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Psychosocial Distress Predicts Persistent HPV Infection after Conization: A Machine Learning Model and Immune Profiling Study

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Abstract

Objectives

To investigate the association between negative emotional states and the persistence of human papillomavirus (HPV) infection following cervical conization for high-grade squamous intraepithelial lesions (HSIL), and to explore potential underlying immune mechanisms.

Methods

We conducted a prospective follow-up study of 333 patients who underwent cervical conization for HSIL at the First Affiliated Hospital of Harbin Medical University between July 2019 and December 2022, with a minimum follow-up of one year. Psychological distress and sleep quality were assessed using the Depression Anxiety Stress Scales-21 (DASS-21) and the Self-Rating Scale of Sleep (SRSS). Patients were categorized based on postoperative HPV persistence or clearance. Feature selection was performed using LASSO regression, and a machine learning-based predictive model was developed. Single-cell RNA sequencing data from the GEO dataset (GSE208653) were analyzed to characterize the immune microenvironment and cell-cell communication.

Results

Among the 333 patients, 68 (20.4%) exhibited persistent HPV infection, while 265 (79.6%) achieved clearance. The persistence group demonstrated significantly higher scores for stress, anxiety, depression, and poorer sleep quality (all $P < 0.05$). LASSO regression identified the absence of anxiety, mild stress levels, and good sleep quality as key protective factors against persistence. A Random Forest model constructed with these features accurately predicted HPV clearance (AUC = 0.93). Single-cell transcriptomic analysis revealed that HSIL progression involves significant T-cell remodeling and enhanced MHC-I/MIF signaling, indicating a concurrent state of immune activation and evasion within the lesion microenvironment.

Conclusion

Poor sleep quality and negative emotional states are associated with an increased risk of persistent HPV infection after conization, whereas psychological well-being and good sleep promote viral clearance. These factors may aid in risk stratification for persistent infection. At the mechanistic level, an MIF/MHC-I signaling-driven immune imbalance in the HSIL microenvironment provides novel insights into HPV immune escape, linking psychosocial factors to local immunobiology.

1. Introduction

Cervical cancer is a major global health issue, ranking among the most prevalent malignancies affecting women. According to the 2022 global cancer statistics from the International Agency for Research on Cancer (IARC), cervical cancer accounted for 6.8% of new cancer diagnoses and 8.1% of cancer-related deaths among women. It is the fourth most commonly diagnosed cancer worldwide. The primary cause of cervical cancer is persistent infection with high-risk persistence of human papillomavirus (HPV) types, particularly HPV-16 and HPV-18. This persistent infection drives cervical carcinogenesis and is a key target for prevention and management within the female reproductive tract [1].The primary cause of cervical cancer is persistent infection with high-risk HPV types, particularly HPV-16 and HPV-18 [2]. This persistent infection drives cervical carcinogenesis and is a key target for prevention and management within the female reproductive tract [3].

Persistent HPV infection following cervical conization is marked by the presence of HPV before the procedure and at the first follow-up visit, usually conducted about six months after surgery [4–6]. Persistent infection is a significant precursor to cervical squamous intraepithelial lesions (SIL), which are classified as low-grade (LSIL) or high-grade (HSIL). Studies show that individuals diagnosed with LSIL have a relatively high chance of HPV clearance through lifestyle changes and medical interventions [7, 8]. In contrast, those with HSIL are at a higher risk of persistent infection and progression to more severe lesions, which could lead to invasive cancer if not managed effectively [9]. The persistence of HPV infection is crucial in cervical cancer development. It is estimated that 70% of women clear the virus within two years of initial infection, but those with persistent infections are at a higher risk of developing precancerous lesions [10].

Recent research suggests that persistent HPV infection is linked to various risk factors, including age, immunosuppression, HPV genotype, co-infections, high-risk sexual behaviors, and smoking [11–17]. Studies also show that depression can impair antiviral immunity through the AVP–AHI1–Tyk2 axis, increasing susceptibility to viral infections in people with depressive disorders [18]. Moreover, individuals living with HIV are more susceptible to HPV and face an increased risk of progression to cervical cancer [19]. Evidence also indicates a link between sleep quality and HPV clearance, suggesting that better sleep may aid viral clearance [20].

Investigating the risk factors for persistent HPV infection is critical for cervical cancer prevention. However, the role of psychological factors—such as depression, stress, and anxiety—along with immune status in the persistence of HPV infection following cervical conization in patients diagnosed with HSIL is not well understood.

This study aims to fill this gap by conducting a longitudinal analysis of patients with cervical HSIL post-conization. Using cell-cell communication analysis and genomic data from HPV-infected samples in the Gene Expression Omnibus (GEO) database, we will examine the correlation between persistent HPV infection and factors like sleep quality, depression, stress, anxiety, and immune response. We have also developed a predictive model to assess the risk of persistent HPV infection following cervical conization.

This study addresses three major research gaps: (1) the lack of prospective studies on psychological factors in post-conization patients; (2) the absence of research connecting psychological risks with local immune microenvironment mechanisms; and (3) the lack of clinical prediction tools for risk stratification. Therefore, we conducted a prospective study integrating psychosocial assessments, machine learning, and single-cell transcriptomics to: (1) determine if psychosocial distress predicts postoperative HPV persistence; (2) develop a clinical prediction model; and (3) elucidate potential immune mechanisms underlying this association.

2.Materials and methods

2.1 Data Sources

Data were obtained from patients who underwent cervical conization for cervical HSIL at the First Affiliated Hospital of Harbin Medical University between July 2019 and December 2022. All participants provided informed consent prior to enrollment in the study. The study protocol received approval from the Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Ethics Approval No.: 2024257). Comprehensive information regarding the study's purpose, procedures, and pertinent details was communicated to all participants to ensure their thorough understanding before inclusion.

Inclusion Criteria: (1) Patients with a preoperative diagnosis of high-risk HPV infection and histopathologically confirmed HSIL through colposcopy-guided biopsy; (2) Patients who underwent either cold knife conization (CKC) or loop electrosurgical excision procedure (LEEP) at our institution; (3) Patients who completed postoperative HPV follow-up assessments for a minimum duration of one year. Exclusion Criteria: (1) Lack of essential clinical data; (2) Non-compliance with scheduled follow-up visits or loss to follow-up; (3) Undergoing subsequent total hysterectomy; (4) Presence of comorbid systemic diseases or other malignant tumors; (5) History of psychiatric or neurological disorders; (6) Unwillingness or inability to participate in questionnaire-based surveys.

Gene expression profiles pertinent to the progression of cervical intraepithelial neoplasia (CIN) were obtained from the GEO database, hosted by the National Center for Biotechnology Information (NCBI). The single-cell RNA sequencing (scRNA-seq) dataset was accessed using accession number GSE20865342, which utilizes the high-throughput sequencing platform GPL24767. This dataset encompasses genomic data from normal cervical tissues infected with HPV (GSM6360682, GSM6360683) and HPV-infected HSIL cervical tissues (GSM6360684, GSM6360685), all of which were downloaded for further analysis.

2.2 Methods

2.2.1 Questionnaire Survey

Demographic and clinical characteristics of the patients were systematically documented, including variables such as age, number of vaginal deliveries, menopausal status, presence of diabetes and

hypertension, smoking history, occupation, education level, and peripheral blood lymphocyte and leukocyte counts. Additionally, data on multiple infections, HPV subtype infections (16/18/52, as well as other high-risk and low-risk HPV subtypes), surgical approach, intraoperative bleeding, and the diameter and depth of resected tissue were collected. Information regarding the use of vaginal packing, hemostatic materials, and petrolatum gauze, as well as pathological grade and margin positivity, was also recorded. All patients were followed for more than one year, during which factors related to sleep, stress, anxiety, and depression were evaluated using the Self-Rating Scale of Sleep (SRSS) and the Depression Anxiety Stress Scales-21 (DASS-21). Postoperative HPV status was assessed at 6-month and 12-month intervals.

The Self-Rating Scale of Sleep (SRSS), developed by Jianming Li, was used to assess sleep quality over the preceding month[21]. The SRSS includes ten items, each rated on a 5-point scale from 1 (no disturbance) to 5 (severe disturbance), with a higher total score indicating more severe sleep disturbances. The possible score range is from 10 (no sleep disorders) to 50 (severe insomnia). Previous studies have demonstrated that the SRSS shows high reliability and validity in Chinese populations, with a reported Cronbach's α of 0.893, indicating strong internal consistency in assessing sleep disturbances in the target population .

The Depression Anxiety Stress Scales-21 (DASS-21)[22] is a shortened version of the original DASS[23], designed to measure levels of depression, anxiety, and stress using three subscales. Each subscale contains seven items, with total scores ranging from 0 to 42. Standard interpretation guidelines suggest that scores exceeding 14 on the stress subscale indicate significant stress, scores above 7 on the anxiety subscale suggest notable anxiety, and scores greater than 9 on the depression subscale reflect clinically relevant depressive symptoms. The DASS-21 has been widely validated in Chinese populations, In this study, the Cronbach's alpha coefficients for the three subscales of the DASS-21 were 0.887, 0.870, and 0.880,respectively.Confirming its reliability and validity in assessing psychological distress in similar cohorts .

2.2.2 Data Processing and Analysis

Patients were stratified into two cohorts based on their postoperative HPV test outcomes: the persistent HPV infection cohort and the HPV clearance cohort. A univariate analysis was conducted to explore the associations between postoperative HPV infection status and a variety of clinical characteristics, psychological distress, and sleep quality. Differences in the levels and distributions of clinical features between the two cohorts were analyzed.

Variable selection was performed using the Least Absolute Shrinkage and Selection Operator (LASSO) regression technique. The R package glmnet was used for LASSO regression, with 10-fold cross-validation to determine the optimal regularization parameter (λ). To address class imbalance within the clinical dataset, an undersampling method was employed, where the majority class was randomly downsampled to match the number of instances in the minority class. This undersampling was carried

out within the training set only to avoid data leakage and ensure that the testing set remained untouched.

Two machine learning algorithms, Random Forest (RF) and Support Vector Machine (SVM), were used to develop predictive models. The R package randomForest was used for the RF model, with parameters set as follows: 500 trees (ntree) and a minimum node size of 5 (nodesize). The dataset was split into a training set (80%) for model development and a testing set (20%) for internal validation. The performance of each model was assessed on the testing set using accuracy, precision, recall, F1-score, and the area under the receiver operating characteristic curve (AUC) as key metrics to evaluate predictive accuracy.

Model validation was conducted through held-out validation, where the testing set, which was never used in training, served to assess model performance. In addition to the primary evaluation metrics, calibration curves were plotted to assess the model's probability calibration. Decision Curve Analysis (DCA) was also performed to evaluate the clinical utility of the models by considering the trade-off between benefit and harm at various threshold probabilities.

For single-cell analysis, we used CellChat to investigate intercellular communication. This approach identifies ligands and receptors that are overexpressed within specific cell populations and deduces cell-state-specific interactions. Data preprocessing steps included quality control, normalization, and dimensionality reduction using the R packages Seurat and scater. Cells were filtered based on criteria such as mitochondrial gene content and the number of genes detected, ensuring that only high-quality cells were included in downstream analysis. Cell type annotation was performed using a predefined list of marker genes from published references for human cervical tissues.

For CellChat analysis, the version 1.1.0 of the database was used, with a significance threshold of $p < 0.05$. Ligand-receptor interactions were explored to elucidate communication patterns within the cervical microenvironment [22].

2.2.3 Statistical Analysis

Statistical analysis of the clinically collected data was executed using SPSS (version 30.0) software. Machine learning algorithms and cell-cell communication analyses were performed using R language (version 4.4.1). Measurement data are presented as mean ± standard deviation ($x \pm s$), while enumeration data are described using case numbers. For categorical data, the chi-square test was applied. For continuous variables, normality was assessed using the Shapiro-Wilk test. If the data were normally distributed, the t-test was employed. For non-normally distributed measurement data, the paired-sample Wilcoxon signed-rank test was utilized for univariate analysis. A p-value < 0.05 was considered statistically significant. The technical roadmap of this study is depicted in Fig. 1.

3. Results

This study encompassed a cohort of 333 patients who underwent cervical conization. Of these, 68 patients were classified into the persistent HPV infection group, while 265 patients achieved HPV clearance and were categorized into the HPV clearance group.

3.1 Univariate Analysis of HPV Infection Status Post-Cervical Conization

3.1.1 Univariate Analysis of Clinical Characteristics

Patients with persistent HPV infection had significantly higher rates of petrolatum gauze use (25.00% vs. 12.08%, p = 0.007) and positive surgical margins (8.82% vs. 3.02%, p = 0.033) compared to the HPV clearance group (Table 1, Fig. 2a). No other clinical characteristics differed significantly (Supplementary Table S1, S2).

3.1.2 Univariate Analysis of Negative Psychosocial States

The proportion of patients with stress, anxiety, and depression was significantly higher in the persistent HPV infection group (51.47% vs. 7.55%, p<0.001) compared to the HPV clearance group (Table 2, Fig. 2b).

3.1.3 Univariate Analysis of Sleep Status

Persistent HPV infection was associated with significantly higher SRSS scores (21.5 ± 5.553 vs. 17 ± 5.307, p<0.001) compared to the clearance group (Supplementary Table S3, Fig. 2c).

3.2 LASSO Analysis of HPV Infection in Patients

In this study, LASSO regression analysis was employed to identify the factors influencing HPV persistence in patients post-cervical conization, with HPV persistence as the dependent variable. A total of 46 potential influencing factors were considered, and the optimal penalty coefficient (λ) was determined through ten-fold cross-validation. As λ was adjusted, the coefficients of the independent variables were progressively compressed, leading to the identification of three significant predictors: SRSS sleep score, mild stress tendency (score 15–18), and absence of anxiety tendency (score 0–7) (Fig. 3a). These predictors were ranked according to their strength of association with HPV persistence, with the absence of anxiety tendency (score 0–7) being the most protective factor, followed by mild stress tendency (score 15–18) and SRSS sleep score (Fig. 3b).

3.3 Development and Validation of a Predictive Model for HPV Infection Risk in Patients

To develop a predictive model for HPV persistence, feature selection was performed using SVM, which achieved the lowest error rate with 32 selected feature variables (Fig. 4a). The model demonstrated excellent stability, with no evidence of overfitting or underfitting. In the validation set, the SVM model

was evaluated using a confusion matrix and Receiver Operating Characteristic (ROC) curve, resulting in an AUC of 0.65 (Fig. 4b, Fig. 4c).

Subsequently, the RF model was employed to explore the relationships between persistent HPV positivity and various clinical features. Risk factors identified included mild-to-severe anxiety, mild-to-moderate depression and stress, use of petrolatum gauze, vaginal packing, and HSIL with glandular involvement. Protective factors included the absence of anxiety, stress, and depression tendencies, HPV18 infection, elevated lymphocyte count, and HSIL without glandular involvement (Fig. 4d).

Using the mean decrease in Gini impurity, the top 10 clinical features influencing persistent HPV positivity were selected and used to develop the RF model (Fig. 4g). This model also exhibited excellent stability with no overfitting or underfitting observed. Evaluation of the RF model in the validation set resulted in an AUC of 0.93, with corresponding metrics of 96% accuracy, 92% precision, 100% recall, and 0.96 F1-score (Fig. 4e, Fig. 4f).

A comparative analysis between the SVM and RF models revealed that the RF model outperformed the SVM model in all performance metrics. Specifically, the SVM model achieved an AUC of 0.65, accuracy of 65%, precision of 46%, recall of 75%, and F1-score of 0.57. In contrast, the RF model demonstrated superior performance, with an AUC of 0.93, accuracy of 96%, precision of 92%, recall of 100%, and F1-score of 0.96 (Fig. 4h). Given that the RF model consistently exceeded the SVM model in all key metrics, it was selected as the optimal predictive model.

3.4 Dynamic Alterations in the Immune Microenvironment of HPV-Infected Normal and HSIL Cervical Tissues

3.4.1 Single-Cell Atlas Characterization of HPV-Infected Normal and HSIL Cervical Tissues

Cell types within HPV-infected cervical tissue were characterized using a t-SNE plot annotated with marker gene expression (Supplementary Fig. S1). A total of ten major cell types were identified (Fig. 5a), including four structural cell types (epithelial cells, fibroblasts, endothelial cells, smooth muscle cells) and six immune cell types (myeloid cells, NK/T cells, neutrophils, B cells, mast cells, plasma cells). Epithelial cells were the most prevalent structural cell type, while T cells dominated the immune microenvironment (Fig. 5b).

3.4.2 Dynamic Changes in Immune Cell Populations during the Progression of Cervical Lesions under HPV Infection

We observed significant shifts in T cell subpopulations between normal and HSIL cervical tissues. In normal tissues, HPV infection led to the identification of four CD8 + and four CD4 + T cell subtypes. In contrast, HSIL tissue exhibited five subtypes for both CD8 + and CD4 + T cells, with notable increases in effective T cells such as CD8 + Tem and CD4 + Th1-like cells, and a decrease in naive T cells (Fig. 5c).

Specifically, CD8 + Tem cells and CD4 + Tem cells showed increased proportions in HSIL, indicating local immune activation and enhanced effector function, while the proportion of naive T cells decreased, suggesting differentiation or recruitment to the lesion site (Fig. 5d).

3.4.3 Cell-Cell Communication among T Cell Subsets in the Immune Microenvironment

The Circos plot illustrates potential cell-cell interactions among major cell types. Its suggest a pronounced enhancement of interactions between immunoregulatory and effector cells in the HSIL context (Supplementary Fig. S2).CellChat analysis revealed significantly enhanced interaction strength in the MIF ($p < 0.01$) and MHC-I ($p < 0.01$) pathways in HSIL compared to normal tissues (Fig. 5e). Key ligand-receptor pairs such as MIF-(CD74 + CXCR4) and HLA-CD8 were predominantly elevated in HSIL (Fig. 5f). These interactions indicate a more intricate immune communication network in HSIL, with enhanced immune activation and inflammatory signaling. The MIF pathway showed heightened involvement of both CD4 + Th1-like and Treg subsets, while the MHC-I pathway highlighted the increased involvement of CD8 + Tem and CD4 + Th1-like cells, reflecting an amplified immune response and regulatory activity in the HSIL environment.

To identify key cellular drivers of these networks, a comparative analysis of the cellular roles within MIF and MHC-I pathways revealed that in HSIL, Treg and Th1-like cells played a central role, with their increased functional overlap reflecting the complex inflammatory microenvironment and immune escape mechanisms characteristic of HSIL (Fig. 5g).

4. Discussion

This study provides three key discoveries: first, it demonstrates that negative psychological states and sleep quality play significant roles in the persistence of HPV infection following cervical conization; second, it identifies glandular involvement in HSIL as a factor correlated with persistent HPV infection; and third, it highlights that psychosocial stress and sleep disturbances impact HPV clearance by affecting immune responses through neuroendocrine pathways. Specifically, stress and poor sleep quality are linked to chronic HPV persistence, potentially through their effects on immune system modulation.

Sleep is an active and essential physiological process crucial for maintaining human health and well-being. In recent years, sleep disorders have emerged as a significant public health concern, playing a critical role in disease prevention and management [23]. Inadequate sleep habits can have widespread negative effects on the body, with established associations with Alzheimer's disease and cardiovascular conditions, and may also influence the development and progression of HPV infection [24].Psychosocial stress, as a whole, exerts profound systemic and local effects on the immune system through the activation of the HPA axis and elevated cortisol levels. Previous studies have shown that chronic stress, through sympathetic nervous system activation, promotes the secretion of catecholamines and cortisol.

These factors regulate the tumor microenvironment through immune pathways, potentially activating immune signaling pathways such as MIF and MHC-I, which in turn affects immune system function and facilitates the persistence of HPV [25–27]. Furthermore, chronic stress is closely associated with cancer progression, particularly in HPV-related carcinogenesis, where psychological stress is thought to promote the continued development of precancerous lesions [28]. This study is the first to suggest that negative psychological states play a significant role in the persistence of HPV infection following cervical conization, highlighting the importance of sleep quality and mental health in clinical management. We recommend that future studies validate these findings through rigorously designed cohort studies and explore the impact of psychological and sleep interventions on HPV clearance rates.

Previous research has indicated that factors such as age, parity, menopausal status, and cone depth may affect persistent HPV positivity post-cervical conization [29]. However, our study did not find significant differences in these variables between the comparison groups ($P > 0.05$). Our study introduces novel findings, such as the link between petrolatum gauze use and HPV persistence, which could be explained by foreign body reactions that lead to chronic inflammation and interfere with immune mechanisms at the cervical site. This differs from prior reports, highlighting the importance of considering local mechanical factors in HPV clearance. Additionally, we identified glandular involvement in HSIL as a significant factor influencing HPV persistence. This finding suggests that lesions extending into glandular structures may alter the local immune microenvironment, further hindering viral clearance. Although the correlation is observational, this provides a new biological hypothesis that warrants experimental verification.

The reason why some HPV infections persist and develop into cancer, while others can be cured is not known, as is the exact transformation path from persistent high-risk infection to HSIL and invasive cancer[2]. This knowledge gap highlights the urgent need for in-depth analysis of potential immune dynamics. Our research began to solve this problem, focusing on T cells. Our analysis shows that they are the dominant group of cells in the immune microenvironment. We observed that these T cell groups have undergone significant changes in their composition and functional status during the progression of the disease. Specifically, T cells in HSIL show higher heterogeneity and activation state - which may be an adaptive response to chronic HPV antigen exposure. However, this activation occurs in a complex regulatory environment, in which there is a contradictory coexistence of pro-inflammatory and immunosuppressive signals.

Within the tumor microenvironment, $CD8^+$ cytotoxic T lymphocytes (CTLs) are crucial for the eradication of tumor cells. The generation of tumor-specific $CD8^+$ T cell responses is contingent upon the effective recognition of tumor antigens presented via MHC-I or human leukocyte antigen (HLA) molecules. Contemporary research on the regulation of MHC-I in cancer predominantly concentrates on genetic alterations or diminished expression of "positive regulatory factors" associated with essential components of the MHC-I antigen processing machinery (APM) [30]. Investigations into the correlation between MHC-I expression and prognosis have demonstrated that the loss of MHC-I correlates with

poorer clinical outcomes across various cancer types, including cervical cancer [31]. In this study, the increased communication probability of MHC-I ligand-receptor pairs across diverse immune subpopulations indicates enhanced cytotoxic immune recognition mechanisms. The MIF is a homotrimeric protein that acts as a pleiotropic pro-inflammatory cytokine, participating in numerous processes such as leukocyte recruitment, inflammation, immune responses, cell proliferation, tumorigenesis, and the counter-regulation of glucocorticoids (GCs) [32]. Depending on the inflammatory context and cell type, MIF interacts with specific receptors or receptor complexes that dictate its functional activity [33]. As a pivotal inflammatory mediator, MIF engages multiple receptors, including CD74—its primary receptor, often in complex with CD44, CXCR2, or CXCR4—to initiate intracellular signal transduction [33–35]. This activation facilitates downstream signaling through pathways such as MAPK/ERK, PI3K/Akt, and NF-κB, thereby regulating a variety of biological processes, including cell proliferation, inflammatory responses, survival, and chemotaxis.we propose that the immune microenvironment within HSIL cervical tissues is characterized by increased activation, intensified cellular communication, and enhanced functional heterogeneity. Enhanced antigen presentation and inflammatory signaling may promote immune recognition and the clearance of infected or aberrant cells. Conversely, an elevated proportion of Tregs and increased regulatory activity may indicate the onset of immune evasion mechanisms [36], thereby fostering conditions favorable to viral persistence and disease progression. Our findings suggest a potential mechanistic pathway in which psychological stress triggers HPA axis activation and sympathetic nervous system excitation, leading to the release of stress hormones like catecholamines and cortisol. These factors, in turn, influence immune responses by upregulating key inflammatory mediators such as MIF and MHC-I. The MIF pathway enhances immune cell recruitment and amplifies inflammatory responses, while MHC-I signaling plays a crucial role in antigen presentation and CD8 + T cell activation. These pathways contribute to immune dysregulation, promoting HPV persistence in a microenvironment characterized by immune activation and immune evasion. We propose that this immune imbalance is a driving mechanism for the development of cervical precancerous lesions and viral persistence. Future research could validate this hypothesis by correlating serum cortisol and MIF levels with single-cell characteristics in HPV-infected tissues.

5. Implications

This study underscores the significant roles of psychological stress and sleep quality in the persistence of HPV infection. We propose a mechanistic model involving stress-induced neuroendocrine activation and its effects on the immune system, particularly through MIF and MHC-I pathways. While promising, these findings require further validation, especially with experimental data on stress hormones, immune signaling, and HPV infection dynamics.

6. Limitations

This study has several limitations that should be considered. First, the single-center design may introduce selection bias, limiting the generalizability of the findings. Second, the sample size was

relatively small, which may restrict the application of more complex machine learning models. Third, the cross-sectional nature of psychological assessments prevents the determination of causal relationships—whether stress causes persistent infection or persistent infection induces stress. Fourth, the single-cell data came from public databases rather than from the same cohort, limiting direct mechanistic associations. Finally, we did not control for important confounding factors, such as sexual behaviors and HPV vaccination history, which could affect both infection persistence and clearance.

7. Conclusions

Our study provides the first evidence that psychosocial distress and poor sleep are independent risk factors for persistent HPV infection after conization. The high-performance RF model offers a potential tool for clinical risk stratification, effectively predicting HPV clearance based on psychological and sleep-related factors. Integrated single-cell analysis further hypothesizes that an MIF/MHC-I-driven immune imbalance may serve as a mechanistic link between psychological stress and local immune evasion, facilitating HPV persistence. These findings strongly advocate for incorporating psychosocial screening and intervention into routine post-conization care to improve HPV clearance rates and reduce recurrence.

Declarations

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Declaration of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Authors statement form

Yuhui Sun conceived and supervised the research. Huixin Chen,Jiao Yu and Liusu Wang retrieved literature, collected datas drafted the manuscript and drew the figures. Hao Yu ,Xiangyu Liu and Tianjiao Zheng provided valuable suggestions for figure design. Shibo Li,Xianglan He and Yu Zhang modified and polished the manuscript. All authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Ethics Statement

This study involved human participants and was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Approval No.: 2024257). All participants were fully informed about the study and provided written informed consent prior to their enrollment.

Consent for publication

All authors agree to the publication of this review in this journal.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1
Comparison of Surgical Characteristics between Patients with Persistent HPV Positivity and HPV Clearance

Surgical Approach	**Persistent HPV positivity**	**HPV turning negative**	***P value***
			0.069
LEEP	27	138	
CKC	41	127	
Intraoperative Bleeding			0.744
< 5ml	52	211	
≥ 5ml, < 20ml	12	37	
≥ 20ml	4	17	
Diameter of resection			0.33
≤ 3cm	65	259	
> 3cm	3	6	
Depth of excision			0.851
≤ 2cm	66	256	
> 2cm	2	9	
Vaginal Packing			0.194
deny	13	71	
yes	55	194	
Use of Hemostatic Agents			0.995
deny	40	156	
yes	28	109	
Use oil yarn			0.007
deny	51	233	
yes	17	32	
Pathological grade			0.109
HSIL does not affect the glands	28	138	
HSIL multiple glands	40	127	
Pathological margin positive			0.033

Surgical Approach	Persistent HPV positivity	HPV turning negative	*P value*
			0.069
deny	62	257	
yes	6	8	

Table 2
Comparison of DASS-21 Severity Levels between Patients with Persistent HPV Positivity and Those Who Cleared the Virus.

Stress (DASS-21 score)	HPV turning negative	Persistent HPV positivity	*P value*
			<0.001
No tendency to stress(0–14)	247	35	
Mild tendency to stress(15–18)	14	25	
Moderate tendency to stress(19–25)	4	7	
Severe tendency to stress(26–33)	0	1	
Extreme stress tendency(≥ 34)	0	0	
Anxiety9(DASS-21 score)			<0.001
No anxiety tendency(0–7)	243	25	
Mild tendency to anxiety(8–9)	14	18	
Moderate tendency to anxiety(10–14)	8	22	
Severe anxiety tendency(15–19)	0	3	
Extreme anxiety tendency(≥ 20)	0	0	
Depression(DASS-21 score)			<0.001
No depressive tendencies(0–9)	245	39	
Mild depressive tendency(10–13)	18	20	
Moderate depressive tendencies(14–20)	2	9	
Major depressive tendencies(21–27)	0	0	
Extreme depressive tendencies(≥ 28)	0	0	

Figures

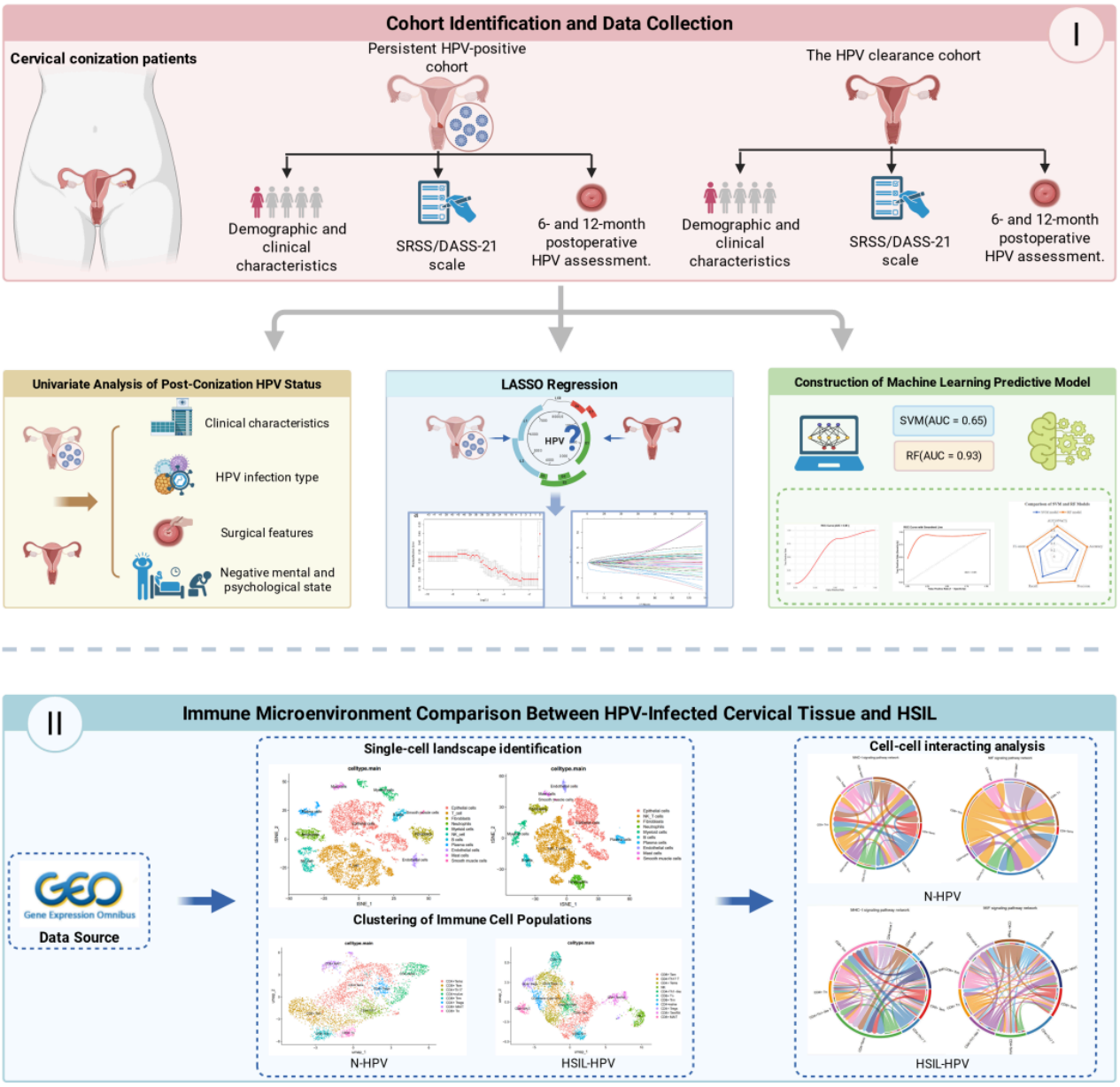


Figure 1

Technical Roadmap

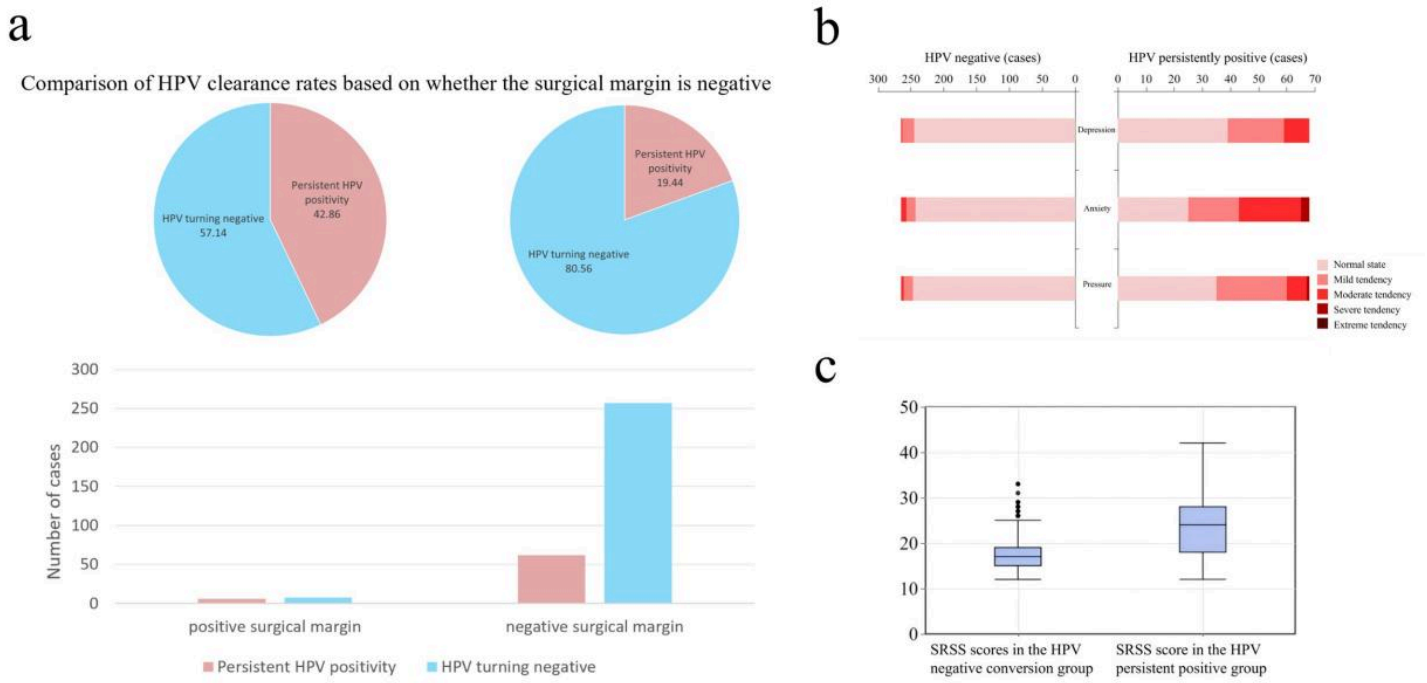


Figure 2

Univariate Analysis of HPV Infection Status Post-Cervical Conization

(a)Comparison of persistent HPV positivity rates between positive and negative surgical margins.(b) Stacked Butterfly Chart of DASS-21 Grading for Patients with Persistent HPV Positivity and Negativity. (c)Boxplot of SRSS scores of patients with persistent positive and negative results

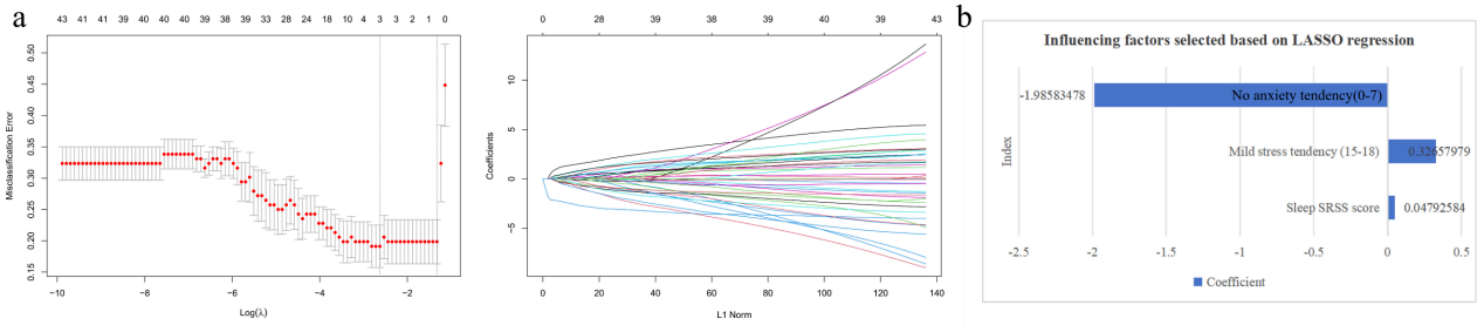


Figure 3

LASSO Analysis of HPV Infection in Patients

(a)Clinical feature selection based on LASSO regression.(b)Box plot of SRSS scores for patients with persistent positivity and those who turned negative.

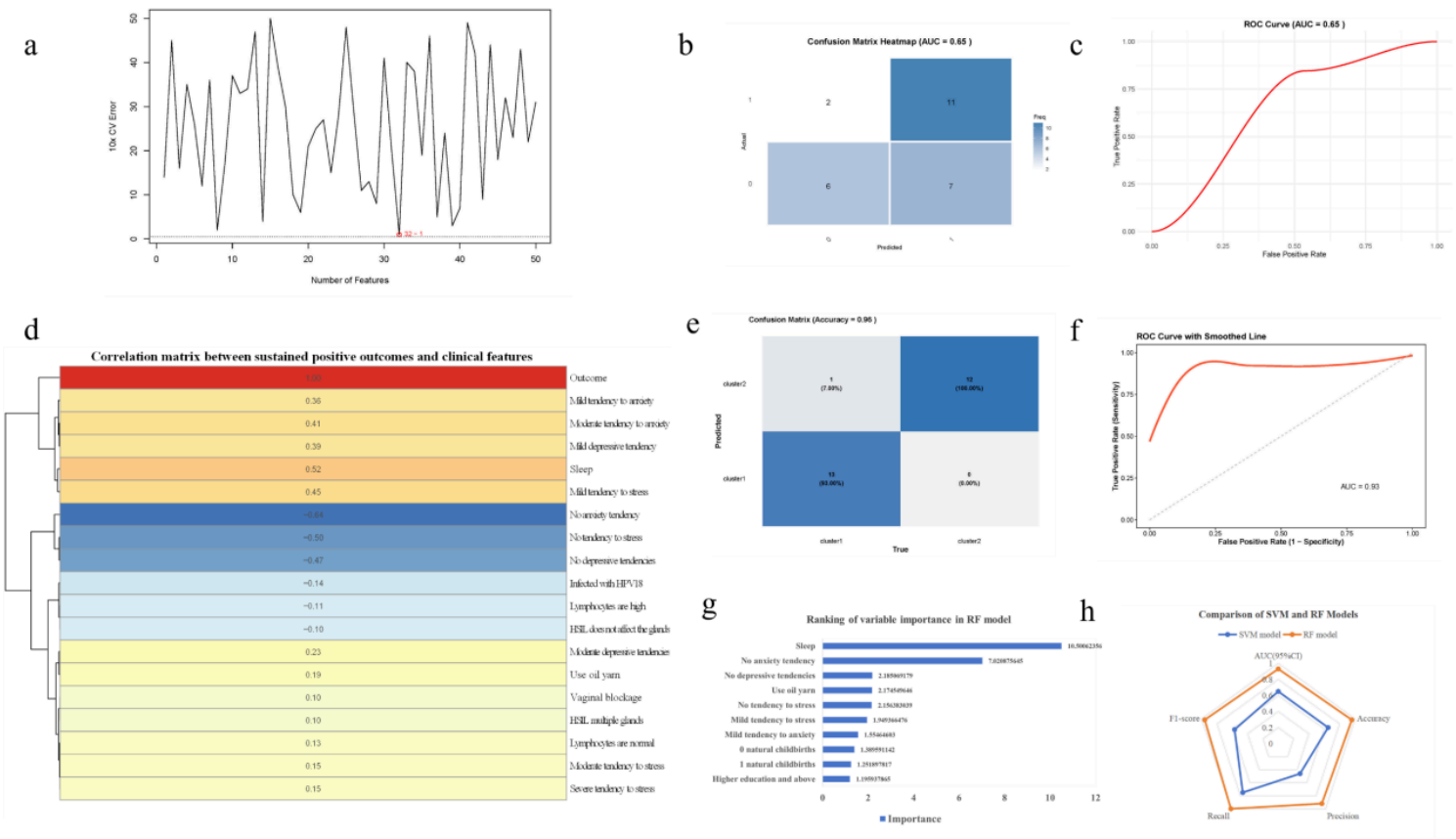


Figure 4

Development and Validation of a Predictive Model for HPV Infection Risk in Patients

(a)Feature variable selection using SVM model.(b-c)Confusion matrix and ROC curve of the SVM model on the test set.(d)Correlation matrix between persistent HPV positivity and clinical characteristics after cervical conization.(e-f)Confusion matrix and ROC curve of the RF model on the test set.(g)Ranking of variable importance in RF model.(h)Radar Chart for Comparison of SVM Model and RF Model.

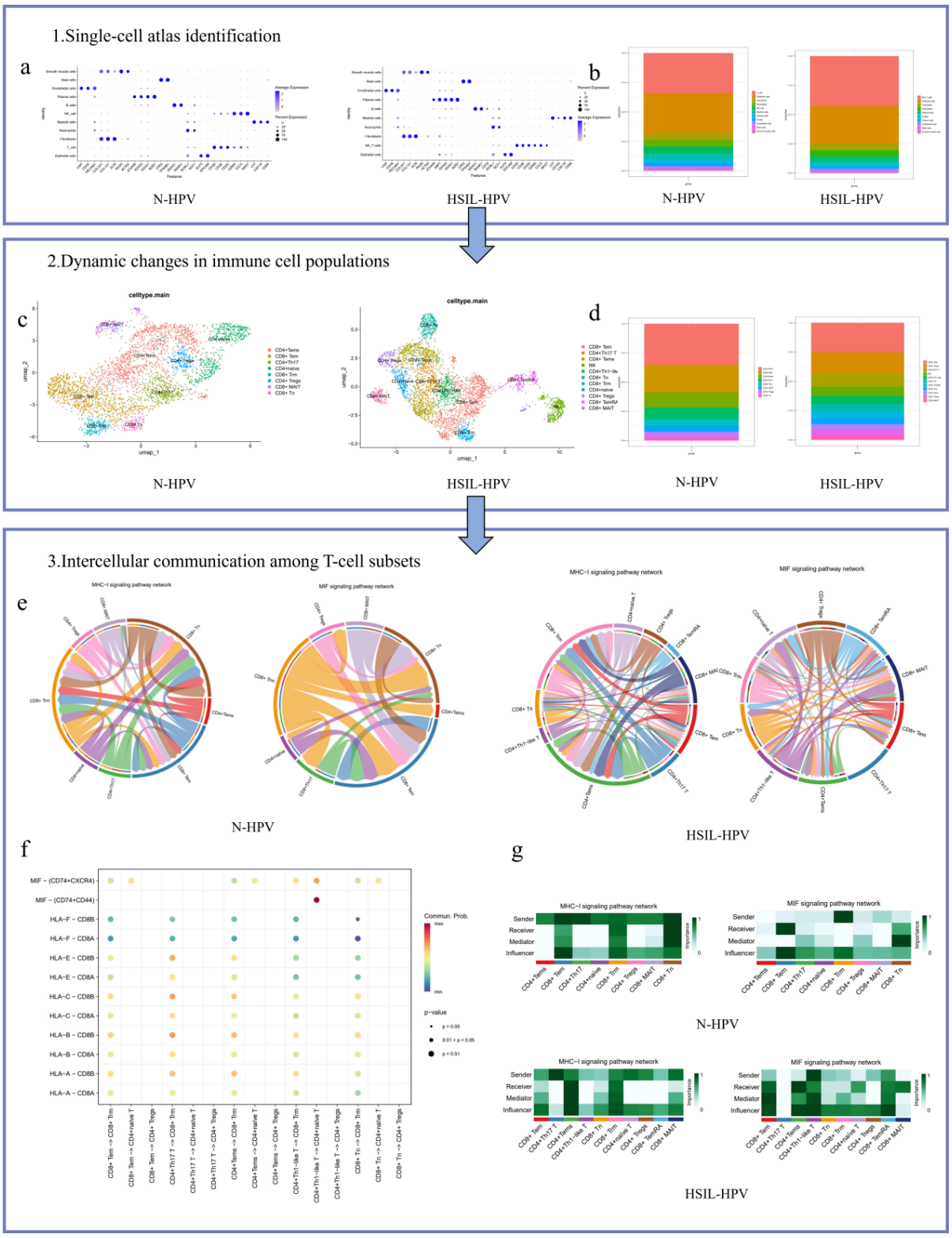


Figure 5

Visualization of cell communication in normal cervical tissue infected with HPV and HSIL cervical tissue

(a)Dot plot of marker gene expression for cell types in HPV-infected normal cervical tissue and HSIL cervical tissue.(b) Proportion of different cell types in HPV-infected normal cervical tissue and HSIL cervical tissue.(c)Re-clustering UMAP plot of T lymphocyte subpopulations.(d)Composition and

proportion of T cell subpopulations in normal cervical tissue and HSIL cervical tissue with HPV infection. (e)Cell interaction network of MHC-I signaling pathway and MIF signaling pathway in normal cervical tissue and HSIL cervical tissue with HPV infection.(f)Ligand-receptor interaction analysis of MHC-I and MIF signaling pathways in the HISL-HPV immune ecosystem.The Y-axis represents specific ligand–receptor pairs, the X-axis denotes "from → to" communication relationships between cell subpopulations, the color corresponds to communication probability, and the point size reflects statistical significance (p-value).(g)Cell communication network system analysis: Identifying dominant senders, receivers, mediators, and influencers in the MHC-I and MIF communication network between cells.

Supplementary Files

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- Appendices.docx