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ORIGINAL ARTICLE

Upregulated Expression of TAP1 Promotes Progression of HPV+ Cervical Squamous Intraepithelial Lesions

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Abstract

There are still a significant number of cervical squamous intraepithelial lesions (CSIL) that progress to cervical cancer in the short term, despite the great improvements in HPV vaccines. Transporter associated with antigen processing 1 (TAP1) is a molecule involved in the processing and presentation of major histocompatibility complex class I (MHC-I) restricted antigens. Previous studies have shown that TAP1 is aberrantly expressed in a variety of cancers and is involved in cancer immunity. However, the function of TAP1 in CSIL remains unclear. In this study, 130 HPV-infected patients were examined to investigate the role and possible mechanism of TAP1 in CSIL. In addition, we found that TAP1 was up-regulated and positively correlated with P16 and Ki67 in cervical squamous cell carcinoma (SCC) from the TCGA and GTEx databases, and the TAP1-high group showed a worse prognosis. TAP1 was also found to be up-regulated in LSIL, HSIL and SCC in 130 clinical samples and was positively correlated with P16 and Ki67. We confirmed that TAP1 promotes the progression of CSIL and facilitates the proliferation and migration of cervical SCC through a combination of bioinformatics analysis and experiments. Therefore, a novel and potential mechanism was proposed that TAP1 regulates the malignant progression of CSIL by inhibiting tumour immune activation. Our study provides a new perspective for the treatment and diagnosis of cervical SCC.

Keywords: TAP1, HPV+, cervical squamous intraepithelial lesions, invasion, migration, immunological function

Introduction

Cervical squamous-cell carcinoma(CSCC, **Table 1**) is the second most common cancer in women worldwide and the fourth leading cause of cancer death in women, with approximately 604,000 new cases and 342,000 additional deaths worldwide in 2020[1]. Cervical squamous cell carcinoma originates from the squamous epithelium of the cervical columnar junction and develops progressively through low- and high-grade squamous intraepithelial lesions. Human Papillomavirus (HPV) infection is the major cause of cervical squamous intraepithelial lesions and even carcinogenesis[2]. In the process of persistent HPV infection, the imbalance of oncogene, tumor suppressor gene and immune microenvironment plays an important role in the prognosis of cervical squamous intraepithelial lesions[3].

TAP1 and TAP2, two members of TAP, are molecules involved in the processing and presentation of MHC-I-restricted antigens, including tumour-associated antigens[4]. Medeiros[5]found that women with HPV infection have higher TAP-2 mRNA and protein levels, which may affect the transport of HPV peptides from the cytoplasm to the endoplasmic

reticulum, thereby increasing susceptibility to the development of high-grade cervical lesions. TAP1 is highly expressed in most cancers and has a clear prognostic value[6], but its role in cervical squamous intraepithelial lesions is unclear. P16/CDKN2A, a surrogate immune marker for HPV infection, is ofen used as an adjunct diagnosis in equivocal cases of cervical intraepithelial lesions, and in combination with KI67 has high sensitivity and specificity for the diagnosis of HSIL and LSIL[7]. As early as 2007, Yildiz[8] has demonstrated that strong and full-thickness staining of p16 in the cervical epithelium was highly suggestive of HSIL, whereas weak or patchy staining was more suggestive of LSIL.

It is known that the host immune system can control the development of cervical squamous intraepithelial lesions, while major histocompatibility class I(MHC-I) molecules play a critical role in anti-tomour immune response during persistent HPV infection[9]. T cell-specific anti-tumour and anti-viral immune responses require the MHC-I environment of tumor and virus-related antigens[10]. Antigen presentation proteins (TAPs) can recognise MHC-I molecules and are responsible for loading HPV polypeptides onto MHC-I molecules, which contributes to successful HPV eradication[11]. To investigate the role and immune function of TAP1 in cervical squamous intraepithelial lesions, we analysed the expression of TAP1 protein and mRNA levels in cervical squamous intraepithelial lesions, and the relationship between TAP1 and P16, KI67, invasion ability, and immune infiltration.

Materials and methods

1. Clinical Samples and Ethics Statement

The research was conducted to gather data from 130 female patients with cervical biopsy in Bishan Hospital of Chongqing Medical University between January 2019 and December 2019. The participants were aged 22 to 77 years, with an average age of 43.26 ± 10.09 years.The histological examination yielded four groups of cases: 29 cases of cervical chronic inflammation (CCI, Table 1), 34 cases of low-grade squamous intraepithelial lesions (LSIL, Table 1), 37 cases of high-grade squamous intraepithelial lesions (HSIL, Table 1) and 30 cases of squamous cell carcinoma (SCC, Table 1). The inclusion criteria were as follows: The patients were HPV positive, with the biopsy tissue sample measuring > 2 mm. The exclusion criteria were cervical biopsy specimens measuring < 2 mm or biopsy specimens rich in a large amount of mucus.

The study was approved by the Medical Ethics Committee of Bishan Hospital of Chongqing Medical University (approval number: 2019-ky-07). Each patient provided a sample for acquisition and gave informed consent for the study in accordance with the approved guidelines.

2. Data sources and processing

The data sources for this study were the TAP1 mRNA expression in tumour and corresponding normal tissues from the The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases (https://portal.gdc.cancer.gov/). The data were used to verify the expression of TAP1 in cervical SCC and the R V4.0.3 software package was employed to analyse the correlation between TAP1 and P16, Ki67 based on the TCGA data. The R package PROGENy was utilised to calculate the activation scores of 14 typical pathways between the TAP1-high and -low expressed group. Metascape (http://metascape.org/gp/index) was employed for function enrichment analysis, resulting in the identification of pathway and process enrichment analysis outcomes. Additionally, DisGeNET[12] was utilized for further analysis.

3. Immunohistochemical staining

The primary antibody, TAP1 immunohistochemical reagent, was purchased from the Santa Cruz company and has a concentration of 1.0 mg/μl. The solution was then diluted to 10 ng/μl with nuclease-free water. The primary antibodies P16 and Ki67, as well as the general secondary antibody, were purchased from the Maixin company. The staining procedure was conducted in strict accordance with the instructions provided. The interpretation of the results is as follows: TAP1 was observed to be expressed in both the nucleus and cytoplasm, with the presence of brown granules. The immunohistochemical (IHC) score was calculated by multiplying the intensity of the staining by the proportion of positive cells. The intensity of the staining was quantified as indicated below: Scores of 0, 1, 2, and 3 were assigned to indicate the intensity of staining, with 0 representing negative

results, 1 indicating weak staining, 2 signifying moderate staining, and 3 denoting strong staining. The proportion of positive cells was scored as follows: The following scale is used to quantify the proportion of positive cells: 0: <10%, 1: 10% to 25%, 2: 26% to 50%, 3: 51% to 75%, 4: >75%.

4. Total RNA Extraction and qRT-PCR

The tissue was extracted using the RNAiso Plus reagent (Takara, Kusatsu, Japan) in accordance with the manufacturer's instructions. The RNA was reverse-transcribed into complementary DNA using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara). Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using TB Green Premix Ex Taq II (Takara) in a final volume of 10 μL on a CFX96TM Real-Time PCR Detection system (Bio-Rad Laboratories, Hercules, CA, USA). The target messenger RNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to glyceraldehyde 3-phosphate dehydrogenase. The polymerase chain reaction (PCR) primer sequences utilized for TAP1 are as follows: The forward primer sequence was 5′-TGCCCCGCATATTCTCCCT-3′, while the reverse primer sequence was 5′-CACCTGCGTTTTCGCTCTTG-3′.

5. Cell culture

The HPV E6/E7-positive SiHa cell line was procured from the Shanghai Hongshun Biotechnology Company. The SiHa cells were cultured in a medium of RPMI-1640 (GIBCO, Shanghai, China), which was supplemented with 10% fetal bovine serum (GIBCO, Shanghai, China). The cells were incubated in a humidified atmosphere at 5% CO_2 at 37°C.

6. Cell transfection

The recombinant vector containing the target gene must be reconstructed. Transfect SiHa cells with recombinant vectors and virus packaging plasmids for the packaging and production of the virus. The virus solution should be collected, concentrated and purified, and then used to infect and screen cells. Finally, the virus titer must be accurately determined through quantitative PCR. The target sequence is as follows: The forward sequence is as follows: 5′-GAGTGAGAATCTGA

GCTTATTTC-3′, siR-TAP1 Forward: 5′-CCGGTGGAATAAGCTCAGATTCTCAC

TCTTCAAGAGAGAGTGAGAATCTGAGCTT ATTTTTTTTG-3′, Reverse: 5′-GAA

TTCAAAAAAAATAAGCTCAGATTCTCACT CTTCAAGAGAGAGGTGAGAATCTGAGCTT ATTCCA-3′.

7. In Vitro Cell Migration Assay

SiHa cells were transfected with TAP1 overexpression plasmid and interference plasmid, respectively. After 24 hours of culture, the cells were washed with PBS and suspended in serum-free medium to adjust the cell concentration to $1x10^6$ cells. In a 24-well plate, 700 microlitres of conventional culture medium containing 10 % serum were added to a well, and then the migration chamber was replaced in the well (Millipol, PI8P01250). First, 200 microliters of cell suspension were added to each chamber. The plate was incubated at 37 °C for 24 hours. After the culture was completed, the medium in the chamber was removed and the chamber was gently rinsed with PBS twice. Cells were fixed with formaldehyde (3%) at room temperature for 20 minutes, then washed with PBS and permeabilized with 100 % methanol at room temperature for 20 minutes. After removing methanol and washing with PBS, the solution was stained with 1 % crystal violet at room temperature for 20 minutes. The excess crystal violet was removed and the cells were washed with PBS. Finally, the cells in the chamber were counted under light microscope.

8. Wound-Healing Assay

SiHa cells were seeded in six-well plates and transfected with a plasmid encoding TAP1 overexpression and a plasmid encoding TAP1 interference, respectively. Following a 24-hour incubation period, the cells were observed to have adhered to the surface of the culture vessel, with a cell confluence of approximately 80%. Each hole was then scratched with a capillary (width within one-third of the field of view), serum-free medium was added, and the Olympus IX50 inverted system microscope (Olympus, Inc., Center Valley, PA) was used to take pictures of the exfoliated area every day for three consecutive days.

9. Analysis of immune function

The cervical SCC RNAseq level was obtained from the TGCA database. The immune infiltrating cells (B cells, CD8 + T cells, CD4 + T cells,

neutrophil dendritic cells, dendritic cells and macrophages) and immune checkpoints (SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, LAG3 and PDCD1LG2) were analysed between the TAP1 high expression group and the TAP1 low expression group. Furthermore, the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm was employed to forecast the potential efficacy of TAP1-targeted immunotherapy.

10. Statistical analysis

Spss22.0 software was used for statistical analysis. Multiple group comparisons of two dimensions were performed using Welch one-way ANOVA and Games-Howell test. The difference between two groups was measured by t-test. Correlations between two variables were analysed by Spearman. The difference was statistically significant ($P<0.05$).

Results

1. TAP1 expression is upregulated and positively correlated with P16 and Ki67 in cervical squamous cell carcinoma

In order to observe the difference in the expression of TAP1 in cervical cancer and adjacent tissues, we obtained TAP1 mRNA data from 253 patients with cervical cancer and 22 patients with adjacent tissues from the TCGA and GTEx database. It was found that TAP1 was higher expressed in cervical cancer tissues(Fig1 A) and the TAP1-high group showed a worse prognosis by Wilcoxon analysis using R software package(Fig1 B). Moreover, TAP1 in cervical SCC was positively correlated with P16 and Ki67(Fig1 C,D).

2. TAP1 expression is upregulated in LSIL,HSIL and SCC,and positively correlated with P16 and KI67

We further verified 130 clinical samples with high-risk HPV infection, and divided them into 4 groups according to the pathological diagnosis results. Immunohistochemical staining and qRT-PCR were performed to analyze the expression and correlation of TAP1, P16 and Ki67 in the 4 groups. From the results of IHC scores(Fig1 E) and RT-PCR, the expression of TAP1 protein(Fig1 F) and mRNA levels(Fig1 G) in LSIL, HSIL and SCC groups was significantly higher than that in CCI group, and the expression of TAP1 in SCC group was higher than that in LSIL group, but there was no significant difference between HSIL group and LSIL group, the same was true between HSIL group and SCC group. In addition, the expression of TAP1 was positively correlated with P16 and Ki67(Fig1 H,I). These results are consistent with the results of the TCGA database, suggesting that TAP1 may play an important role in the progression of cervical squamous intraepithelial lesions.

Figure1

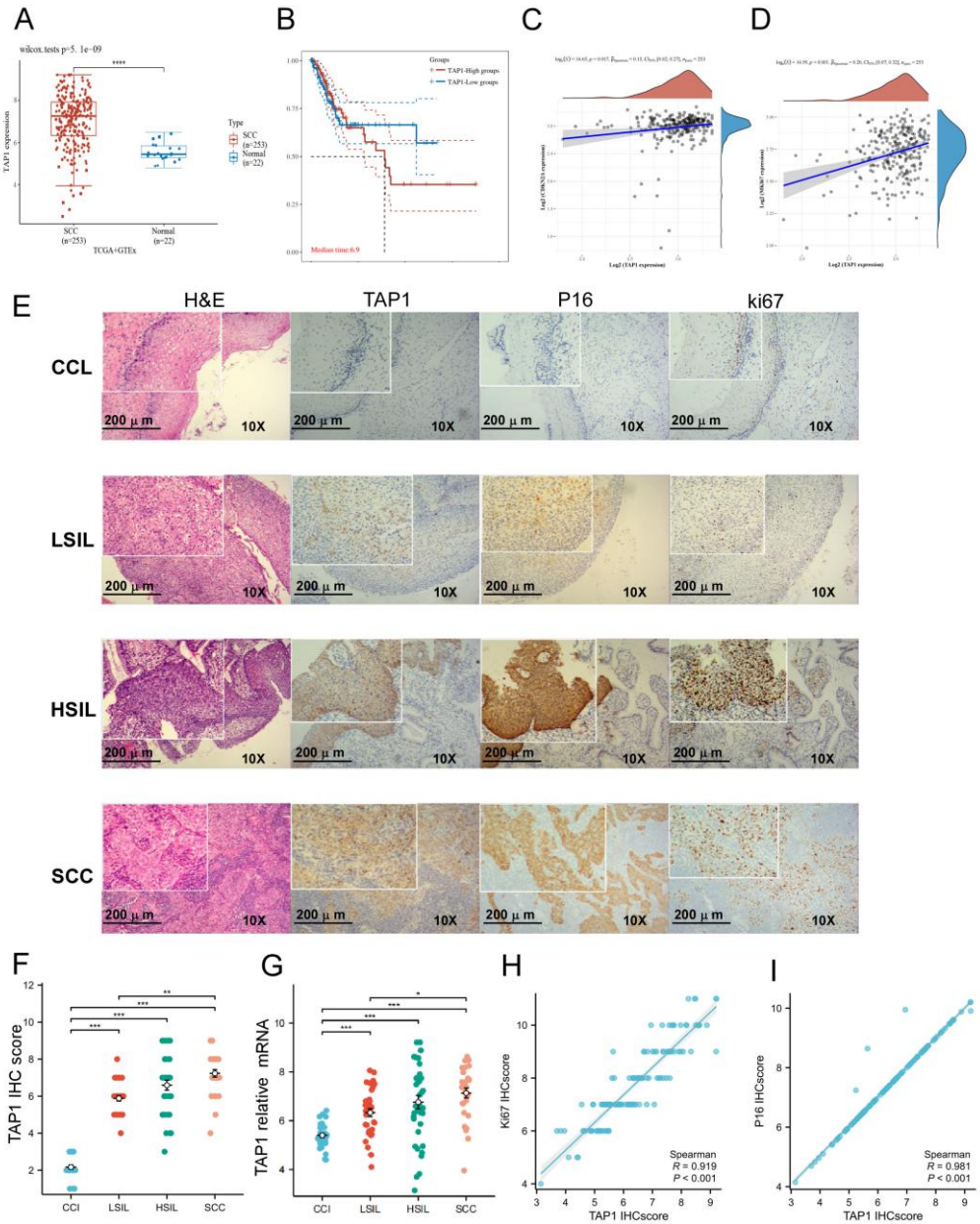

Figure 1. The expression of TAP1 in cervical squamous epithelial lesions. (A: TAP1 was higher expressed in cervical cancer tissues from the TCGA and GTEx database. B: TAP1-high group showed a worse prognosis. C, D: TAP1 in cervical SCC was positively correlated with P16 and Ki67. E: Immunohistochemical results of TAP1, P16 and Ki67 in CCI, LSIL, HSIL and SCC groups. F, G: The expression of TAP1 protein and mRNA levels in LSIL, HSIL and SCC groups was significantly higher than that in CCI group, and the expression of TAP1 in SCC group was higher than that in LSIL group. H, I: The expression of TAP1 was positively correlated with P16 and Ki67.)

3. **TAP1 promotes invasion and migration of cervical squamous cell carcinoma**

To elucidate the biological function of TAP1 in regulating the invasion and migration of cervical SCC and the related pathways involved, we conducted in Vitro Cell Migration Assay and Wound-Healing Assay, and analyzed the activation status of 14 typical pathways using R package PROGENy, and performed functional enrichment analysis using Metascape. Compared with the TAP1-low group, the JAK−STAT, Trail, NFkB, TNF-α, and PI3K pathways are significantly activated in TAP1-high group(Fig2 A), and cell adhesion molecules are enriched in the regulation of TAP1 on cell invasion and migration(Fig2 B). TAP1 was transfected to determine whether TAP1 can regulate the

biological function of cervical SCC and establish TAP1-over and Si-TAP1 cells stably. We further demonstrated that the ability of cell migration and invasion in TAP1-over group was significantly higher than that in Si-TAP1 group by In Vitro Cell Migration Assay and Wound-Healing Assay(Fig2 C,D,E,F).These results suggest that TAP1 may promote distant metastasis of cervical SCC.

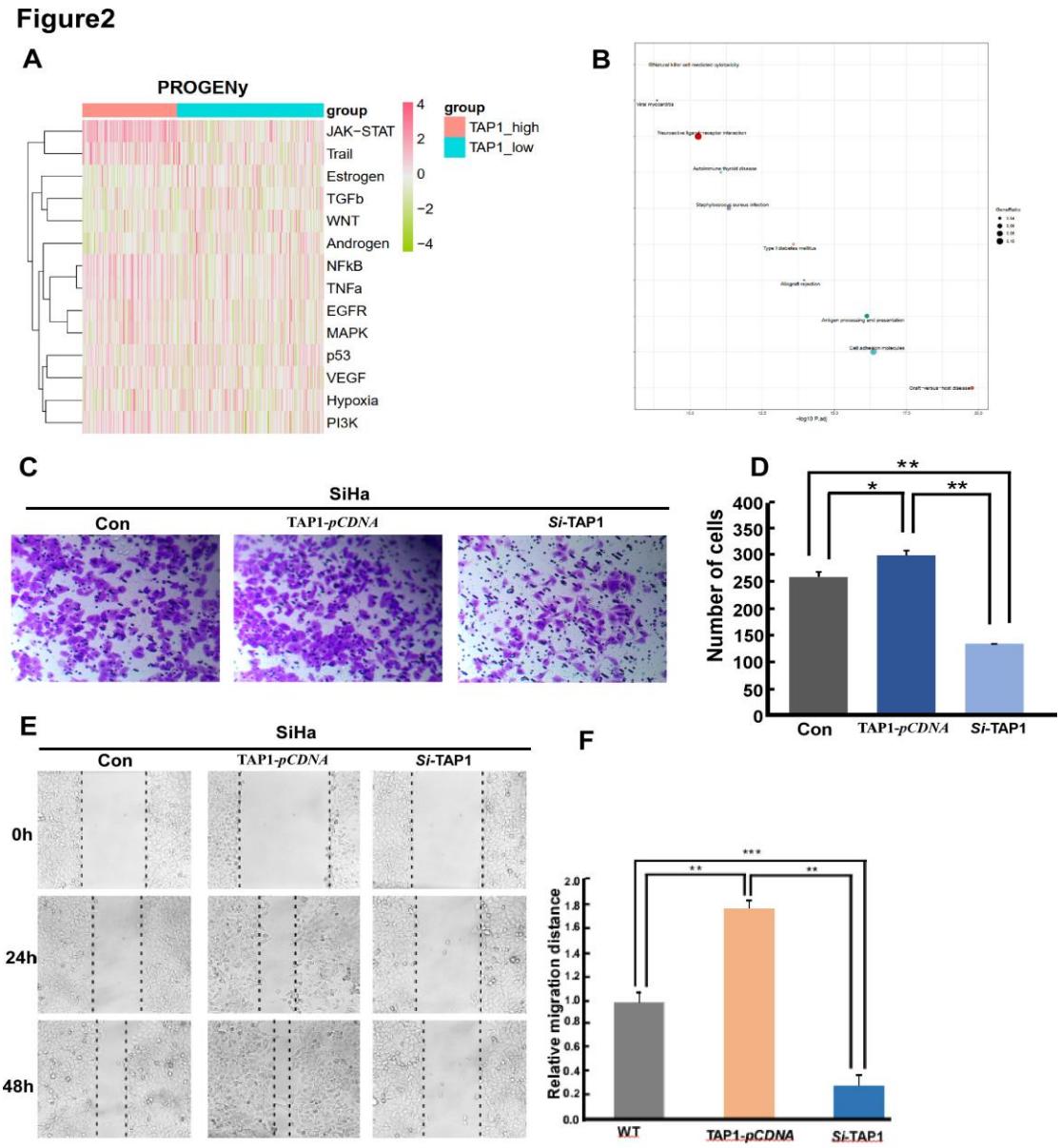

Fig 2. TAP1 promotes invasion and migration of cervical squamous cell carcinoma. (A: the JAK−STAT, Trail, NF-kB, TNF-α, and PI3K pathways are significantly activated in TAP1-high group by PROGENy. B: cell adhesion molecules are enriched in the regulation of TAP1 on cell invasion and migration. C,D,E and F: the ability of cell migration and invasion in TAP1-over group was significantly higher than that in Si-TAP1 group by In Vitro Cell Migration Assay and Wound-Healing Assay.)

4. The immunological function of TAP1 may inhibit tumor immunity, promoting the proliferation and migration of cervical SCC

Tumor microenvironment(TME) is involved in multiple components of surrounding tumor cells, particularly various immune cells[13]. And the immunological infiltration characteristics are related to the biological activity of tumors and the response to immune checkpoint inhibitor (ICI) treatment. Despite the fact that PD-1 has ushered in a new era of cancer treatment as one of ICIs, its efficacy is still limited due to the complexity of tumor immune escape mechanisms. Therefore,

identifying new tumor immune-related genes is crucial for understanding the regulation mechanism of tumor immune microenvironment and establishing an immune-based prognostic model for tumors.

To elucidate the immunological role of TAP1 in cervical squamous epithelial lesions, we analyzed the correlation between TAP1 and immune infiltrating cells in cervical SCC based on TGCA data, and investigated the distribution differences of immune checkpoint-related molecules between the TAP1-H group and TAP1-L group, and predicted the immune therapeutic effect of TAP1 using the TIDE algorithm.

TAP1 expression showed a significant correlation with immune infiltrating cells. There was a positive correlation between B cells, CD8+ T cells, CD4+ T cells, neutrophils and dendritic cells with TAP1 (Figure 3A). From the expression distribution of immune checkpoint genes in the TAP1-H group and the TAP1-L group (Figure 3B), it was found that the immune checkpoint genes in the TAP1-H group were significantly higher than those in the TAP1-L group, except for SIGLEC15. These results suggest that high expression of TAP1 not only increases tumour immune infiltration, but also promotes tumour immune escape, inhibits tumour immunity and thereby promotes the invasive and metastatic biological behaviour of tumours. Based on the prediction of the immunotherapeutic response of TAP1 by the TIDE algorithm (Figure 3C), the TIDE score of the TAP1-H group is lower than that of the TAP1-L group, suggesting that the TAP1-H group may have a better immunotherapeutic effect. However, compared to the TAP1-L group, the difference in immunotherapy is not significant.

Figure 3

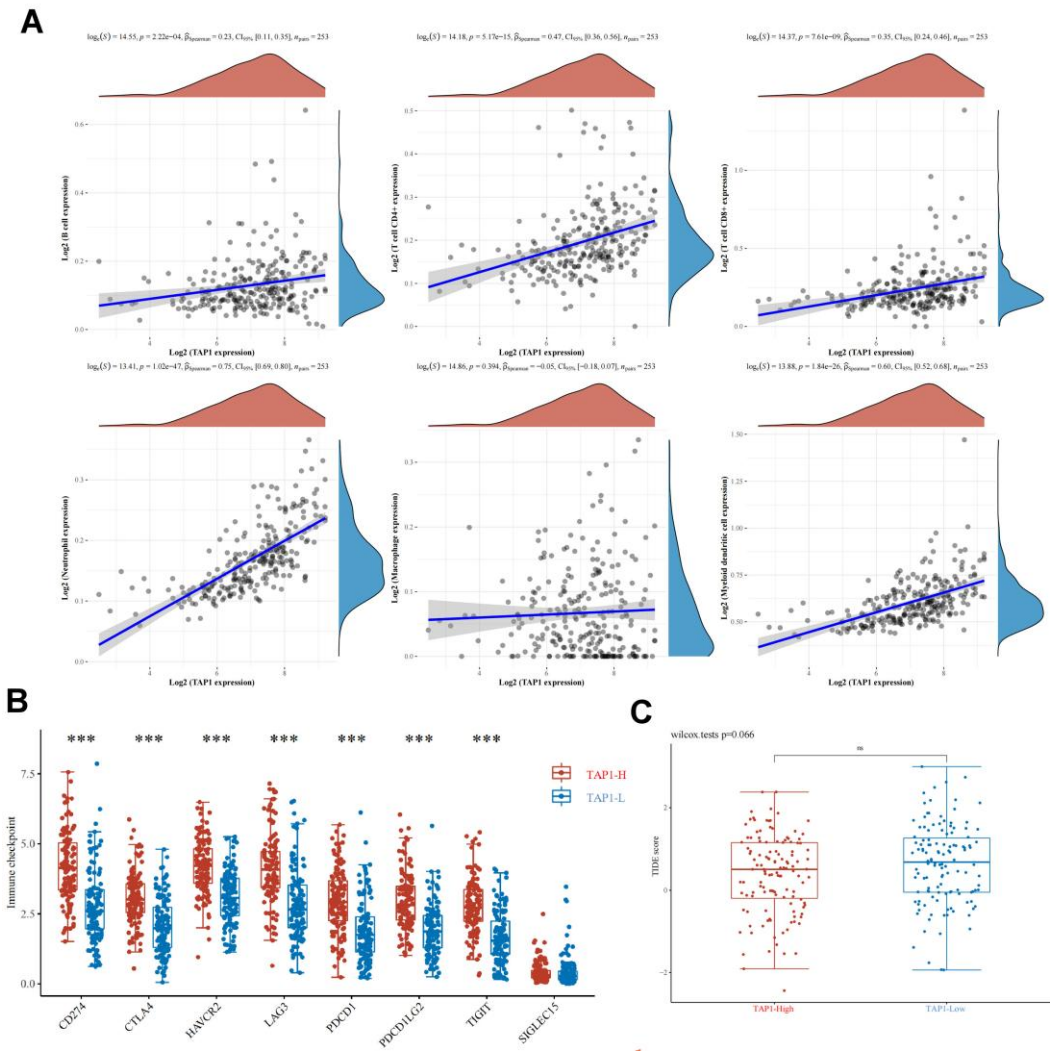

Figure 3. The immunological function of TAP1. (A: There was a positive correlation between B cells,

CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells with TAP1. B: the immune checkpoint genes in the TAP1-H group were significantly higher except for SIGLEC15. C: The TIDE score of TAP1-H group was lower than that of TAP1-L group, but there was no statistical significance.)

Discussion

Cervical squamous cell carcinoma (CSCC) is a common form of cervical cancer and is mainly caused by high-risk human papillomavirus (HR-HPV) infection[14]. Currently, the main treatment for CSCC is surgery and radiotherapy, but these methods cannot completely cure patients with advanced stage disease[15]. Immunotherapy has gradually become an important direction in the treatment of CSCC. A typical example is that immunotherapy represented by anti-PD-1 monoclonal antibody has brought great changes in the field of cancer treatment. Unfortunately, only some patients are able to achieve long-term efficacy and long-term survival from anti-PD-L1 treatment[16]. Therefore, research into more accurate immunotherapy methods and biomarkers is considered important for effective CSCC treatment.

Recently, emerging studies have shown that transporter associated with antigen processing 1 (TAP1) plays a critical role in innate immune antigen presentation. Specifically, when antigen is presented by MHC-I, TAP1 transports the antigenic peptide from the surface of the endoplasmic reticulum (ER) to the ER lumen[17]. However, the role of TAP1 in tumours seems to remain controversial. This is due to the function of TAP1 in antigen presentation, with high TAP1 expression corresponding to the appearance of more neoantigen epitopes that promote recognition by phagocytes, T cells and other cells to inhibit tumour growth, invasion and metastasis[18]. In contrast, most cancer studies have suggested that TAP1 is an oncogene. Theoretically, downregulation of TAP1 expression could lead to a reduction of the MHC-I complex on the cell membrane, allowing tumour cells to evade recognition by cytotoxic T cells and ultimately achieve immune escape[19]. Kasajima[20]et al. demonstrated an association between downregulation of TAP1 expression and loss of inflammatory response, suggesting a poor prognosis in colorectal cancer patients. Leone[21]et al. found that downregulation of TAP1 expression leads to empty loading of MHC-I molecules, suggesting that tumour cells evade NK cell recognition by expressing HLA molecules on the surface. However, the mechanisms by which TAP1 affects tumour biological behaviour differ in different tumour environments. Overexpression of TAP1 in ovarian cancer patients is associated with poor prognosis[22], and promotes tumour migration and metastasis in vivo and in vitro. Wang[23] et al. demonstrated the promoting role of TAP1 in the migration and metastasis of clear cell renal cell carcinoma (CCRCC, Table 1) by in vitro experiments, and found that the high expression of TAP1 in CCRCC suggested a poor prognosis. Meanwhile, TAP1 expression in early CCRCC (stage 1 and stage 2) was found to increase with tumour stage, indicating that TAP1 was involved in other mechanisms besides antigen presentation in CCRCC development. TAP1 was highly expressed in the side population (SP, **Table 1**) of pancreatic ductal adenocarcinoma (PDAC, **Table 1**), while SP cells were more resistant to chemotherapy than other tumour cells. Notably, SP cells are considered as cancer stem cell candidates, which is a promising therapeutic method to develop more effective targeted therapy for PDAC patients[24]. Likewise, Zhou[25]*et al* suggested that TAP1 may play a vital role in mediating chemoresistance to Hedgeg signalling in hepatocellular carcinoma. Tabassum[26]*et al* also discovered that TAP1 was significantly overexpressed in liver, ovarian, lung, and breast cancers compared with the corresponding normal tissues. Henle's team[27]found that TAP1 was low or negatively expressed in early-stage breast cancer, but was significantly overexpressed in advanced breast cancer regardless of HER-2, estrogen, and progesterone levels. Besides, previous studies discovered that the overexpression of TAP1 in advanced breast cancer was associated with the infiltration of immune cells into the tumour tissue, which could secrete IFN-γ, leading to the upregulation of TAP1 in the tumour cells. Bioinformatic analysis showed that the most probable transcription factor of TAP1 was IRF1, which was upregulated under IFN-γstimulation[23]. Although IRF2 was a transcriptional repressor, it could activate the expression of TAP1[28]. Gameiro[29] described

that the expression of TAP1 in the head and neck squamous cell carcinoma(HNSCC, **Table 1**) was associated with HPV status, and the expression of TAP1 was higher in HPV+HNSCC than in HPV-HNSCC, which was owing to the fact that HPVE6/E7 oncoproteins could escape immune surveillance. These findings suggested that overexpression of TAP1 may inhibit tumour immunity and promote tumour immune escape, leading to tumour progression. However, the role of TAP1 in cervical intraepithelial lesions remains unclear.

With the objective to further investigate the functional role of TAP1 in cervical squamous intraepithelial lesions, we conducted bioinformatic and immunological functional analyses based on multiple databases, and verified the correlation between TAP1 expression and invasion and metastatic ability by in vitro experiments. Our results indicated that TAP1 was upregulated at both protein and mRNA levels in LSIL, HSIL and SCC compared with the CCI group, and was positively correlated with P16 and KI67; the expression of TAP1 in the SCC group was higher than that in the LSIL group. Consistent with the data from bioinformatics analysis, the data from TCGA analysis revealed that the expression of TAP1 in cervical SCC was significantly higher than that in the adjacent normal tissues, and was positively correlated with P16 and KI67. P16 has been well-established as a surrogate marker for HPV infection in promoting the development of cervical squamous cell carcinoma [4]. Ki67[30], a cell proliferation marker, has been shown to increase in expression with the severity of cervical squamous intraepithelial lesions, indicating a higher malignant potential and increased invasive ability. There was a positive correlation between the expression of TAP1 and both of them, which suggested that upregulation of TAP1 could promote the proliferation, invasion and metastasis of cervical squamous epithelial lesions.

In addition, it was showed that the TAP1 overexpression group exhibited stronger invasion and migration abilities in vitro experiments. We also found that the JAK/STAT signalling pathway was more active in the TAP1 high-expression group in CSCC. The JAK/STAT pathway plays an important role in tumour proliferation, invasion, and migration. There is increasing evidence that IL-10 can activate JAK1 and JAK3, which in turn activate STAT3 and STAT1, thereby suppressing the immune response and promoting tumour cell invasion and metastasis[31]. Moreover, the pro-tumorigenic signal IL-6 enable inhibit the function of APC including TAP1 and TAP2 by activating the JAK/STAT pathway, promoting the differentiation of Treg cells and ultimately promoting tumour growth[32]. Interestingly, we found that TAP1 was mainly enriched in cell adhesion function by gene function enrichment analysis. In conclusion, it is reasonable to explain that up-regulation of TAP1 expression can inhibit tumour immunity and promote tumour proliferation, invasion and metastasis, which is closely related to the activation of tumour-promoting factors in the JAK/STAT pathway and changes in cell adhesion.

Analysis of the immune function of TAP1 in CSCC showed that the expression of TAP1 was correlated with tumour immune infiltrating cells, and the expression of immune checkpoint genes was higher in the TAP1-H group. Except for macrophages, TAP1 was positively correlated with immune regulatory cells, including B cells, CD8 + T cells, CD4 + T cells, neutrophil dendritic cells, and dendritic cells, and the expression of immune checkpoint genes was significantly increased in the TAP1-H group except for SIGLEC15. High levels of TAP1 expression are associated with better response to immune checkpoint inhibitors based on Tumour Immune Dysfunction and Exclusion (TIDE). In conjunction with the existing literature, functional enrichment results, and the current findings, one potential explanation is that the upregulation of TAP1 expression facilitates the infiltration of more immune cells into the tumour microenvironment. However, the overexpression of TAP1 increases the expression of the oncogene E6/E7, which enables tumour cells to evade the surveillance of immune cells, thereby promoting the occurrence and progression of the tumour[33]. In previous studies, hypermethylation of gene promoters has been associated with downregulation of TAP1 protein expression in esophageal squamous cell carcinoma, colon carcinoma[34], renal cell carcinoma and melanoma. Hasim's research[35] demonstrated that the methylation level of the TAP1 gene in CSCC tissues was significantly lower than that in cervical intraepithelial neoplasia (CIN) and

normal tissues, which may also be one of the reasons for up-regulation of TAP1 expression. Another experiment indicated that TAP1 is overexpressed in various tumors and suggests a worse prognosis, it has been reported in the literature that TAP1 transcription is decreased in tissue culture models[36], indicating that TAP1 function is more complex and requires further research to understand its function and mechanism of occurrence. However, there are also shortcomings in our current experiments, such as we did not classify HPV types and further verify the mechanism, this work will be further improved in our next experiment. Based on our experimental results and literature reading, we mapped the possible mechanism of TAP1 promoting the progression of cervical squamous epithelial lesions (Figure 4).

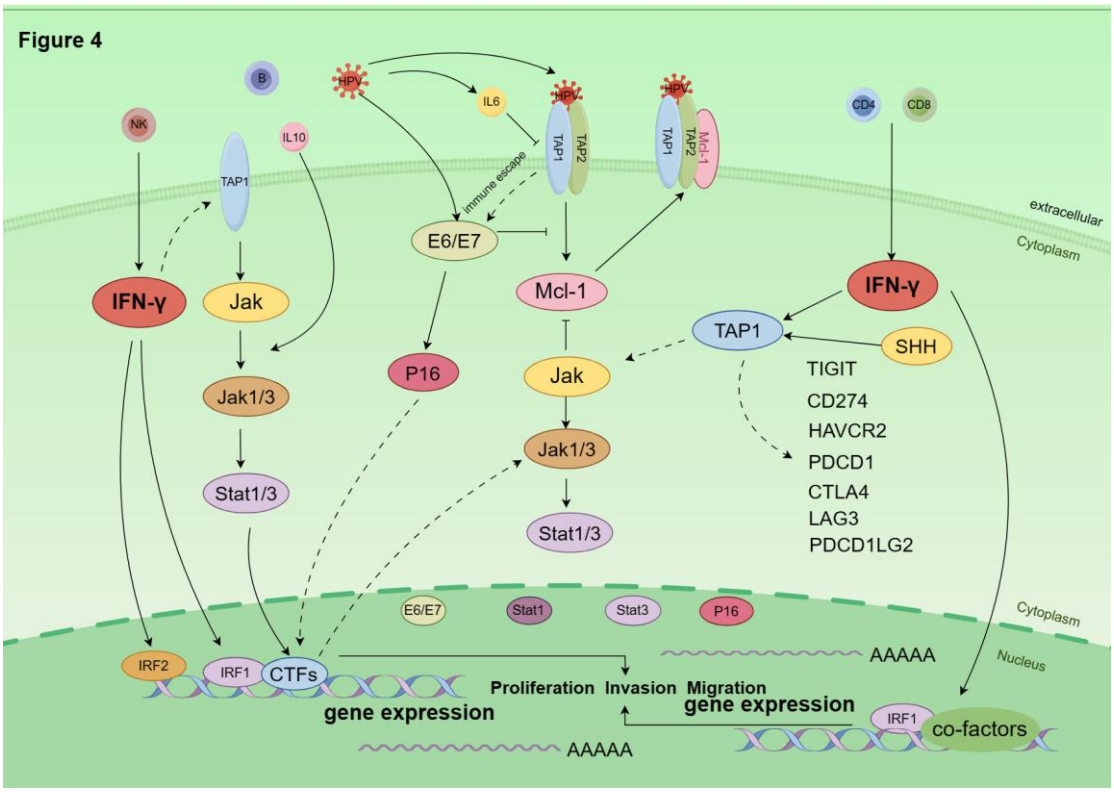

Figure 4. The possible mechanism of TAP1 promoting the progression of cervical squamous epithelial lesions. After human infection with HPV, the increased secretion of IFN-γ promotes the up-regulation of TAP1 expression and activates the JAK / STAT pathway, which leads to the overexpression of E6 / E7, thereby inhibiting tumor immunity and leading to tumor progression.

Table 1. Glossary of abbreviations

Abbreviation	English full title
TAP1	transporter associated with antigen processing 1
CCI	cervical chronic inflammation
LSIL	low-grade squamous intraepithelial lesions
HSIL	high-grade squamous intraepithelial lesions
CSCC	cervical squamous cell carcinoma
CIN	cervical intraepithelial neoplasia
IHC	immunohistochemical
MHC-I	major histocompatibility complex class I
CCRCC	clear cell renal cell carcinoma
SP	side population cells
PDAC	pancreatic ductal adenocarcinoma
HNSCC	the head and neck squamous-cell carcinoma

Conclusion

We revealed that TAP1 was up-regulated in cervical squamous cell carcinoma and positively correlated with P16 and Ki67, indicating poor prognosis by analysis of the TCGA and GTEx databases. Meanwhile, TAP1 expression was also upregulated in LSIL, HSIL and SCC and positively correlated with P16 and Ki67 in 130 clinical samples. In conclusion, we confirmed that TAP1 can promote the progression of cervical squamous intraepithelial lesions and inhibit tumour immunity. Thus, TAP1 has a promising investigative prospect in the two-way track of clinical and mechanism research in response to HPV + cervical squamous intraepithelial lesions.

Author Contributions: J.C. organized and wrote the manuscript; Q.W. performed the experiment and statistical analysis; MS.L, X.L. and E.H. performed the experiment; FG.H. designed the structure and framework of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and material

Not applicable.

Declarations

Conflict of interest

The authors declare no relevant competing interests to disclose.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Bishan Hospital of Chongqing Medical University, the approval number of the Medical Ethics Committee is 2019-ky-07. Each patient provided sample acquisition and informed consent for the study in accordance with approved guidelines.

Consent for publication

Not applicable.

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