EXPRESSION OF NESTIN IN CERVICAL CARCINOMA AND ITS CLINICAL SIGNIFICANCE

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ABSTRACT

Objective*: Cervical cancer has become the second most common malignancy of women in the world with its high incidence, high invasiveness, high metastasis, and gradual rejuvenation. The prognosis of patients with cervical cancer has closely related to its stage. For invasive metastasis, the prognosis is relatively poor, and the prognosis of those with limited tumor is relatively good.*

Methods*: At present, the treatment of cancer is gradually becoming an era of individualized treatment, which emphasizes systemic therapy. Detection of specific tumor molecular markers is an important basis for identifying individual differences in tumor patients, and it is the premise and basis for individualized treatment of tumors. This paper has selected suitable drugs for patients.*

Results*: This paper has also randomly selected pathological specimens of 80 newly diagnosed cervical cancer patients with pathologically confirmed and complete clinical data in our hospital as the study group. Immunohistochemistry has used to detect the expression of nesting in cervical cancer tissue and to investigate its expression in cervical cancer. 60 patients are selected and pathological specimens of newly diagnosed cervical cancer patients were divided into two groups according to the efficacy of chemotherapy: chemotherapy effective group and chemotherapy ineffective group, 30 cases in each group. The nestin expression level of interstitial markers has compared between the two groups.*

Conclusions*: Through the comparison of nestin expression levels of interstitial markers between the two groups. This paper provides personalized predictive indicators for patients with personalized adjuvant chemotherapy, worthy of clinical application.*

Keywords*: Cervical neoplasms, N-cadherin, Cisplatin chemotherapy, Immunohistochemistry.*

DOI: 10.19193/0393-6384_2018_6_258

Received *January 30, 2018;* ***Accepted*** *March 20, 2018*

Introduction

Cervical cancer is the highest incidence of gynecological malignancy in China. According to World Health Organization estimates, there are 500,000 new cases of cervical cancer worldwide each year, compared with 131,500 in China, accounting for about one-third of the total number of new cases. About 200,000 women die from cervical cancer each year in the world.

In China, about 53,000 people die of cervical cancer each year, ranking the second in women's cancer deaths. Cervical cancer is a malignant tumor that occurs in the uterine vagina and cervix. Cervical cancer metastasis can spread directly to neighboring tissues and organs, down to the vaginal condyle and vaginal wall[1]. It can invade the uterus body upwards, and can invade the pelvic tissue on both sides. It can invade the bladder forward and can invade the rectum. It can also be transferred to the paracervical, intraorbital, extra-iliac, inguinal lymph nodes through lymphatic vessels, and can even be transferred to the supraclavicular and other lymph nodes throughout the body. Hematogenous metastasis is rare, common sites of metastases are lung, liver, and bone. When the symptoms of cervical cancer appear three months later, two-thirds of patients have advanced cancer. The most common symptoms of cervical cancer are increased leucorrhea and vaginal bleeding[2]. Leucorrhea can be m-tang-like or pink, and there is odor; vaginal bleeding begins after sexual intercourse, after defecation,

or after gynecological examination. Vaginal bleeding after menopause should be noted.

Cervical cancer has characterized by its high morbidity, high invasiveness and high metastasis, as well as its tendency helps to become younger. Studies have confirmed that the persistent infection high-risk human papilloma virus (HPV) is the first cause of the development of cervical cancer[3]. In Western countries, screening for cervical cancer using liquid-based cytology based on cytology across the country has resulted in a significant reduction in the incidence of cervical cancer. However, for high-grade lesions or cervical cancer, the sensitivity of cytological detection is limited. Therefore, repeated detection is required. In China, however, the incidence of cervical cancer tends to be younger[4]. A cervical cancer with a poor prognosis is usually associated with pelvic lymph node involvement. It may be that the tumor cells have metastasized. In the initial stage of invasion and metastasis of tumor cells, epithelial cells undergo morphological transformation and shift to mesenchymal cell morphology, resulting in epithelial-mesenchymal transition (EMT)[5]. EMT is associated with tumor spread, tumor metastasis, and tumor spread[6-7]. However, it is still unclear how the ability of cervical cancer cells to invade surrounding tissues and metastases is still unknown, and the role of EMT in cervical cancer cells and tissues is still unknown.

The pathological specimens of 80 newly diagnosed cervical cancer patients with pathologically confirmed and complete clinical data in our hospital have randomly selected as the study group. Nestin expression in the cervical cancer tissue has detected by immunohistochemistry to investigate its expression in cervical cancer. The clinical significance and the relationship between staging and lymph node metastasis are very important. Collect the pathological specimens of patients with primary cervical cancer who have treated with chemotherapy containing cisplatin for 1-2 cycles. Divide into two groups according to the efficacy of chemotherapy: chemotherapy effective group and chemotherapy ineffective group, 30 cases in each group[8]. The expression of nestin in the mesenchymal marker has used to investigate the correlation between nestin expression in cervical cancer tissue and the efficacy of cisplatin chemotherapy.

Table

The Stages of Cervical Cancer Stages Of Cervical Cancer

Cervical cancer pathological staging

Many factors must be considered before deciding on the best form of treatment, including the size of the tumor, the age of the patient, and overall health status. The treatment of cervical cancer includes surgical resection, radiation therapy, and chemotherapy[9]. The treatment options for different stages of cervical cancer are different. The pathological stage of common cervical cancer is as follows.

Phase 0: The treatment plan for stage 0 cervical cancers is highly controversial. Generally, it is believed that if there are residual lesions on the edge of the cone section, a cervical cone resection must be performed. However, if patients do not consider the birth of children, most doctors will recommend hysterectomy.

Phase 1: Cancer cells have confined to the uterine cervix. Since the first period, cancer has begun to invade and it has divided into two phases, IA and IB, depending on the extent of its violation.

Phase 2: Cancer cells have invaded the upper two-thirds of the vagina or connective tissues near the uterus.

Phase 3: Cancer cells have invaded the lower third of the vagina or have invaded the pelvic cavity.

Phase 4: Cancer cells have broken through the genital organs, or they have already exceeded the scope of the pelvic cavity. They directly invade the rectum or bladder, and even have distant metastases.

After the third and fourth period (including recurrence): the standard treatment for advanced cervical cancer, and the same treatment as 2B, direct high-dose radiation therapy with external radiation and prophylactic methods. However, chemotherapy can temporarily control the development of the disease, prolong the patient's survival and reduce the patient's pain. Nowadays, there are quite a number of clinical researches on chemical therapy and radiation therapy[10]. The Chemotherapy and radiation therapy are implemented simultaneously. Chemotherapy has performed before radiation therapy, or after chemotherapy has added after radiation therapy.

However, so far, there is no any evidence that the treatment has the best effect, and the preliminary results of all studies are yet to confirm further by the clinic.

After the uterus eradication surgery, it usually takes one to two weeks for hospitalization. A few days after the operation, there will be difficulty in urination, abnormal peristalsis, and pain in the lower abdomen, which will usually return to normal within one to two months. If women remove the uterus, menstruation will not come again. If you do not remove the ovary, you will not experience physiological changes in menopause because the ovaries still produce hormones[11, 12]. However, if the ovaries have removed or the function is impaired due to treatment, physiological changes in menopause will occur. Sexual abilities and libido will not change due to resection of the uterus, but simply because there is no way to become pregnant, there may be Very strong sense of loss (Table 1).

FIGO Stage	UICC		
	T	N	M
0	Tis	N0	M0
IA1	T1a1	N0	M0
IA2	T1a2	N0	M0
IB1	T1b1	N0	M0
IB2	T1b2	N0	M0
IIA	T2a	N0	M0
IIB	T2b	N0	M0
IIIA	T3b	N0	M 0
IIIB	T1	N1	M0
	T2	N1	M0
	T3a	N1	M0
	T3b	N	M0
IVA	T4	N	M0
IVB	T	N	M1

Table 1: Cervical Cancer: FIGO and UICC Staging.

Immunohistochemical Method for Detection of Nestin in Cervical Cancer Tissue Mesenchymal Markers

Materials and Methods

Expression of N-cadherin: 80 pathological specimens of newly diagnosed cervical cancer patients with pathologically confirmed and complete clinical data in our hospital are selected as the study group randomly. Nestin is detected by immunohistochemical staining for cervical cancer tissue[13, 14]. The expression of Nestin and N-cadherin is used to explore the clinical significance of its expression in cervical cancer, and staging, lymph node metastasis. The experiment has approved by the Ethics Committee of Nanxishan Hospital of Guangxi Zhuang Autonomous Region.

Clinical data collection

This group has selected 80 cases of cervical cancer patients in Guangxi from December 2016 to December 2017 in our hospital, who are aged from 23 to 68 years, and the mean age is 44. All patients have diagnosed with pathological diagnosis, including 74 cases of squamous cell carcinoma. Eight cases of adenocarcinoma have classified according to FIGO, 8 cases in stage IIa, 19 cases in stage IIb, 25 cases in stage IIIa, 16 cases in stage IIIb, and 12 cases in stage IVa. All patients have diagnosed as having squamous cell carcinoma of the cervix by histopathological diagnosis. The weight was greater than 45 kg. The expected survival time was greater than 6 months.

The patient agreed to receive the treatment plan. Exclude patients who have treated in a foreign hospital. In this study, 80 patients completed all treatments as planned and received regular checks.

Reagents and methods

Murine anti-human monoclonal N-cadherin antibodies (ZM-0094, Zymed), E-cadherin (610181, BD), SP kits, and DAB substrate chromogenic reagents were purchased from Beijing Zhongshan Jinqiao Company. All specimens were fixed in 10% formalin, paraffin-embedded, and serially sectioned at 3 μm in thickness. Immunohistochemistry has performed according to the conventional general-purpose SP method. Blank controls have designed and PBS has used instead of primary antibody.

Judgment criteria

The immunohistochemistry results were judged using a double-blind method and were independently evaluated by two pathologists in a double-blind manner. Under the microscope, 4 high-power fields were randomly selected for counting, and a comprehensive scoring was performed using the positive cell area and staining intensity[15]. According to the percentage of positive cells, scores were: 0 for no positive cells, 1 for positive cells, 1% for 1%, 0% for 10% to 50%, and 3 for 51% to 100%; staining intensity Scoring: Not colored 0 points, light yellow 1 point, brown yellow 2 points, and brown 3 points. The two scores are mul-

tiplied, 0 to 4 are divided into low expressions, and 6 to 9 are divided into high expressions. We also do another work, the positive staining of N-cad has localized on the cell membrane and near cytoplasm of the cytoplasm. The specific manifestation was that the cell membrane was brownish brown or cytoplasm brownish yellow. The semi-quantitative treatment has performed on the combined staining intensity and positive cell rate: Under the microscope (x400), visual fields with non-overlapping, and positive expression rates were concentrated. The results were judged: the percentage of positive cells counted in the total number of cells, 0 to 4% was 0; 5% to 25% was 1; 26 to 50% was 2; 51% to 75% was 3,> 75% is 4 points.

The staining intensity has scored as the staining intensity exhibited by most of the positive cells: light yellow, 1 point without obvious particles, 2 points for yellow-brown and visible particles, and 3 points for dark brown with visible particles or densely stained particles. After adding the scores together, the total score is counted as 0 - 1 for negative (-), 2-3 for negative (+), and 4-7 for strong positive (++-+++). The patient's clinical and pathological data have not displayed during cell counting. Specific clinical records can be found in the appendix.

Statistical Methods

Statistical software is used for data analysis. The comparison of the rates between the two groups has performed using the foursquare test $\chi 2$ test. The relationship between N-cadherin and E-cadherin has analyzed using the Spearman correlation coefficient (rs). Kaplan-Meier survival curves have used for univariate analysis. Log-rank test has used to analyze differences in survival curves. Multivariate survival analysis has performed using the Cox model.

Results

N-cadherin

Expression of N-cadherin in NPC tissues and chronic cervicitis tissue showed that the expression of N-cadherin was absent or lowly expressed in epithelium of 30 cases of chronic cervicitis (Figure 1), and only 1 case showed high expression; 122 The expression level of N-cadherin in NPC tissue was increased, and the expression was located in the cytoplasm and (or) nucleus. The expression of N-cadherin was brownish-yellow.

The total positive rate of N-cadherin was 59.0% (72/122). After statistical analysis, there was a significant difference in N-cadherin expression between the two groups ($\chi^2 = 29.910$, $P < 0.001$, Fig. 2-5). In addition, vascular endothelial cells were positive for N-cadherin protein[15].

Fig. 1: Schematic diagram of minimal invasive cancer treatment.

The stromal cells are the main components of the interstitial and connective tissues and are dispersed in the extracellular matrix. The characteristics of stromal cells include a loose structure and relatively non-polar cells. Figure 2 shows that the mesenchymal cells are connective tissues.

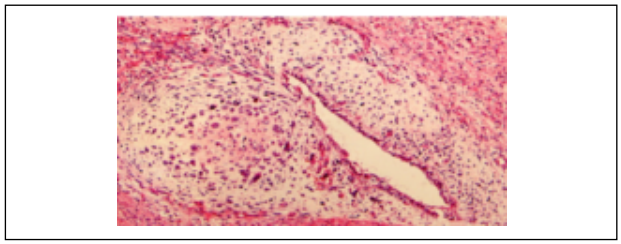

Fig. 2: The mesenchymal cells are connective tissues.

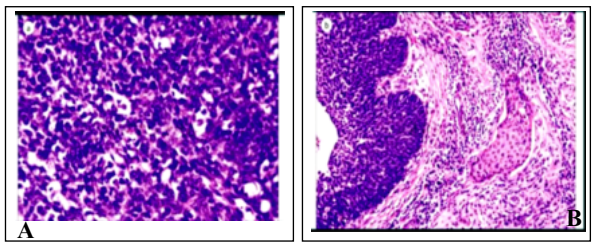

Fig. 3: Epithelial-mesenchymal transition, EMT.

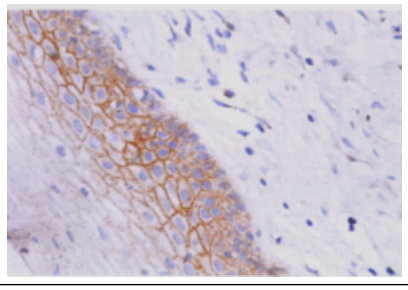

Fig. 4: Cytoplasmic expression of Ncadherin in cervical cancer tissue.

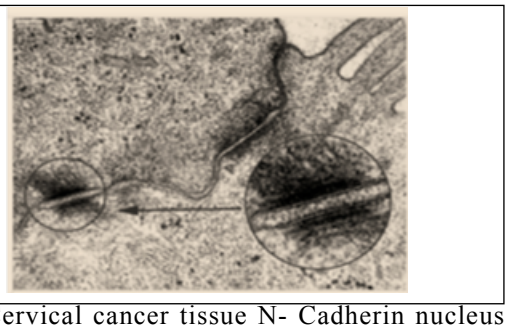

Fig. 5: Cervical cancer tissue N- Cadherin nucleus expression.

Relationship between N-cadherin expression and clinicopathological parameters of NPC

Table 2 shows that N-cadherin protein is expressed in the patient's age (P = 0. 664), histological type (P = 0. 103), T stage (P = 0. 054), and M stage (P = 0.078) were not significantly different. There was no statistical difference between groups. Significance; Correlation with patient gender (P=0.024), N-phase (P=0.002), clinical stage (P=0.000), and recurrence (P=0.025).

E-cadherin	*n*	N-cadherin			
		Low expression	High expression	*r*	*p*
Low expression	62	24	38		
High expression	18	11	7	-0.196	0.032

Table 2: N-cadherin protein expressed in the patient's age.

Correlation between N-cadherin and E-cadherin expression

The E-cadherin protein expression was previously reported by us. Statistical analysis showed that in 122 NPC tissues, 45 co-expressors of N-cadherin and E-cadherin were present; 77 cases were inconsistent; N-cadherin was highly expressed and E-cadherin was lowly expressed in 61 cases; Ncadherin was lowly expressed and E -cadherin high expression in 16 cases. Spearman correlation analysis further confirmed that there was a significant negative correlation between N-cadherin expression and E-cadherin expression (rs = - 0.198, P = 0.029, Table 3).

FIGO Stage		N-cadherin (n, %)			
		Low expression	High expression	χ2	P
Age					
<48	43	17	26	0.192	0.647
>=48	37	13	24		
Histochemistry type					
differentiated	17	10	7	2.664	0.11
Undifferentiated	63	29	34		
T stage					
T1+T2	38	19	19	3.69	0.056
T3+T4	42	14	28		
N stage					
N0+N1	76	32	45	15.16	0
N2+N3	4	1	3		
Clinical stage					
I+II	21	14	7	3.214	0.081
III+IV	59	19	40		

Table 3: N-cadherin protein is expressed in the patient's age.

The relationship between N-cadherin expression and survival in NPC patients

Kaplan-Meier analysis and Log-rank test were used to investigate the effect of Ncadherin expression and other clinicopathological parameters (including patient gender, age, T stage, N stage, M stage, clinical stage, and recurrence) on survival. Univariate analysis found that N-cadherin expression could affect overall patient survival. The overall survival rate in the N-cadherin low expression group was 78.9% (95% CI was 0. 726 to 0. 851). The overall survival rate of the expression group was 62.2% (95% CI was 0. 549 to 0. 696), and the difference was statistically significant (P = 0.006, Figure 6).

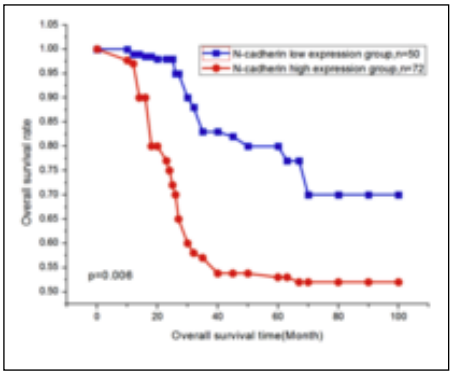

Fig. 6: N-cadherin expression affect overall patient survival.

In addition, N stage (P = 0.029), M stage (P <0.001), clinical stage (P = 0.004), and recurrence (P <0.001) were also significant for patient survival. influences. Cox model analysis showed that M staging (P = 0.001), clinical staging (P = 0.012), and recurrence (P <0.001) independently affected the patient's survival prognosis and Ncadherin expression (P = 0.296). It is not used as an independent influencing factor.

Correlation Between Nestin Expression in Stromal Markers and Cisplatin Chemotherapy Efficacy

Collect pathological specimens of patients with cervical cancer who are treated with chemotherapy containing cisplatin for 1-2 cycles of chemotherapy. Divide into two groups according to the efficacy of chemotherapy: chemotherapy effective group and chemotherapy ineffective group, 30 cases in each group[16]. The expression of nestin in the mesenchymal marker has used to investigate the correlation between the expression level of Nestin in cervical cancer tissue and the efficacy of cisplatin chemotherapy.

The effect of monotherapy is not satisfactory. The best effect is cisplatin. More effective drugs DDP, CTX, IFO, etc., the reaction rate from 6% to 25%. The use of two or more anticancer drug treatments is more effective than single drug chemotherapy. The effective rate of DDP combined with other drugs is over 32%.

General Information Collect 80 cases of cervical cancer (diagnosed by pathology)

Patients are undergoing surgical treatment in our hospital from 2016 to 2017 at random. The minimum age is 23 years and the maximum age is 68 years. There were 42 cases of squamous cell carcinoma, 16 cases of adenocarcinoma, and 2 cases of cervical alveolar rhabdomyosarcoma.

Materials ATP-TCA[17] kit provided by Huzhou Haichuang Biotechnology Co., Ltd. Chemotherapeutics: Paclitaxel (TAX, Bristol-Myers Squibb), Cisplatin[18] (DDP, Jiangsu Haosen Pharmaceutical), Mouse anti-Human Monoclonal N-cadherin Antibody (ZM-0094, Zymed), E-cadherin (610181, BD Corporation). All are general clinical preparations. Its concentration has determined by the effective blood drug concentration of the clinical drug. The specific test concentration is shown in Table 4.

Drug	TAX	DDP	CBP	Topotecan	GEM	Docetaxel	CTX	ADM
100%PPC (ug/ml)	13.8	6.3	25	0.75	25	10	3	3

Table 4: Test Concentrations of Common Chemotherapeutic Drugs.

Methods

ATP-TCA procedure: Take fresh tumor tissue specimens that have been surgically removed, remove blood clots and necrotic tissue on the surface, cut and centrifuge, add tissue digestive enzyme solution, centrifuge after full digestion, remove supernatant, and collect cells. In this paper, we can adjust the cell concentration to 10-300000/ml, add 96-well cell culture plate, set in 37 °C, 5% CO2 incubator for 4h, and add chemotherapy drugs. Each drug set 5 concentrations (200%,100%, 50.0%, 25.0%,12.5%PPC), blank control (without tumor cells) and control wells (with tumor cells but no chemotherapeutic drugs) at 5%CO2, 37°C, 95% Incubate for 5-7 days under humidity. The tumor cell ATP extract has added and mixed for 15 min at room temperature. 0.05 ml of the mixture has placed on a microplate fluorescence analyzer[19]. Calculate the inhibition rate of each chemotherapeutic agent by the following formula.

Inhibition rate = (1-test well ATP amount / control ATP amount) × 100%

Inhibition rate ≥ 70% is highly sensitive; 50% ~ 70% is sensitive; 30% ~ 50% are low sensitivity; <30% is drug resistance; clinical inhibition can be recommended when the inhibition rate is ≥ 50%.

Statistical methods all data in this experiment were statistically analyzed using SPSS 13.0 software.

ATP standard curve

ATP standard curve is used for quality control, used for luminescence test when each system is used and verification when the experimental results are abnormal. Measure the luminescence average at each concentration, and use the LG logarithm value of the diluted concentration as the independent variable. Determine the linearity of the LG logarithm value of the measurement result as the dependent variable by drawing a standard curve. The ATP standard concentration is 10-11~10-6 mol. Within the range of /ml, there is a good linear correlation between the two. The correlation coefficient is r ≥ 0.975, t test t, P <0.001 (Fig 7).

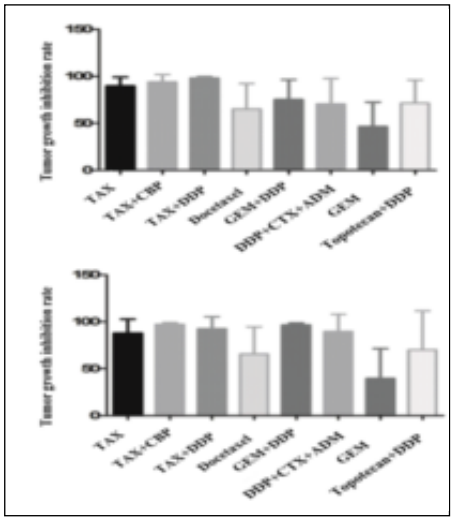

Fig 7: Different drugs inhibit cervical squamous cell carcinoma and adenocarcinoma.

ATP bioluminescence assay results

Of the total 41 patients collected, 41 were evaluable. The total assessability rate is approximately: 100%. The mean inhibition rates of drugs on cervical squamous cell carcinoma and adenocarcinoma at peak plasma concentrations are shown in Table 5. The inhibitory rate of the drug on cervical squamous cell carcinoma and adenocarcinoma in the plasma peak concentration is shown.

Pathological drugs	TAX	TC	TP	Docetaxel	GEM+DDP	DDP+CTX+ ADM	GEM	Topotecan+ DDP
Squamous cell carcinoma	9.71	3.89	8.07	5.17	5.28	0.62	6.6	1.56
	87.98	97.29	92.62	65.58	96.6	89.7	39.7	70.13

Table 5: Mean inhibition rates of drugs on squamous cell carcinoma and adenocarcinoma at peak plasma concentrations (%).

In addition, the sensitivity of cancer cells in 1 case of cervical alveolar rhabdomyosarcoma: TAX single drug is highly sensitive, TAX + CBP program is sensitive, DDP + CTX + ADM program is low sensitive, and the rest are drug resistance.

The above test results showed that: In the peak plasma concentration, the inhibition rate of chemotherapy drugs for cervical squamous cell carcinoma are as follows. TAX + DDP> TAX + CBP> TAX> GEM + DDP> Topotecan + DDP> DDP + CTX + ADM> Docetaxel> GEM. At the peak plasma concentrations, the inhibition rates for chemotherapeutic drugs for cervical adenocarcinoma are as follows. TAX + CBP> GEM + DDP> TAX + DDP> DDP + CTX + ADM> TAX> Topotecan + DDP> Docetaxel> GEM. However, the difference in the sensitivity of different histopathological drugs in cervical cancer was statistically significant according to SPSS software P=0.3360(P>0.05). The difference was not statistically significant.

The average tumor growth inhibition rate (%) of the eight groups of drugs collected from the data collected at different concentration gradients is shown in Table 6.

Concentration drug	TAX	TC	TP	Docetaxel	GEM+ DDP	DDP+CTX +ADM	GEM	Topotecan+ DDP
200%PPC	96.98	97.95	99.31	81.94	81.57	80.64	50.57	76.08
100%PPC	89.31 71.44	94.49	96.71	65.3	77.94	75.11	26.61	71.41
50%PPC	54.43	79.05	86.14	49.74	69.76	66.72	26.51	59.7
25%PPC	42.12	59.07	68.37	40.19	58.02	48.37	26.54	45.52
12.5PPC		46.35	48.93	34.24	44.62	32.09	24.42	36.42

Table 6:Mean tumor growth inhibition rates for eight groups of drugs at different concentration gradients.

The comparison of the eight groups of chemotherapeutic drugs against cervical cancer cells in vitro was shown in Figure 8.

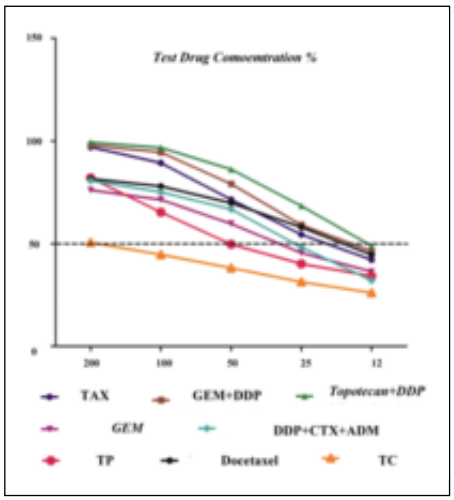

Fig 8: Eight groups of chemotherapeutic drugs test results on cervical cancer cells .

The test results showed that the sensitivity of the eight groups of chemotherapy drugs in this trial has positively related to the concentration of the drug. As the concentration of chemotherapeutic drugs increases, the sensitivity rate to tumor cells gradually increases.When the concentration was 200% PPC, the inhibition rates of chemotherapeutic drugs were as follows,

TAX+DDP>TAX+CBP>TAX>Docetaxel>GEM+DDP>DDP+CTX+ADM>Topotecan+DDP>GEM. From the trend of the overall tumor inhibition rate, the inhibitory effects of the eight groups of chemotherapy drugs on individual cervical cancer

cells were significantly different (P<0.001). The inhibition rates were: paclitaxel + cisplatin> paclitaxel + carboplatin> paclitaxel > Gemcitabine + Cisplatin> Cisplatin + Cyclophosphamide + Doxorubicin> Topotecan + Cisplatin> Docetaxel> Gemcitabine.

Discussion

Expression and other clinicopathological parameters (including patient gender, age, T stage, N stage, M stage, clinical stage, and recurrence) influence patient survival. Univariate analysis found that N-cadherin expression could affect overall patient survival. The overall survival rate in the N-cadherin low expression group was 78.9% (95% CI was 0.726 to 0.851) and it was high. The overall survival rate of the expression group was 62.2% (95% CI was 0. 549 to 0. 696), and the difference was statistically significant (P = 0.006, Figure 5). In addition, N staging (P = 0.029), M staging (P <0.001), clinical staging (P = 0.004), and relapse (P <001) also has a significant impact on the patient's survival. Cox model analysis showed that M staging (P = 0.001), clinical staging (P = 0.012), and recurrence (P <0.001) independently affected the patient's survival prognosis and Ncadherin expression (P = 0.296). Recent studies have shown that EMT is involved in the early invasion and metastasis of malignant tumors[20]. In the process of EMT, the inhibition of E-cadherin expression by epithelial markers and the up-regulation of N-cadherin expression in the mesenchymal marker (E-cadherin to N-cadherin transition) are considered important features of EMT[22, 23]. E-cadherin plays a role in suppressing the invasion of tumors. N-cadherin, an important member of the cadherin gene family that mediates calcium-dependent adhesion, has initially identified as a cell adhesion molecule expressed in nervous tissues, which mediates cell division and maintains adult tissue integrity during embryonic development. In recent years, N-cadherin has found to over expresse in a variety of malignant tumors, such as gastric cancer, pancreatic cancer and breast cancer, and promotes tumor invasion and metastasis[24- 26]. The inhibition or inactivation of Ncadherin can inhibit the migration of tumor cells and the formation of metastasis. These data fully show that Ncadherin plays an important role in the occurrence and progression of tumors.

Recently, Maeda et al.[27] suggested that TGF-β could induce increased nuclear expression of N-cadherin, which contributes to the movement and migration of mammary epithelial cells. In NPC tissues, the regulatory mechanism of Ncadherin cytoplasmic-nuclear translocation expression needs to be further explored. Invasion and metastasis of tumors are multi-factor and multi-step complex processes, among which EMT is one of the major factors in the invasion and metastasis of tumor cells. Studies have pointed out that there is evidence of EMT occurring at the edge of tumor invasion[28, 29].

During the EMT process, malignant epithelial cells acquire higher motor and invasive ability, and are more easily isolated from the primary site and further infiltrated and spread to other tissues. Interestingly, we observed that the overexpression of N-cadherin is evident at the invasive edges of certain NPC tumors, and that these positive tumor cells mostly assume the form of a fusiform interstitium. We have reported for the first time that spindle cell carcinomas in NPC tissues are closely related to the occurrence of EMT[30]. The conversion of E-cadherin to N-cadherin is considered an important molecular feature of EMT. This phenomenon has observed in some malignant tumors such as prostate, pancreatic, and synovial sarcoma[31]. This group found that N-cadherin expression was negatively correlated with Ecadherin expression. The results suggest that overexpression of N-cadherin is likely related to the occurrence of EMT in NPC tissue. As we expected, N-cadherin overexpression has closely related to lymph node metastasis, suggesting that its upregulation is likely to be one of the early events that promote cervical lymph node metastasis in NPC patients. In addition, we also found that patients with N-cadherin overexpression are prone to local recurrence. Next, the survival analysis showed that the survival time of N-cadherin nucleus in NPC patients was significantly lower than that of the low expression group, suggesting that the high expression of N-cadherin has negatively correlated with the patient's survival time. It suggests that N-cadherin may be one of the effective indicators to evaluate the prognosis of patients. However, through multivariate analysis we found that N-cadherin expression does not affect patient prognostic factors. Therefore, the results of this study suggest that for the evaluation of N-cadherin expression and prognosis of patients, we should consider comprehensively other relevant clinicopathological parameters. In conclusion, the study in this study showed that N-cadherin expression is abnormally up-regulated in human NPC tis-

sues, and its high expression is closely related to the patient's poor prognosis, which is likely to be one of promising therapeutic targets for adjuvant therapy. Of course, the specific molecular mechanisms regulating the abnormal expression of N-cadherin still need further study.

The nuclear localization of N-cadherin in human tumor tissues has not report. Our study found that N-cadherin has abnormally expressed in NPC tissues and localized in the cytoplasm and nucleus. However, the expression of N-cadherin was absent or weakly expressed in the epithelium of chronic inflammatory tissues of the cervix. Numerous studies have shown that nuclear translocation plays an important role in tumor progression and metastasis. We do not know the specific molecular mechanism regulating the nuclear translocation of N-cadherin. Recently, Maeda et al.[32] suggested that TGF-β could induce increased nuclear expression of N-cadherin, which contributes to the movement and migration of mammary epithelial cells. In NPC tissues, how Ncadherin regulates cytoplasmic-nuclear translocation expression requires further exploration.

Invasion and metastasis of tumors are multifactor and multi-step complex processes, among which EMT is one of the major factors in the invasion and metastasis of tumor cells. Studies have pointed out that there is evidence of EMT occurring at the edge of tumor invasion[33]. During the EMT process, malignant epithelial cells acquire higher motor and invasive ability, and are more easily isolated from the primary site and further infiltrated and spread to other tissues. Interestingly, we observed that the overexpression of N-cadherin is most evident in the invasive edges of certain NPC tumors, and most of these positive tumors exhibit a fusiform interstitial morphology. We have reported for the first time that spindle cell carcinomas in NPC tissues have closely related to the occurrence of EMT. The conversion of E-cadherin to N-cadherin is considered as an important molecular feature of EMT. This phenomenon has found in some malignant tumors, such as prostate, pancreatic, and synovial sarcoma.

This group found that N-cadherin expression has significantly negatively correlated with Ecadherin expression. The results suggest that overexpression of N-cadherin is likely related to the occurrence of EMT in NPC tissue. As we expected, N-cadherin overexpression has closely related to lymph node metastasis, suggesting that its upregulation is likely to be one of the early events that promote cervical lymph node metastasis in NPC patients. In addition, we also found that patients with N-cadherin overexpression are prone to local recurrence. Next, the survival analysis showed that the survival time of N-cadherin nucleus in NPC patients was significantly lower than that of the low expression group, suggesting that the high expression of N-cadherin has negatively correlated with the patient's survival time. It suggests that N-cadherin may be one of the effective indicators to evaluate the prognosis of patients. However, through multivariate analysis we found that N-cadherin expression does not affect the patient's prognostic factors. Therefore, the results of this study suggest that for the evaluation of N-cadherin expression and prognosis of patients, we should consider comprehensively other relevant clinicopathological parameters. In conclusion, the study in this study showed that N-cadherin expression is abnormally up-regulated in human NPC tissues, and its high expression is closely related to the patient's poor prognosis, which is likely to be one of promising therapeutic targets for adjuvant therapy.

Of course, the specific molecular mechanisms regulating the abnormal expression of N-cadherin still need further study.

Conclusions

At present, there are reports that the positive expression of N-cadherin is located in the cytoplasm of tumor cells. However, the nuclear localization of N-cadherin in human tumor tissues has not reported. Our study found that N-cadherin has abnormally expressed in NPC tissues, and its expression has localized in the cytoplasm and nucleus. However, in the chronic epithelial tissues of cervical chronic inflammation, the expression of N-cadherin was absent or weakly expressed. Numerous studies have shown that nuclear translocation plays an important role in tumor progression and metastasis. However, we do not know the specific molecular mechanisms regulating the nuclear translocation of N-cadherin. Recent studies have shown that EMT is involved in the early invasion and metastasis of malignant tumors. In the process of EMT, the inhibition of E-cadherin expression by epithelial markers and the up-regulation of N-cadherin expression in the mesenchymal marker (E-cadherin to N-cadherin transition) are considered as important features of EMT. E-cadherin plays a role

in suppressing the invasion of tumors. N-cadherin, an important member of the cadherin gene family that mediates calcium-dependent adhesion is identified as a cell adhesion molecule initially, which has expressed in nervous tissues, through mediating the separation of cells during embryonic development and maintaining the integrity of adult tissues. In recent years, N-cadherin has found to over express in a variety of malignant tumors, such as gastric, pancreatic, and breast cancers, and to promote tumor invasion and metastasis.

The inhibition or inactivation of Ncadherin can inhibit the migration of tumor cells and the formation of metastasis. These data fully show that Ncadherin plays an important role in the occurrence and progression of tumors. The correlation between the expression of nestin in cervical carcinoma and the efficacy of cisplatin chemotherapy has not reported in China. This article selected the clinical data of the hospital in the past two years to discuss the expression of nestin in the cervical cancer tissue from the level of genes. Theaim is to find the significance of the expression and the correlation with the efficacy of cisplatin chemotherapy, the detection of interstitial marker nestin is helpful to predict the sensitivity of patients to cisplatin chemotherapy, providing predictive indicators for personalized adjuvant chemotherapy in patients with advanced cervical cancer.

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Acknowledgements

Thanks to the Guangxi Zhuang Autonomous Region Department of Health for funding the research funded by self-financing. Title: Relationship between nestin expression in cervical cancer and chemotherapy efficacy (Contract No.: z2014184).

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