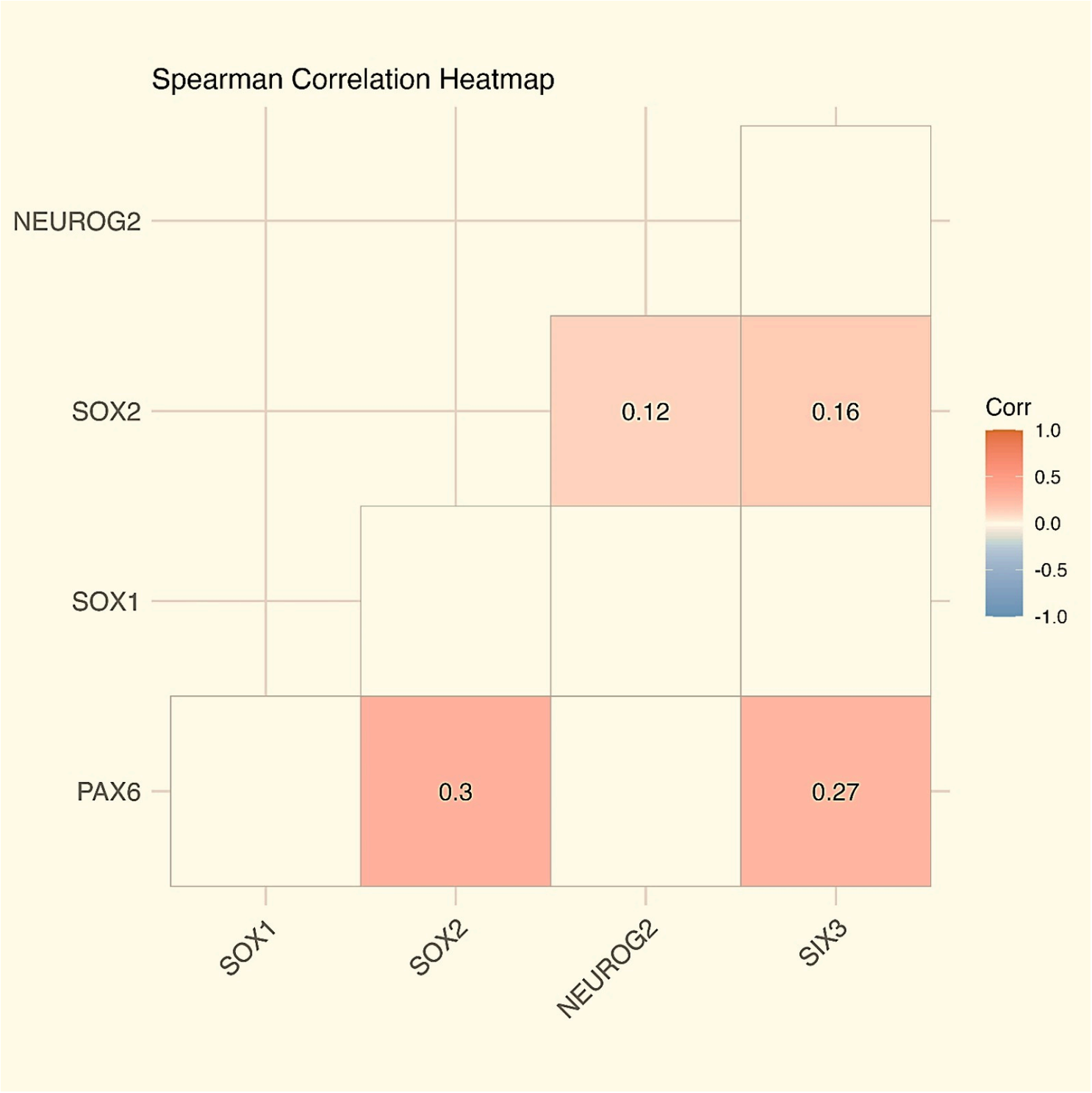


Figure 6

Co-expression matrix of PAX6 and selected developmental regulators in primary cervical tumours. Spearman rank correlation matrix showing pairwise expression relationships among PAX6, SOX1, SOX2, NEUROG2, and SIX3 in 304 TCGA-CESC primary tumours. Numerical values represent Spearman correlation coefficients (ρ). Blank cells indicate associations that did not meet the prespecified FDR-adjusted significance threshold ($p < 0.05$) and do not indicate zero correlation or missing expression data.