nm23-H1, c-erbB-2 and p53 Protein Expression in Cervical Carcinoma and their Clinicopathological Significance

Manhua Liu[1], Yizhong Feng[2], Fang Han[3], Ying Cheng[1], XiaoHua Ji[1], Qian Tao[1]

1 The Second Affiliated Hospital of Nantong Medical College, Natong 226001, China
2 Suzhou Medical University
3 Nantong Cancer Hospital, Natong, China

Abstract **Objective** To analyze p53, c-erbB-2 and nm23 protein expression in cervical carcinoma and their relationship with clinicopathological status. **Methods** A total of 40 cases uterine cervix carcinoma, 10 cases of dysplasia and 10 cases of cervicitis sections were stained using monoclonal antibodies to nm23-H1, c-erB2 and p53. The clinicopathological and prognostic significance of the expression of the three genes were further analyzed by chi-square test. **Results** The expression rate of nm23-H1 was 41.14% in cervical carcinoma and 5.00% in cervicitis(P<0.01), which showed relationship with pathological types: the expression rate of nm23-H1 in adenocarcinoma was 83.33% which is higher than in squamous cell carcinoma (32.35%)(P<0.05). (2)The expression rate of c-erbB2 was 37.50% in cervical carcinoma. No expression was detected in cervicitis and dysplasia of cervical epithelium. Over expression of c-erB2 in squamous cell carcinoma was related to relapse. (3) The expression rate of P53 was 24.14% and it was not expressed in cervicitis. One case of positive expression was detected in serious dysplasia of cervical epithelium. The expression was related to histological grade. There was no relationship between the expression of nm23-H1 and c-erbB2, nm23-H1 and p53, but the expression of p53 is related to that of c-erbB2 (P<0.05). Survical follow-up data showed that relapse rate of positive expression of c-erbB2 was higher than negative expression of c-erbB2. **Conclusions** Higher expression of nm23-H1 may occur in early development stage of adenocarcinoma. This maybe due to the anti-tumor reaction against metastasis; expression of c-erbB2 was a marker of cell's malignant. Over expression in squamous cell carcinoma was related to relapse; Expression of P53 was related to the development of Cervical Carcinoma; Monitoring the expression of c-erbB2 P53 in squamous cell carcinoma may be of prognostic value.

Key words Cervix neoplasms; C-erbB2; nm23-H1; p53; Gene expression

The nm23 is murine metastasis suppressor gene found by subtraction cloning. The c-erB2 gene has high similarity with epidermal growth factor. p53 is one of the most rigorously investigated tumor suppressor gene. It is also the most commonly mutated gene in human tumors causing frequently by an amino acid substitution. Although the roles of the three genes played in the development of tumor are different, they are all located in chromosome 17. It is not clear whether their protein expression patterns are related to the development of cervical carcinoma. To find their clinicopathological and prognostic significance, we detected the three gene's protein expression by immunohistochemistry.

MATERIALS AND METHODS

Clinical data

40 samples of cervical carcinoma section were obtained from Second Affiliated hospital of Medical college of Nantong and Nantong Cancer Hospital between 1993 and 1998. All patients were radical hystereclomy and pelvic lymphography. According to FIGO standard, 13 of them were in grade Ⅰ, 22 of them were in grade Ⅱ and 5 of them were in grade Ⅱb. According to histological standard, 9 of them were in stage Ⅰ, 21 of them were in stage Ⅱ and 11 of them were in stage Ⅲ. Patients were between 36 and 71 years old, the average age is 59.5. 10 cases of dysplasia of cervical epithelium and 20 cases of cervicitis were selected as control. Among the cervical carcinoma resections, 34 were squamous cell carcinoma and 6 were adenocarcinoma.

Methods

The mouse monoclonal antibodies c-erbB-2, p53 and nm23-H1 were products of Symed. We carried on antigentic renovaion according to

requiremgent of regent, and then went about immunohistochemical staining by the means of SP. The operation sequence of immunolbelling technique see instructions of regent.

Positive staining was scored when (1) brownish yellow color was displayed in cell membrane with c-erB-2 detection (2) brownish yellow clump accumulate in cell plasma with nm23-H1 detection and (3) p53 is located within cell nucleus.

The x2 tests for linear association were applied when comparing protein expression with clinicopathological features and post-operative relapse. All patient survive through the post-operative period of 2 years.

RESULTS

Protein expression of nm23-H1 in cervical carcinoma

The rate of positive expression of nm23-H1 in cervical carcinoma is 40.00%, and this expression has no relationship with the ages of patients but show relation with clinical and pathological classification($p<0.05$). Of the cervicitis, one case show positive expression. In dysplasia of cervical epithelium, none of the sample show positive expression. The results were listed in table 1 and 2.

Protein expression of c-erbB2 in cervical carcinoma

The positive protein expression rate of c-erbB2 in cervical carcinoma is 37.50%. The expression is not related to the age of the patients pathological grade($p>0.05$) but show positive correlation with pathological types. It's expression rate in squamous cell carcinoma is 32.35% which is lower than the rate in adenocarcinoma(66.67%), but the different expression in the two type of carcinoma is not statistically important($p>0.05$). None sample of the control of cervicitis and dysplasia of cervical epithelium show positive staining.

Protein expression of p53 in cervical carcinoma

The rate of positive expression of p53 in cervical carcinoma is 25.00%. The expression is not related to the age of the patients but correlated positively with pathological grades. It expression is also related to pathological type. Expression in squamous cell carcinoma is higher than in adenocarcinoma but the difference is not statistically significant.

Relationship of c-erB2, nm23-H1 and p52 protein expression in cervical carcinoma

Comparison between protein expression of c-erbB2, nm23-H1 and p53 show that there is no significant difference between the three genes ($p>0.05$).

Expression of the three genes and their relation with tumor relapse

In our experiment, most patients were in an early stage of cancer development (stage Ⅰb and Ⅱa). 3 of them was in pelvic lymph node metastasis in stage Ⅱ. In the 4 cases of relapse, 1 was in the stage of clinical Ⅰb, 2 were in the stage of Ⅱb and both were squamous cell carcinoma, 3 show c-erB2 positive staining. 2 show p53 expression and none show nm23-H1 expression.

Table 1 Three kinds genes expression relationship with pataient and clinical pathology

type	n	c-erbB-2	nm23(+)	p53(+)
age				
<50	11	5	4	4
>50	29	10	12	6
clinical dassification				
Ⅰb	13	5	10**	2
Ⅱa+ Ⅱb	27	10	6	8
pathological grade				
Ⅰ	9	2	4	0
Ⅱ	21	8	6	4
Ⅲ	11	5	6	7*
pathological type				
squamous cell carcer	34	11	11	9
adenocarcinoma	6	4	5*	1
lymph node metastasis	3	1	0	2

* $P<0.05$ ** $P<0.01$

Table 2 Three kinds genes expression in cervical cancer and cervicitis

Gene protein	carcinoma(n)		Cervicitis(n)		p
type	+	−	+	−	
c-erbB2	15	25	0	20	<0.001
p53	10	30	0	20	<0.001
nm23	16	24	1	19	<0.001

DISCUSSION

nm23 was cloned by Steeg[1] in 1998. The gene is involved in the process of malignant car-

cinoma metastasis. Its expression level in lowmetastatic cell line is 10 fold higher than that in high metastatic cell line. The gene is thought to be a metastasis suppressor gene. In our experiment, we use mouse monoclonal antibody to detect the protein expression of nm23-H1 and found that its expression rate is 40.00%. The expression show relationship with clinical and pathological grade and the earlier the stage stage was, the higher the expression rate would be. The expression rate in adenocarcinoma is higher than in squamous cell carcinoma. The expression rate in adenocarcinoma is higher than earlier report[2]. Since adenocarcinoma has a tendency toward local invasion and lymph node metastasis, the higher expression rate in adenocarcinoma in our experiment imply that it is expressed in the early stage of cancer, and the expression level in the early stage of malignant carcinoma is high. Expression level is negatively correlated with clinical stage. This result is consistent with the results of Schneider and Harlozinska, in which nm23 expression level in ovarian cancer is higher than that in borderline ovarian neoplasms and benign ovarian neoplasms. The ascites cell of cancerous ascites is higher than primary tumor[3,4]. This may be the self defensive reaction of the body to carcinoma metastasis. In the samples of 2 cases of squamous cell carcinoma lymph translocation and 4 cases of squamous cell carcinoma relapse, no nm23-H1 expression was detected.

By immunohistochemistry means, Sato[5] showed that the positive protein expression rate of c-erbB2 is 28.6 0 %, but this c-erB2 over-expression have no prognostic significance. In our experiment, the total expression rate is 37.50%. The expression rate in poorly differentiated tissue is higher than that in well differentiated tissue, and higher in adenocarcinoma than in squamous cell carcinoma, but the difference is not statistically significant. c-erbB2 is not expressed in cervicitis and dysplasia. The difference is obvious(p <0.01). The reason may be that c-erbB2 is expressed after the cell become malignant and the expression may be the marker of this malignant changing. In the 2 cases of squamous cell carcinoma metastasis, one over expressed c-erB2 and in the 4 cases of sqamous cell carcinoma relapse, 3 over expressed the c-erB2, which showed that overexpression of c-erbB2 means a poor prognosis. This result is similar with that of Hale[6].

The total detection of expression of p53 is 25.00%. The expression rate showed correlation with clinical classification and there is no difference between the two control group.(p>0.05) This result is similar with that of Holm[8], and there is no detection in cervicitis.

Although it is reported that expression pattern c-erB2(+)/nm23(-) is an important factor in prognosis evaluation, our result show no relationship among the expression of c-erbB2/nm23, nm23/p53 and c-erbB2/p53. However the three genes were co-expressed in cervical carcinoma, this may be due to that this group is in the early clinical stage, which means that c-erbB2 and nm23-H1 is highly expressed in early cervical carcinoma. The expression level of nm23-H1 decrease with the development of disease, that imply a poor prognosis. We believe that the roles of nm23-H1 and p53 are different in different organs and in different carcinomas. Independent expression of p53 and c-erB2 are both useful in prognosis[7,9,10].

4 cases of squamous cell carcinoma in the 40 cases of cervical carcinoma relapse with 2 years, the relapse rate is 10.00%. In c-erB2 positive expression cases, the relapse rate is 20.00%, and in c-erB2 negative, the relapse rate 4.00%. The difference is statistically not obvious. The total relapse rate of squamous cell carcinoma positive p53 adding c-erB2 expression is 23.53% which is significantly higher than that of the control. P53/c-erbB2 is of prognostic value.

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