



Original Research

Expression and prognosis of CyclinA and CDK2 in patients with advanced cervical cancer after chemotherapy

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Abstract: This study aimed to investigate the expression and prognosis of CyclinA and CDK2 in patients with advanced cervical cancer after chemotherapy. The patient history of 108 patients with advanced cervical cancer admitted to our hospital from December 2013 to January 2016 was selected as a cervical cancer group. 54 normal healthy people admitted to our hospital for physical examination in the same period were selected as the control group. Western blotting and RT-PCR were used to detect the difference between CyclinA and CDK2 proteins and mRNA expression between the two groups and the correlation between them was analyzed. The expressions of CyclinA and CDK2 in serum and the changes in detection index level of squamous cell carcinoma antigen (SCCA), carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) were observed in cervical cancer group at different stages of treatment. The correlation between the two indexes and SCCA, CEA, VEGF and the 3-year survival and prognostic significance of cervical cancer patients with different CyclinA and CDK2 expressions were analyzed. The relative expressions of CyclinA and CDK2 proteins and mRNA in the cervical cancer group were significantly higher than those in the control group ($P < 0.05$). Pearson correlation analysis showed a positive correlation between CyclinA and CDK2 proteins and mRNA expressions. After treatment, the expressions of CyclinA, CDK2 mRNA and SCCA, CEA and VEGF were significantly lower than those before treatment ($P < 0.05$). The 3-year survival rate of CyclinA and CDK2 in the high expression group was significantly lower than that of the low expression group. CyclinA and CDK2 are highly expressed in advanced cervical cancer. The expression is decreased after chemotherapy. The prognosis of both low expressions is higher and the expression is good. It can be used to predict the efficacy and prognosis of cervical cancer in the clinic.

Key words: CyclinA; CDK2; Advanced cervical cancer; Chemotherapy; Prognosis.

Introduction

Cervical cancer is a kind of gynecological malignant disease including in situ and infiltration disease. With the promotion of screening technology, the efficacy of cervical cancer vaccine against human papillomavirus (HPV) subtypes with a high risk of carcinogenesis has also been improved. However, there are still a large number of women diagnosed with cervical cancer and die of this disease every year (1, 2). In the treatment of cervical cancer, surgery, radiotherapy and chemotherapy are often selected and progress has been made in the clinic. Surgery can achieve the goal of eradication to a certain extent, but the surgical treatment is not suitable for a small number of patients, such as the spread of parametrium and adjacent organs. Therefore, more patients with advanced cervical cancer have a poor prognosis (3, 4). The poor prognosis of most patients with cervical cancer is related to early undiagnosed, clinical-stage, judging prognosis and treatment options. Therefore, screening and diagnosis for cervical cancer are essential for targeted treatment and management to

improve the survival time of patients.

Tumor development is a complex process of multiple genes and stages that are abnormally expressed by excessive differentiation and proliferation of cells and belongs to a kind of periodic disease of cells (5-7). Among them, cyclin-dependent-kinases (CDKs) and cyclinA factors play a positive regulatory role when they participate in the regulation of tumor cell cycle (8, 9). CDKs are a class of protein kinases composed of histidine/threonine that act cooperatively cells with cyclin. The CDK2 gene has high activity in the CDKs family. Its expression regulates the transition from the G1 phase to the S phase and accelerates the progress rate of the S phase and the proliferation efficiency of cells through high expression (10). cyclinA is a member of a family of cell cycle genes with a relative molecular weight of 60×10^3 located on the 4q27 chromosome and plays an important role in all stages of cell synthesis and division (11, 12). Currently, the relationship between Cyclin A, CDK2 genes and advanced cervical cancer has not been reported. In order to understand the expression of Cyclin A and CDK2 genes in advanced cervical cancer

and its relationship with the treatment and prognosis of the disease, we investigated the protein and mRNA expressions of Cyclin A and CDK2 genes and analyzed their changes and survival time during the treatment of patients, so as to explore the influence of Cyclin A and CDK2 genes on the disease progression and prognosis of advanced cervical cancer.

Materials and Methods

Baseline data

The patient history of 108 patients with advanced cervical cancer admitted to our hospital from December 2013 to January 2016 was selected as a cervical cancer group. Among them, the age was 36–63 years old, the average age was (49.44±3.42) years old and BMI was (18.93±1.83) kg/m². Disease types were adenocarcinoma in 28 cases, adenosquamous carcinoma in 63 cases and squamous cell carcinoma in 17 cases. Cell classification was 11 patients with G1, 41 patients with G2 and 56 patients with G3. 54 normal healthy people admitted to our hospital for physical examination in the same period were selected as the control group. The age was 36–62 years old, the average age was (49.27±3.29) years old and BMI was (18.96±1.88) kg/m².

All patients with advanced cervical cancer were followed up regularly or reexamined in the clinic by the end of the chemotherapy treatment plan. The follow-up frequency reached an average of once every 3 months. The interval between follow-up should not be too long. The first follow-up after discharge was about 1 month. The data did not lose the case as of February 2019, with a total follow-up of 3 years.

Inclusion and exclusion criteria

Inclusion criteria: Patients with advanced cervical cancer diagnosed by CT, colour doppler ultrasonography or biopsy were included. Exclusion criteria are a) exclusion of patients with pelvic or lymph node metastasis before the test; b) exclusion of patients receiving other immunochemotherapy; c) exclusion of patients in comorbid with hematopoietic or immune system disorders and other organ dysfunction; and d) exclusion of patients who are pregnant or lactation at this stage. The experiment was conducted with ensuring that all patients and their families agree to carry out the study and sign informed consent. All the research processes have been approved by the Medical Ethics Committee.

Experimental reagents and materials

Cisplatin was purchased from Tonghua Maoxiang Pharmaceutical Co., Ltd., approval number: SFDA Approval No. HTC22966. The RNA extraction kit was purchased from Omega, USA. Trizol reagent and reverse transcription kit were purchased from Bei Jing Think-Far Technology Co., Ltd. SYBR Green PCR Master Mix was purchased from Shanghai Xibainuo Biology. All the primers and sequencing were delivered to Shanghai Sangon Biological Engineering Co., LTD. BCA kit and SDS-PAGE were purchased from Shanghai Ruji Biotechnology Co., Ltd. Rabbit anti-human CyclinA, CDK2 primary antibody and horseradish peroxidase were purchased from Bioworld, USA.

Experimental methods

Chemotherapy methods

All patients with advanced cervical cancer were treated with 50 mg/m² of cisplatin for chemotherapy. To prevent side effects such as nephrotoxicity, the subjects were given adequate hydration and diuretic before chemotherapy. 0.9% sodium chloride injection was diluted to 500 ml. Intravenous infusion of 50 mg/m² was conducted in each time, the frequency of chemotherapy was 1 time/week and 4 weeks was a course of chemotherapy.

Detection of the expression of CyclinA and CDK2 mRNA by RT-PCR

Blood samples from patients with advanced cervical cancer were collected and precipitated for 1h before treatment for 1 week, during chemotherapy and at the first time of follow-up, and then centrifuged at 3000 r/min and 4 °C for 5 min. The extraction and reverse transcription of total miRNA were conducted according to the manufacturer's instructions. Purity and concentration were measured by ultraviolet spectrophotometer. B-actin was set as a reference gene. The reaction volume was 5 ul SYBR Green PCR Master Mix, 0.5 ul cDNA, 10 ul of each 0.25 ul upstream and downstream primers and 4.0 μL of double-distilled water. The conditions and steps were as follows: 3 multiple pores were set for each sample and gene and 10 ul of the reaction mixture was incubated in a lamina Lucida at 95 °C for 5 min, denatured at 95 °C for 15 s and annealed, extended at 60 °C for 60 s. Finally, detection of the melting curve was conducted at 60–95 °C. After a total of 40 cycles, data detection of each sample was collected and analyzed. The corresponding Ct values of each gene were calculated and the relative expression of the two was calculated by using 2^{-ΔΔCt}.

CyclinA upstream primer was 5'-GGGATGGCATT-TGGGTG-3' and downstream primer was 5'-TCGAC-TGGGAGAGGAGAGATG-3'; CDK2 upstream primer was 5'-TGCCTTAAACACTCACCTTC-3' and downstream primer was 5'-CTTCAGTCTCCCAGCC-TATT-3'; β-actin upstream primer was 5'-CCTG-TACGCCAACACAGTGC-3' and downstream primer was 5'-ATACTCCTGCTTGCTGATCC-3'.

Detection of the expression of CyclinA and CDK2 proteins by Western blotting

After the PMSF+RIPA cell lysis buffer was added, lysed and centrifuged in each group of tissues, the total protein was collected and the concentration was measured by BCA. The equal amount of protein and buffer mixture was boiled in water at 100 °C for 5 min. Protein separation requires two steps of 10% SDS-PAGE electrophoresis concentration and separation. Electrical transfer to PVDF membrane. 5% skim milk was blocked at room temperature for 2 h and the membrane was washed by TBST. Rabbit anti-human CyclinA, CDK2 primary antibody (1:500) was added and incubated overnight at 4 °C, and the membrane was washed by TBST. Horseradish peroxidase (1:1 000) was labeled at room temperature for 1 h and the membrane was washed by TB-ST. Finally, exposure, color and fixing were conducted. ImageJ determined the density of its zone to calculate and analyse. The expression levels of

CyclinA, CDK2 protein = the pixel gray of CyclinA, CDK2 protein expression in each cell - the expression pixel gray of internal control β -actin

Detection of serum indexes

The serum was taken in the same way as in 2.4.2. Serum VEGF levels were determined by enzyme-linked immunosorbent assay after serum collection. The normal value was 0-142 pg/mL. Serum CEA, SCCA levels were detected by chemiluminescent microparticle immunoassay. The normal reference range of CEA and SCCA was ≤ 5 ng/mL and ≤ 5 ng/mL. The measured value was positive above the normal range.

Observation indexes

The following steps were performed: a) Western blotting and RT-PCR were used to detect the difference of CyclinA and CDK2 protein and mRNA expression in the two groups; b) The correlation of CyclinA, CDK2 protein and mRNA expression was analyzed; c) The expression of CyclinA and CDK2 in serum and the changes of detection index level of squamous cell carcinoma antigen (SCCA), carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) were observed in cervical cancer group at different stages of treatment; d) The correlation between the two indexes and SCCA, CEA, VEGF was analyzed; and e) The correlation between high and low classification and 3-year survival prognosis of patients with different CyclinA and CDK2 expression in cervical cancer group was analyzed.

Statistical methods

In this experiment, SPSS 19.0 statistical software (Beijing Net Number Times Technology Co., Ltd.) was applied for statistical analysis of the data. The measurement data were expressed by mean number \pm standard deviation. The changes in detection indexes in different periods of treatment were compared by one-way ANOVA. T-test was used between the groups. Pearson was used to analyzing the correlation between the two proteins and miRNAs and the relationship between them and SCCA, CEA and VEGF. Kaplan-Meier survival curves were used to map the prognosis of patients. The log-rank test was used for analysis. In this experiment, Graphpad Prism8 was used to draw operational picture samples. The difference was statistically significant with $P < 0.05$.

Results

Detection of the expression of CyclinA and CDK2 proteins between the two groups by Western blotting

The results of Western blotting detection showed that the relative expression of CyclinA and CDK2 proteins in the cervical cancer group were significantly higher than those in the control group based on Figure 1 ($P < 0.05$).

Detection of CyclinA and CDK2 mRNA expression between the two groups by RT-PCR

The results of RT-PCR detection showed that the relative expression of CyclinA and CDK2 mRNA in the cervical cancer group were significantly higher than

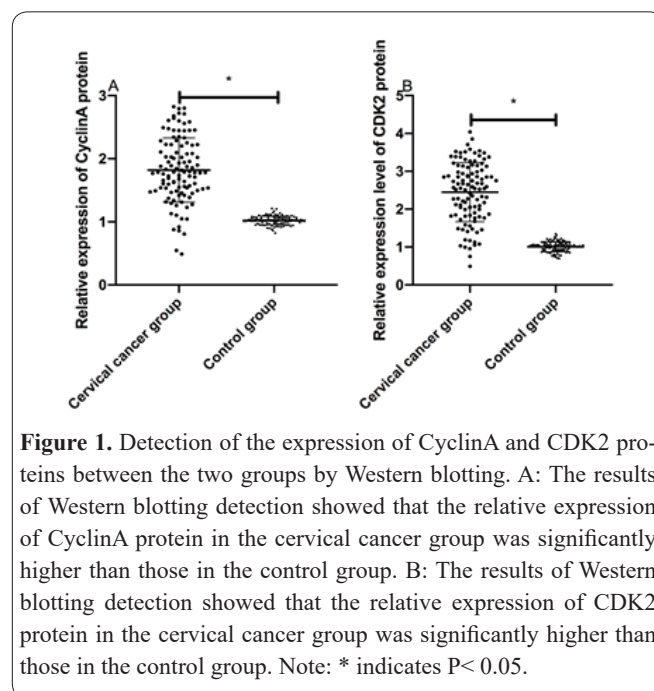


Figure 1. Detection of the expression of CyclinA and CDK2 proteins between the two groups by Western blotting. A: The results of Western blotting detection showed that the relative expression of CyclinA protein in the cervical cancer group was significantly higher than those in the control group. B: The results of Western blotting detection showed that the relative expression of CDK2 protein in the cervical cancer group was significantly higher than those in the control group. Note: * indicates $P < 0.05$.

those in the control group ($P < 0.05$) according to Figure 2.

The relationship between CyclinA, CDK2 protein and mRNA expression

Pearson correlation analysis showed a positive correlation between CyclinA protein and mRNA expression ($r = 0.816$, $P < 0.001$). There was a positive correlation between CDK2 protein and mRNA expression ($r = 0.887$, $P < 0.001$) based on Figure 3.

Serum CyclinA and CDK2 mRNA expression in different periods of treatment

CyclinA and CDK2 mRNA expression decreased gradually with treatment and the detection was lowest after treatment ($P < 0.05$) based on Table 1.

Serum SCCA, CEA, VEGF expression in different periods of treatment

SCCA, CEA and VEGF expression decreased gradually with treatment and the detection was lowest

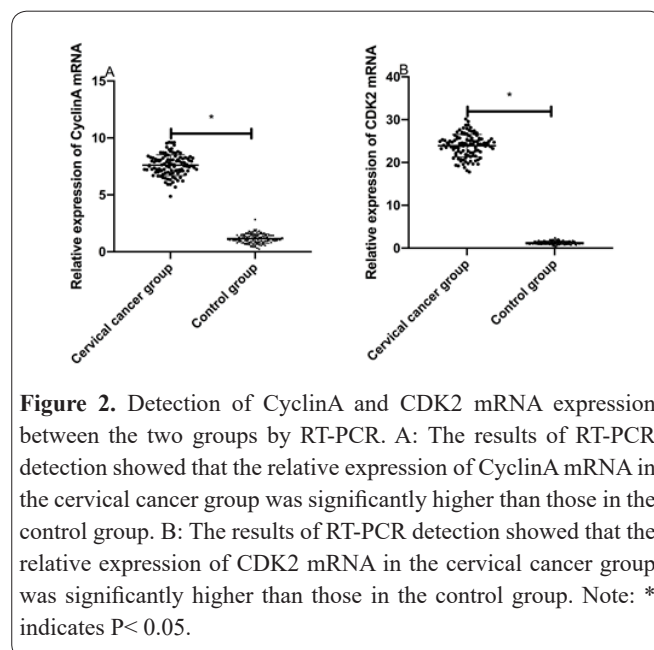


Figure 2. Detection of CyclinA and CDK2 mRNA expression between the two groups by RT-PCR. A: The results of RT-PCR detection showed that the relative expression of CyclinA mRNA in the cervical cancer group was significantly higher than those in the control group. B: The results of RT-PCR detection showed that the relative expression of CDK2 mRNA in the cervical cancer group was significantly higher than those in the control group. Note: * indicates $P < 0.05$.

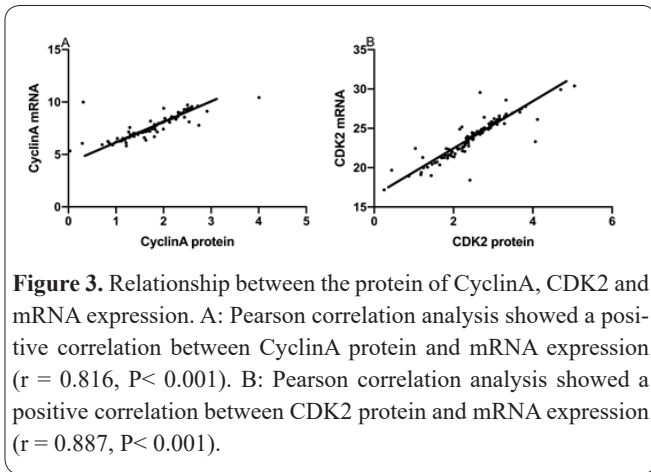


Figure 3. Relationship between the protein of CyclinA, CDK2 and mRNA expression. A: Pearson correlation analysis showed a positive correlation between CyclinA protein and mRNA expression ($r = 0.816, P < 0.001$). B: Pearson correlation analysis showed a positive correlation between CDK2 protein and mRNA expression ($r = 0.887, P < 0.001$).

after treatment ($P < 0.05$). More details are shown in Table 2.

Correlation analysis between the expression of CyclinA in serum and the expression of SCCA, CEA and VEGF

Pearson correlation analysis showed that CyclinA was positively correlated with the expression of SCCA, CEA and VEGF ($r=0.862, P<0.001$), ($r=0.876, P<0.001$), ($r=0.880, P<0.001$) based on Figure 4.

Correlation analysis between the expression of CDK2 in serum and the expression of SCCA, CEA and VEGF

Pearson correlation analysis showed that CDK2 was positively correlated with the expression of SCCA, CEA and VEGF ($r=0.849, P<0.001$), ($r=0.901, P<0.001$), ($r=0.741, P<0.001$) according to Figure 5.

Correlation between different CyclinA, CDK2 expression and 3-year survival prognosis in patients with advanced cervical cancer

According to the optimal cutoff of CyclinA and CDK2 expression after treatment, 52 patients ($CyclinA \geq 5.62$) were divided into CyclinA high expression group and 56 patients were CyclinA low expression group. 55 patients ($CDK2 \geq 16.82$) were divided into CDK2 high expression group and 53 patients were the CDK2 low expression group. The Kaplan-Meier survival curve showed that the 3-year survival rate of the CyclinA high expression group (21.15%) was significantly lower than that of a 3-year survival rate of in the low

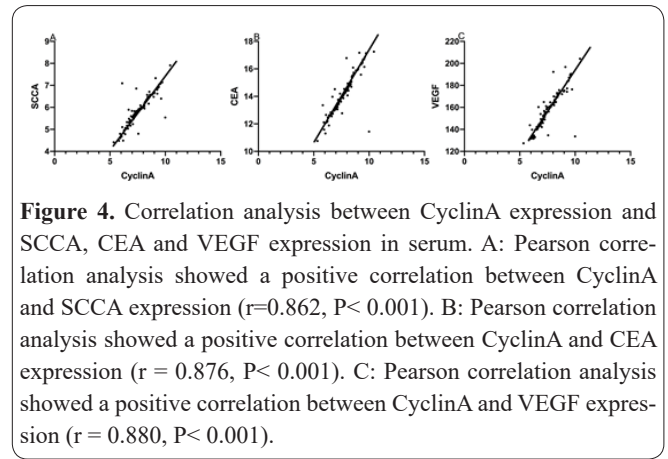


Figure 4. Correlation analysis between CyclinA expression and SCCA, CEA and VEGF expression in serum. A: Pearson correlation analysis showed a positive correlation between CyclinA and SCCA expression ($r=0.862, P < 0.001$). B: Pearson correlation analysis showed a positive correlation between CyclinA and CEA expression ($r = 0.876, P < 0.001$). C: Pearson correlation analysis showed a positive correlation between CyclinA and VEGF expression ($r = 0.880, P < 0.001$).

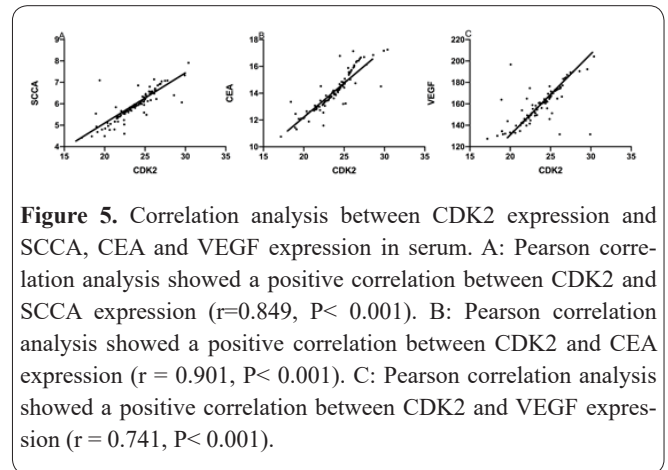


Figure 5. Correlation analysis between CDK2 expression and SCCA, CEA and VEGF expression in serum. A: Pearson correlation analysis showed a positive correlation between CDK2 and SCCA expression ($r=0.849, P < 0.001$). B: Pearson correlation analysis showed a positive correlation between CDK2 and CEA expression ($r = 0.901, P < 0.001$). C: Pearson correlation analysis showed a positive correlation between CDK2 and VEGF expression ($r = 0.741, P < 0.001$).

expression group (42.86%). The 3-year survival rate of the CDK2 high expression group (18.18%) was significantly lower than that of the 3-year survival rate in the low expression group (47.17%). More details are shown in Figure 6.

Discussion

At present, the prevalence of cervical cancer is only behind breast cancer in the malignant tumor of the female population. With the constant changes in living pressure and environment, the patient's age gradually tends to younger women (13). Due to the insipid nature of early symptoms of cervical cancer, when abnormal phenomena such as abdominal pain and bleeding occur, the disease usually progresses to the middle and late stage, accompanied by metastasis of pelvic, abdominal

Table 1. Serum CyclinA and CDK2 mRNA expression in different periods of treatment.

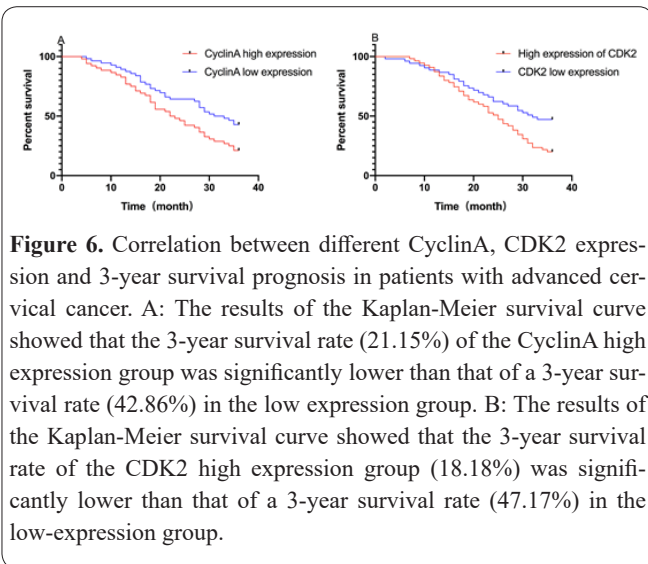
Grouping	Before treatment	During treatment	After treatment	F	P
CyclinA	7.62±1.01	6.24±0.93*	5.62±0.91**	125.200	<0.001
CDK2	23.42±2.64	19.34±2.41*	16.82±2.34**	196.900	<0.001

Note: * represents $P < 0.05$ compared with before treatment and ** represents $P < 0.05$ compared with before and during treatment.

Table 2. Serum SCCA, CEA, VEGF expression in different periods of treatment.

Grouping	Before treatment	During treatment	After treatment	F	P
SCCA (ng/mL)	5.92±0.67	4.18±0.55*	2.01±0.31**	1467.000	<0.001
CEA (ng/mL)	13.92±1.51	8.92±0.89*	4.92±0.56**	1946.000	<0.001
VEGF (pg/mL)	157.34±16.83	149.29±16.19*	136.17±16.01**	46.150	<0.001

Note: * represents $P < 0.05$ compared with before treatment and ** represents $P < 0.05$ compared with before and during treatment.



and lymph node, leading to multiple pain symptoms all over the body, and even death in severe cases (14, 15). In terms of treatment, a small number of cervical cancer patients with low sensitivity to radiotherapy drugs can increase the tumor and sensitivity through the combination of efficacy after chemotherapy, so as to kill tumor cells in different ways and inhibit their repairability (16). Studies on tumor cells have found that the interphase and the mitotic phase can be identified through continuous mitosis, cytokinesis and synthesis. The development of cancer can be induced when the cycling activity is abnormal (17, 18). The expression of CyclinA and CDK2 factors, which often appear to regulate tumor cell cycle changes, are selected before and after the treatment of advanced cervical cancer to analyze their prognosis and therapeutic efficacy.

In order to explore the mechanism of CyclinA and CDK2 participating in the regulation of advanced cervical cancer, the expression of CyclinA and CDK2 were firstly explored and found that the relative expressions of CyclinA, CDK2 protein and mRNA in patients with cervical cancer in this study were significantly higher than those in normal healthy people. The analysis of the relationship between the two proteins and miRNAs revealed that the expression trends of both proteins and miRNAs were the same. These results indicated that both expressions are stable in the body of cervical cancer patients and their expression rises with the increase of the severity of cancer. Studies have shown that high expression of CyclinA can cause a wide range of solid or blood system tumors and cell cycle regulation out of control, promote abnormal changes in cell proliferation ability and induce the development of tumors (19). CDK2 has been reported to have abnormal expression levels in most malignant tumors. Its activity is mostly activated after binding with CyclinA and CyclinA affects its expression (20-22). The results of the expression change in this study have been confirmed. Both of them may have the effect of regulating the proliferation of tumor cells in cervical cancer. In order to explore the relationship between efficacy and the two, we studied the correlation between CyclinA, CDK2, serum cancer cell antigen and angiogenesis index expression of patients in different periods of chemotherapy and found that each test index decreased with the progress of treat-

ment when the treatment operation was continuously completed. CyclinA and CDK2 were positively correlated with each index. SCCA belongs to a serine protease inhibitory glycoprotein located in the cytoplasm of squamous epithelial carcinoma. Its acidic products are mostly present in non-keratinous cancer cells.

The expression of peripheral blood in tumor patients is increased by a large amount of secretion into the blood circulation (23). CEA has a higher positive rate in the later stage of cervical cancer stage when the cell carcinoma is serious (24). VEGF as a specific endothelial cell division element, vascular endothelial cell division and proliferation in vitro can be enhanced by VEGF stimulation and microvascular permeability and endothelial cell gene levels in vivo are improved (25). In addition, some studies have found (26) that chemotherapy can reduce the volume of local lesions by killing tumor cells, inhibiting their repair and hindering the amplification of local lesions. Combined with the results and data, the results showed that chemotherapy inhibited the continuous differentiation and proliferation of tumor cells, reduced the blood transport rate and malignant change rate of cancer cells and achieved the effect of controlling the transformation of the tumor cell cycle. After understanding the effects of the two treatments, the influence on the prognosis of patients was explored and found that when CyclinA and CDK2 expression levels were at a high level, the 3-year survival rate of patients with advanced cervical cancer was worse than that of patients with lower levels of CyclinA and CDK2.

Previous studies have found that Cyclin A overexpression is associated with the prognosis and efficacy of many tumors. Liver cancer cells control the patient's disease deterioration by maintaining the sustained action of Cyclin A to form cells in an uncontrolled manner (27). The survival time of patients with positive expression of CDK2 was lower than that of patients with negative levels of non-small cell lung cancer (28). Previous studies have found that (29) the high expression of Cyclin A and CDK2 is caused by the activation of CDK2 after the combination of Cyclin A and CDK2. The cells are induced to develop from G2 phase to M phase and simultaneously act in G1 phase and S phase. The above literature further illustrated the role of Cyclin A and CDK2 in predicting the outcome of cervical cancer. The treatment options for patients with advanced cervical cancer were determined by the detection of both co-expression trends (28-45).

In summary, CyclinA and CDK2 are highly expressed in advanced cervical cancer. The expression is decreased after chemotherapy. The prognosis of both low expressions is higher and the expression is good. It can be used to predict the efficacy and prognosis of cervical cancer in the clinic. However, the problems in this experiment have not been solved. The serum detection markers that we selected for the control of cervical cancer progression have certain limitations and the possibility of error. More clinical indicators often used to diagnose cervical cancer can be selected to provide more data support.

Authors' contributions

YG wrote the manuscript. HW analyzed and interpreted the patient general data. AZ performed PCR and

Western blot. TY was responsible for observation indicators analysis. All authors read and approved the final manuscript.

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