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Expression and Role of STK11 protein in Cervical Cancer

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Abstract: Objective: This study explores STK11 expression and mechanism in cervical cancer tissue. **Methods:** STK11 expression in cervical cancer was retrieved from Xiantao Academic Database and The Human Protein Atlas. UALCAN was used to analyze its correlation with patients' pathological characteristics. KM Plotter provided gene expression data and survival statistics for cervical cancer, which underwent COX regression to determine STK11 expression's relationship with overall survival and calculate patients' risk ratio. TIMER was used to investigate associations between STK11 expression and immune cell infiltration in cervical cancer. STRING constructed the STK11-related protein-protein interaction network. Finally, immunohistochemistry verified STK11 protein expression in 90 cervical cancer tissues and 26 adjacent tissues, analyzing correlations with prognosis and clinical characteristics. **Results:** STK11 protein expression was significantly lower in cervical cancer tissue than normal cervical tissue, correlating with recurrence ($P < 0.05$) but not with overall survival, age, cancer type, clinical stage, pathological grade, or lymphatic metastasis ($P > 0.05$). Additionally, STK11 expression in cervical cancer positively correlated with B cell and CD4+ T cell infiltration ($P < 0.05$). **Conclusion:** Low STK11 protein expression in cervical cancer associates with disease occurrence and development, potentially serving as an early diagnostic indicator and novel therapeutic target for cervical cancer.

Keywords: STK11; Cervical Cancer; Express; Role.

1. Introduction

Cervical cancer is recognized by the World Health Organization as the fourth most critical malignancy endangering women's lives and stands as a prominent cause of cancer-related fatalities among females [1]. In 2020, 604,127 new cases of cervical cancer globally, with over 341,831 resulting in death, which represents nearly 8% of all cancer-related mortalities in women annually [2]. Recent years have witnessed substantial advancements in both the prevention and management of cervical cancer as well as its precancerous lesions on a global scale. The incidence and mortality rates of cervical cancer have shown a significant downward trend in most developed countries, but the overall prognosis for cervical cancer patients in economically underdeveloped developing countries remains poor [3,4]. The lack of early and distinctive clinical symptoms often leads to diagnoses occurring at more advanced stages, and the treatment outcomes for individuals with advanced or recurrent cervical cancer continue to be less than favorable in clinical settings.. Therefore, further research on diagnostic markers, potential therapeutic targets, and prognostic indicators for cervical cancer is crucial.

STK11, also known as Liver Kinase B1 (LKB1), plays an important role in regulating cell growth, metabolism, proliferation, and apoptosis, primarily by participating in the regulation of gene expression and signaling pathways, thus playing a key role in the occurrence and development of cancer [5,6]. Studies have shown that mutations in the STK11 gene are associated with the occurrence of cervical cancer [7]. However, the precise mechanisms by which the STK11 gene contributes to the onset and progression of cervical cancer remain to be elucidated. This study analyzes the expression of STK11 protein in cervical cancer tissues using bioinformatics methods and validates it through immunohistochemical experiments, analyzing the role of STK11 in the occurrence and development of cervical cancer and its relationship with the prognosis of cervical cancer patients.

2. Materials and Methods

2.1. Clinical Specimen

This study selected 90 cases of cervical cancer patients as research subjects, with an average age of 47.44±9.02 years. Among them, there were 83 cases of squamous carcinoma and 7 cases of non-squamous carcinoma, including 26 cases with adjacent cervical tissue. The pathological grading included 24 cases of grade I+II and 66 cases of grade III. The clinical staging comprised 70 cases of stage I+II and 20 cases of stage III+IV. There were 71 cases without lymphatic metastasis and 19 cases with lymphatic metastasis, with 58 cases having no recurrence and 32 cases having recurrence. The tissue samples were obtained from the cervical cancer tissue microarray (HUteS154Su01) of Shanghai Xinchao Biotechnology Co., Ltd., and were diagnosed by two associate chief physicians in a double-blind manner. The clinical data of the patients from whom the tissue samples were taken were complete, and none had received drug treatment or radiotherapy before surgery, nor did they have malignant tumors in other locations. The ethics committee granted approval for this study.

2.2. Data Collection and Analysis

2.2.1. STK11 Gene Expression Data Analysis in Cervical Cancer and Adjacent Tissues

Use the Xiantao Academic Database (https://www. xiantao. love/) to check the expression levels of STK11 in cervical cancer samples. Since the TCGA database only contains 3

cases of adjacent samples from cervical cancer, in order to obtain more accurate analysis results, this study selected 10 cases of normal cervical tissue from the GTEx database and 33 cases of adjacent samples from TCGA cervical cancer as controls for differential expression analysis of STK11.

2.2.2. STK11 Gene Expression in Cervical Cancer Tissues of Different Grades

The Human Protein Atlas website (https://www.proteinatlas.org) utilizes the integration of various omics technologies, including transcriptomics, antibody-based imaging, and mass spectrometry proteomics, to map all proteins in human cells, tissues, and organs. This study obtained the protein expression results of STK11 antibody detected by immunohistochemistry in cervical cancer tissues of different grades from The Human Protein Atlas website, analyzing the protein expression and localization of STK11 in cervical cancer tissues of different grades.

2.2.3. STK11 Expression Vs. Clinical Characteristics in Cervical Cancer Patients

UALCAN, a web - based tool, enables in - depth analysis of TCGA and MET500 datasets. This database analyzes the expression levels of STK11 in cervical cancer tissues and normal cervical tissues, as well as its relationship with the pathological features of cervical cancer.

2.2.4. STK11 Expression's Prognostic Value in Cervical Cancer Patients: Database Analysis

Utilizing the online platform KM Plotter (http:// kmplot.com), which provides gene expression datasets along with survival information pertaining to cervical cancer patients, we conducted an analysis to evaluate how varying levels of STK11 gene expression influence the prognosis of individuals diagnosed with cervical cancer. The patients' samples were divided into two groups based on the median STK11 gene expression levels: a low expression group and a high expression group. COX regression analysis was used to assess the relationship between STK11 gene expression levels and the overall survival of cervical cancer patients, as well as the hazard ratios (HRs), 95% confidence intervals (95% CI), and log-rank P values, to statistically analyze the prognostic value of STK11 in cervical cancer.

2.2.5. STK11 Protein Expression Vs. Prognosis Correlation in Clinical Cervical Cancer Patients

Based on the expression levels of the STK11 protein, 90 cervical cancer patients were categorized into two categories: the STK11-negative and STK11-positive expression groups. Kaplan-Meier survival analysis and Log-rank test were used to explore the association between STK11 protein expression and overall survival (OS) in cervical cancer patients.

2.2.6. STK11 Expression and Tumor Immune Cell Correlation

TIMER (https://cistrome.shinyapps.io/timer/) is an interactive portal that provides a comprehensive analysis of the infiltration levels of different immune cells. The relationship between STK11 expression and immune cell infiltration levels in cervical cancer is studied using the TCGA database through the gene module of the TIMER online database.

2.2.7. STK11 Gene Regulatory Network

The STRING online database (https://string-db.org/) is primarily a database for studying interactions between known proteins and predicted proteins. A PPI network was developed for the STK11 gene utilizing data from the database, the 10 interaction genes most relevant to the STK11 gene were obtained.

2.3. Immunohistochemical Experiment Verification

The immunohistochemical method for detecting protein expression in tissues is strictly conducted according to the reagent instructions. Tissue chips are dried, dewaxed, and subjected to antigen retrieval, followed by the addition of the primary antibody, incubated overnight at 4°C in a humid box. After adding the secondary antibody, it is incubated at room temperature for 45 minutes, followed by blocking, secondary antibody binding, DAB chromogenic reaction, hematoxylin counterstaining, and mounting. Clear images of representative high-power fields are selected for analysis, and a comprehensive score is given based on the proportion of positive cells and the intensity of cell staining in high-power fields. Positive cells are scored based on staining intensity: no staining is 0 points, light yellow is 1 point, brownish yellow is 2 points, and brown is 3 points; the proportion of stained cells: proportion ≤10% is 0 points, proportion 11%-30% is 1 point, proportion 31%-60% is 2 points, and proportion ≥61% is 3 points. A product of the scores from the two criteria ≥2 points indicates positive expression of STK11 protein; otherwise, it indicates negative expression of STK11 protein [8].

2.4. Statistical Methods

Using the Wilcox rank-sum test to evaluate the differential expression of genes; the relationship between gene expression and clinical pathological parameters was assessed using the log-rank test, and survival curves were plotted using Kaplan-Meier. A P-value < 0.05 was considered statistically significant.

3. Result

3.1. STK11 Gene Expression in Cervical Cancer

This research employed the Xiantao Academic Database to evaluate and contrast the expression levels of STK11 in both cervical cancer tissues and normal cervical tissues. The findings indicated a significantly reduced expression of STK11 in cervical cancer tissues as opposed to that observed in normal cervical tissues. ($P < 0.01$, Figure 1).

3.2. STK11 Protein Expression and Localization in Cervical Cancer and Adjacent Tissues

The Human Protein Atlas database provides immunohistochemical results using two staining methods (HPA017254 and CAB022105), indicating that STK11 protein expression is significantly lower in cervical squamous cell carcinoma tissues compared to normal cervical squamous cells, with both primarily localized in the cytoplasm (Figure 2). Immunohistochemical validation results: among 90 cervical cancer patients, 58 cases showed positive STK11 protein expression, with a positive expression rate of 64.4%, most staining appearing light yellow and showing low expression; in 26 cases of adjacent cervical tissues, all showed positive STK11 protein expression, with most staining appearing as brownish-yellow granules, and a small portion as brown granules. Positive expression of STK11 protein was not observed in the nuclei of cervical cancer and adjacent

tissues, but positive expression was present in the cytoplasm. This validation experiment further confirms the above results (see Table 1, P < 0.001, Figure 3)

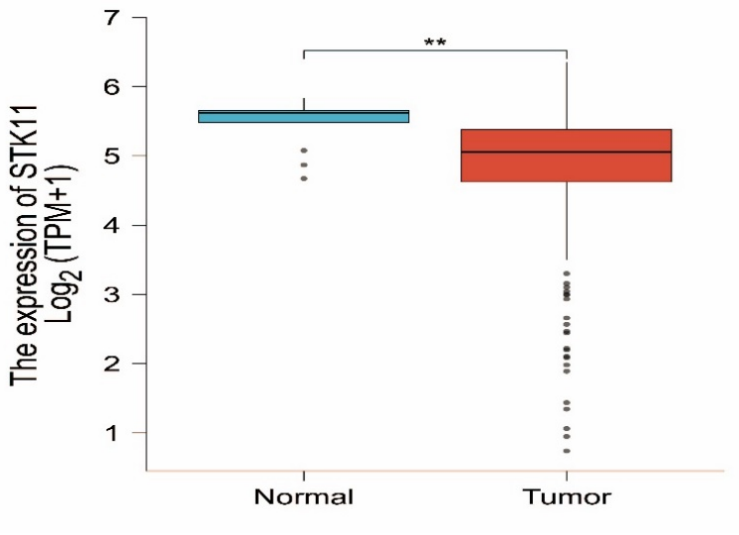

Figure 1. STK11 gene expression in cervical cancer
* P<0.05, ** P<0.01, ***P<0.001

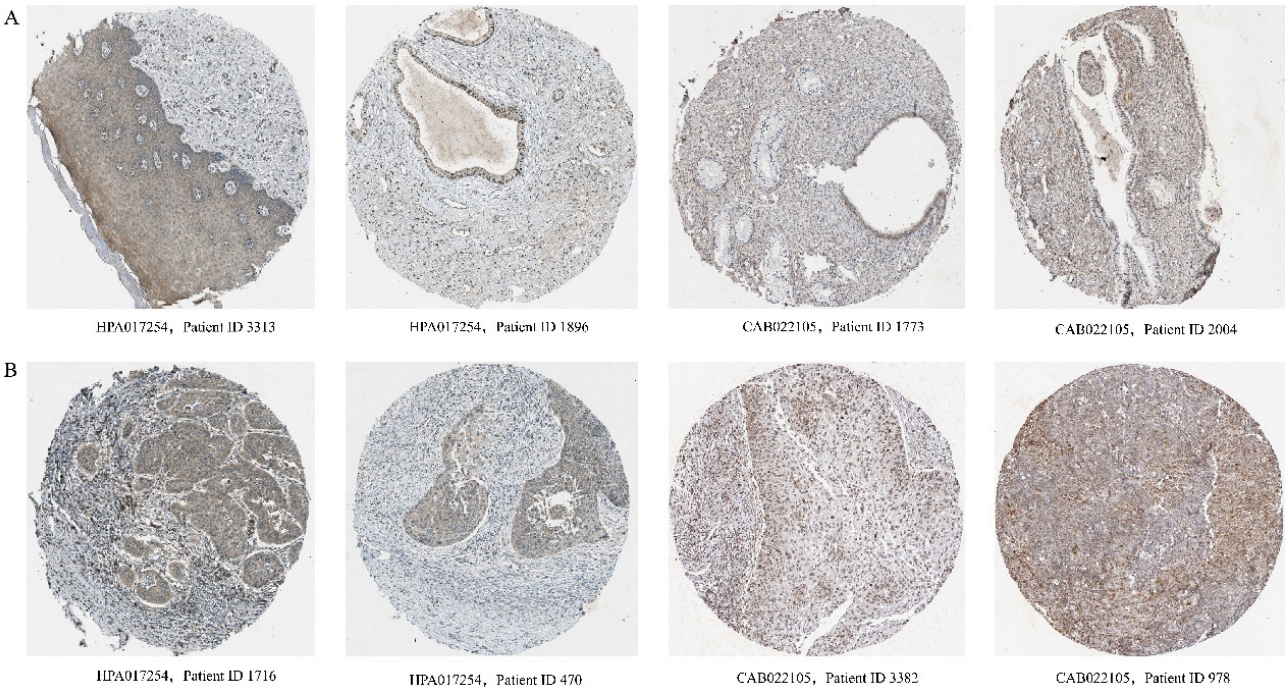

Figure 2. Immunohistochemical staining of STK11 protein in cervical cancer tissues provided by The Human Protein Atlas database (A: normal cervical tissue; B: cervical squamous cell carcinoma tissue)

Table 1. Differential expression of STK11 protein in cervical cancer and adjacent cervical tissues.

Group	Sample size(n)	STK11 expression status		χ^2	*P*
		Positive	Negative		
Cervical cancer	90	58(64.4%)	32		
Paracervical tissue	26	26(100%)	0	12.77	<0.001

3.3. STK11 Expression and its Correlation with Clinical Characteristics in Cervical Cancer Patients

The UALCAN online database analysis results show that the expression level of STK11 is not related to the pathological features of cervical cancer (including tumor subtype, stage, grade, and lymph node metastasis) (P > 0.05). Subsequently, this study used immunohistochemistry (IHC) to detect the expression level of STK11 protein in 90 clinical cases of cervical cancer tissues. The results indicate that the expression level of STK11 protein is related to recurrence, but is not related to age of onset, pathological type, pathological grade, clinical stage, or lymph node metastasis (Table 2).

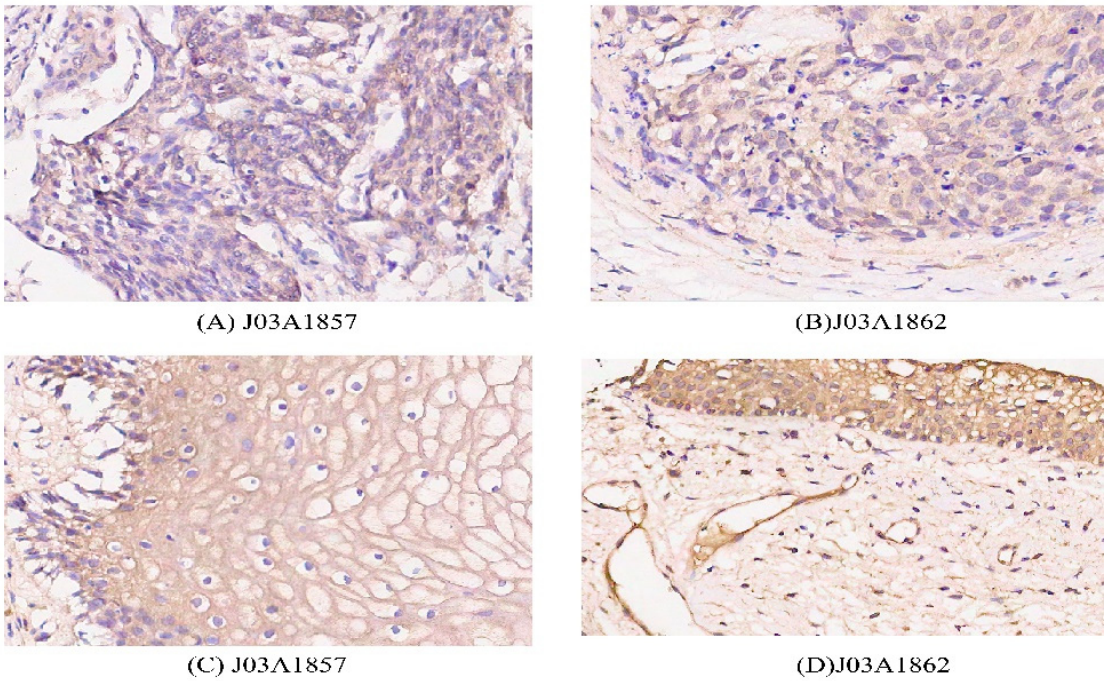

Figure 3. STK11 protein expression in cervical cancer and adjacent cervical tissues. (A) and (B) show the expression of STK11 protein in cervical cancer tissues numbered J03A1857 and J03A1862 (X200); (C) and (D) show the expression of STK11 protein in adjacent cervical tissues numbered J03A1857 and J03A1862 (X200)

Table 2. STK11 protein expression and clinicopathological parameter relationship

	N	STK11 protein expression		*P*
		Positive	Negative	
Age (years)				
≤60	79	52(65.8%)	27(24.2%)	
>60	11	6(54.5%)	5(45.5%)	0.512
Pathological type				
Squamous carcinoma	83	56(67.5%)	27(32.5%)	
Non-squamous carcinoma	7	2(28.6%)	5(71.4%)	0.092
Pathological grading				
I-II	24	15(62.5%)	9(37.5%))	
III	66	43(65.2%)	23(34.8%)	0.816
Clinical staging				
I-II	70	45(64.3%)	25(35.7%)	
III-IV	20	13(65.0%)	7(35.0%)	0.953
Lymphatic metastasis				
YES	19	12(63.6%)	7(36.4%)	
NO	71	46(64.8%)	25(35.2%)	0.895
Relapse				
YES	32	16(50.0%)	16(50.0%)	
NO	58	42(72.4%)	16(27.6%)	0.033

3.4. STK11 Expression and its Relationship with Prognosis in Cervical Cancer Patients

The Kaplan-Meier database analysis results show that the expression level of STK11 protein has no effect on the overall survival rate (OS) of cervical cancer patients ($P > 0.05$, Figure 4). Subsequently, this study used the Kaplan-Meier and Log-Rank test to conduct a univariate analysis of the overall survival period in 90 cases of clinical cervical cancer tissues. There were 58 cases with positive expression of STK11 protein and 32 cases with negative expression. The survival curve results confirmed that the expression level of STK11 protein is unrelated to the OS of cervical cancer patients ($P > 0.05$, Figure 5).

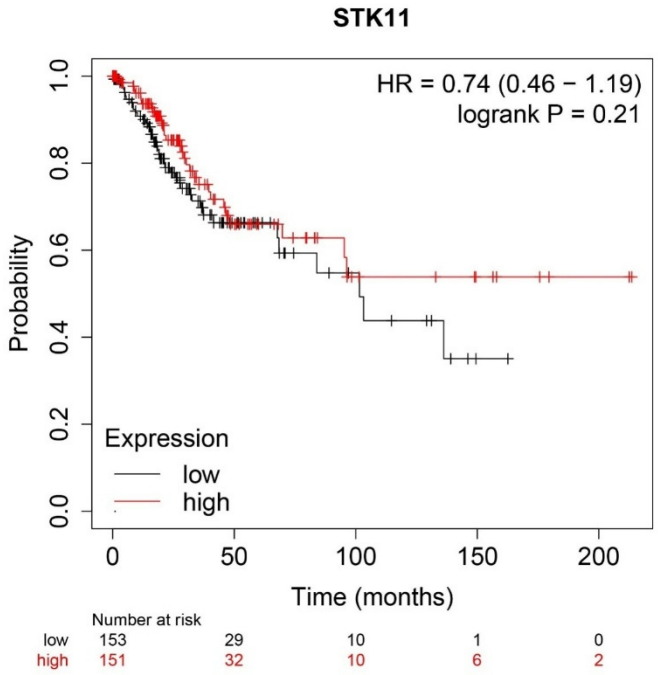

Figure 4. STK11 expression levels and cervical cancer patients' OS relationship

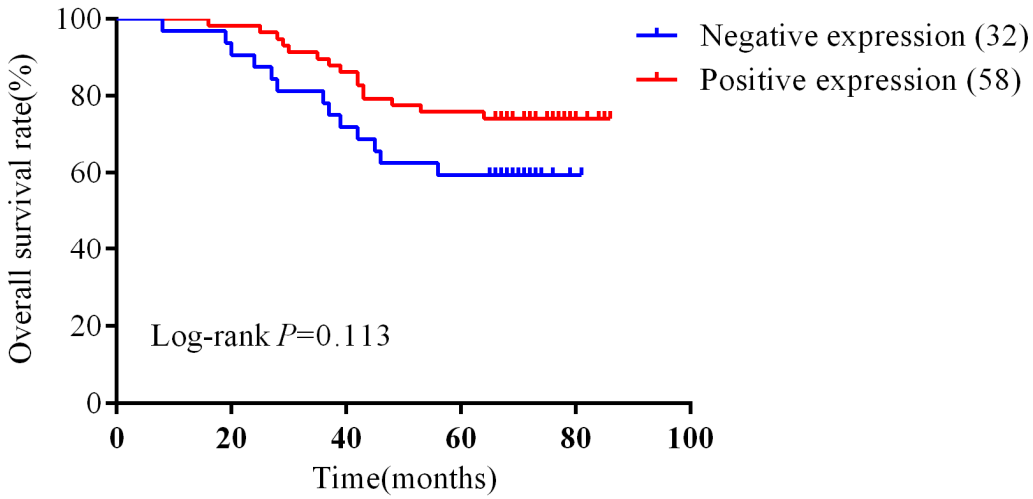

Figure 5. OS survival curve in cervical cancer patients by STK11 expression levels

3.5. STK11 and Immune Cell Infiltration Correlation

TIMER database analysis shows STK11 expression in cervical cancer positively correlates with B cell and CD4+ T cell infiltration, but not with CD8+ T cells, macrophages, neutrophils, or dendritic cells (Figure 6).

3.6. The Regulatory Network of the STK11 Gene

STRING database generated the STK11 gene's PPI network (Figure 7). This protein-protein network has 43 edges and 11 nodes, with the 10 genes most related to STK11 including PPKAA1, TP53, CAB39, and AXIN1, among others.

4. Discussion

STK11, a tumor suppressor gene, has protein function loss or reduction as a key cause of malignant tumorigenesis [9]. STK11 was initially identified as a pathogenic mutation in Peutz-Jeghers syndrome, characterized by benign gastrointestinal hamartomas, with a cancer risk (including tubular gastrointestinal cancer, pancreatic cancer, breast

cancer, gynecological tumors, and other cancers) potentially up to 10 times that of the general population [10,11]. Additionally, somatic mutations or functional loss of STK11 have also been found in several malignancies, such as non-small cell lung cancer, cervical cancer, ovarian cancer, breast cancer, and hepatocellular carcinoma [12]. Despite advancements in diagnostic and therapeutic techniques such as early diagnosis, targeted therapy, and immunotherapy, cervical cancer is frequently identified at a later stage, which is associated with an unfavorable prognosis and a treatment regimen that is comparatively intricate.

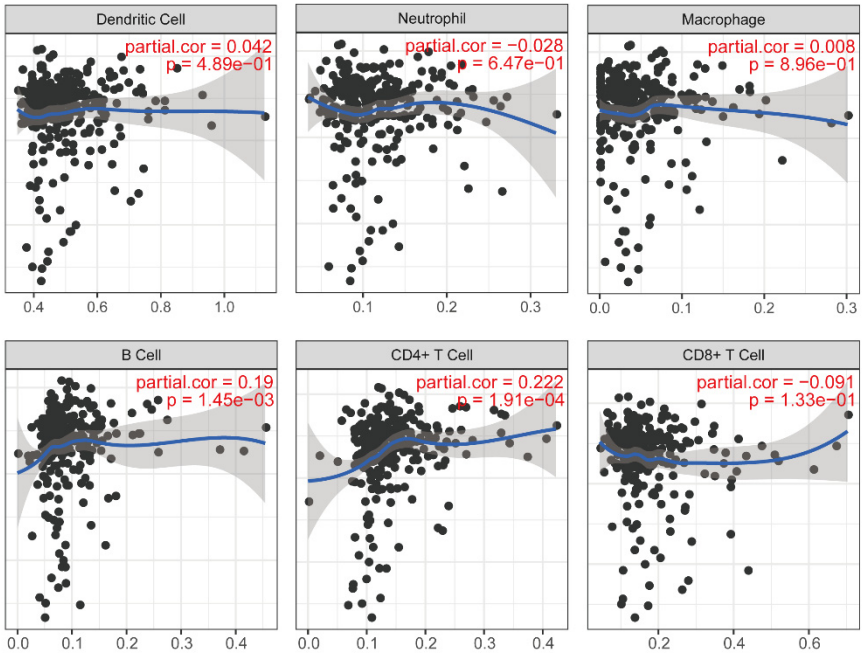

Figure 6. STK11 expression levels vs. immune cell infiltration correlation
Note: Dendritic cell, Neutrophil, Macrophage, B cell, CD4+ T cell, CD8+ T cell.

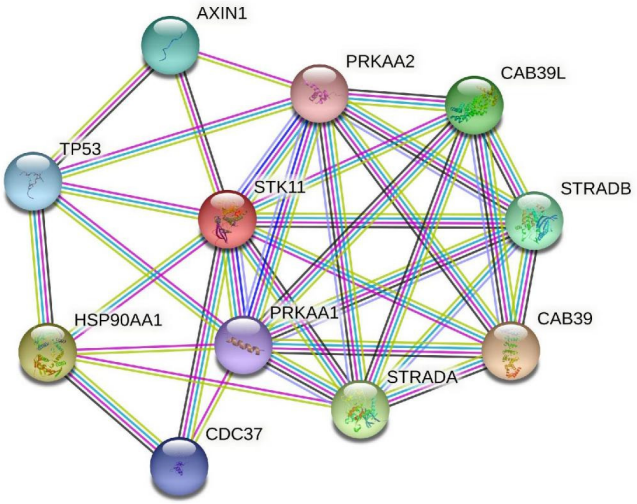

Figure 7. STK11 gene PPI network generated via STRING database

This study employs bioinformatics to analyze the expression and role of STK11 in cervical cancer, validated by immunohistochemical experiments. The STK11 protein is expressed at low levels in cervical cancer tissues. Moderate to high expression in adjacent non-cancerous tissues, mainly cytoplasmic in both cervical cancer and adjacent tissues; associated with patient recurrence but not overall survival, age, pathological type, grade, clinical stage, or lymphatic metastasis. Clinically, the pathological parameters of tumors (pathological grade, clinical stage, lymphatic metastasis, and distant metastasis) should be closely related to the prognosis of cervical cancer patients. However, results from databases and experimental validation show that STK11 protein is not associated with clinical pathological parameters, which may be related to differences in the clinical staging and pathological grading standards used, or STK11 protein expression may indeed not correlate with the aforementioned clinicopathological parameters. However, it cannot be ruled out that its expression level may be associated with the early detection, diagnosis, and treatment of cervical cancer. Thus, this study further verified STK11 protein expression's correlation with cervical cancer patients' prognosis via univariate survival analysis. Results show no correlation with their overall survival, consistent with Kaplan-Meier database findings, indicating STK11 expression does not affect cervical cancer patients' OS. The above clinical experiments further confirm low STK11 expression in cervical cancer, linked to its occurrence and development, potentially serving as a diagnostic biomarker and new therapeutic target.

Next, analyzing STK11 and immune cell infiltration in cervical cancer showed STK11 expression positively correlates with B cell and CD4+ T cell infiltration Previous studies [13-14] have shown that mutations in the STK11 gene in malignant tumors lead to the expression of a large number of cytokines (including IL-3, IL-6, and CXCL7) in tumor cells, recruiting tumor-associated neutrophils, inhibiting T cell cytotoxicity, leading to immune evasion, and weakening the effectiveness of immunotherapy. Furthermore, STK11 loss in tumors reduces T cell infiltration. Thus, reducing intratumoral cytokine and neutrophil secretion can increase T cell infiltration, enhancing tumor therapeutic efficacy. Currently, no research reports have been found regarding the correlation between STK11 and immune infiltration in cervical cancer, but these results have comprehensively

analyzed the important role of STK11 protein in cervical cancer, indicating that STK11 protein expression is related to the occurrence and development of cervical cancer and closely related to tumor-infiltrating immune cells, providing certain guidance for immunotherapy and targeted therapy in cervical cancer.

Although STK11 is related to the occurrence and development of cervical cancer, the specific regulation of its pathogenesis remains unclear. This study constructs a PPI network, showing that the STK11 gene is most closely related to 10 genes, including TP53 and CAB39. TP53, as a tumor suppressor gene, is commonly mutated in cancers, leading to the inactivation of TP53 protein (p53). Research by Liu et al.[15] indicates that the TP53 gene is overexpressed in cervical cancer through microRNA-3647-5p, thereby inhibiting the proliferation of cancer cells and promoting their apoptosis. Research by Li Zhiqiang et al [16] found that TP53 is expressed at low levels in cervical cancer tissues, significantly correlating with tumor staging, pathological differentiation, and lymph node metastasis, and can assess the risk of recurrence in cervical cancer patients post-surgery. Additionally, the binding of TP53 to STK11 is also associated with improved patient survival rates and good responses to standard chemotherapy and immunotherapy [17]. CAB39, through the upregulation of miR-451a overexpression, shows significantly reduced expression levels in cervical cancer, and the downstream proteins of CAB39, p-P13K and p-AKT, are downregulated, leading to a significant increase in G1 phase arrest and apoptosis of cervical cancer cells, thereby significantly reducing the proliferative capacity of cervical cancer cells[18]. The above research results suggest that the STK11 gene may indirectly regulate the occurrence and development of cervical cancer by affecting the regulatory network of related genes.

In summary, the STK11 gene plays a key role in the occurrence and development of cervical cancer, and research on the STK11 gene can provide a theoretical basis for the diagnosis and treatment of cervical cancer.

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