*Chinese Journal of Cancer Research 10(1):68-70, 1998.*

# EXPRESSION OF c-erbB-2 AND PCNA IN CERVICAL ADENOCARCINOMA AND ITS SIGNIFICATION

Huang Yong 黄勇　Cai Shumo 蔡树模　Yu Shaoyin 俞绍音　Shi Daren 施达仁

*Cancer Hospital, Shanghai Medical University, Shanghai 200032*

**Objective: To investigate the significance of c-erbB-2 and PCNA expression in adenocarcinoma. Methods: Expression of c-erbB-2 and PCNA in 74 cases of adenocarcinoma of the uterine cervix was examined by immunohistochemistry. Results: The expression of c-erbB-2 was detected in 34 cases. The positive staining of c-erbB-2 was associated with increased lymph node metastasis (57.1% Vs 24.0%, P=0.041) and lower 5-year survival rate (32.4% Vs 58.9%, P=0.008). The average PCNA labeling index (PCNA LI) was 40.6% (0.1%-91.4%). High PCNA LI was associated with lymph node metastasis (56.4% Vs 38.5%, P=0.016) and lower 5-year survival rate (28.7% Vs 64.4%, P=0.005). Positive staining of c-erbB-2 were associated with high PCNA LI (44.7% Vs 34.6% P=0.003). Conclusions: c-erbB-2 and PCNA LI were associated with malignant biological behavior and poor prognosis of patients with cervical adenocarcinoma.**

**Key words: Uterine cervix neoplasms, Adenocarcinoma, c-erbB-2 PCNA, Prognosis, Immunohistochemistry.**

The percentage of adenocarcinoma in invasive cancer of uterine cervix has increased to about 20%.[1] The prognosis of adenocarcinoma was poor then that of squamous histology. Accurately predict the prognosis is important to improve prognosis. We examined the expression of c-erbB-2 and proliferating cell nuclear (PCNA) antigen in cervical adenocarcinoma by immunohistochemistry, analyzed the relationship between the protein expression and prognosis.

Accepted August 4, 1997

## MATERIALS AND METHODS

### Patients Selection

Seventy-four patients with invasive adenocarcinoma of the cervix treated in cancer hospital, shanghai medical university from January 1985 to December 1990, whose fromalin fixed, paraffin enbaded specimens was persevered and suit to immunostaining. The age of patients was 25 to 74 years (average 54.4±10.4 years). Histologicaly these carcinomas consisted of 60 pure adenocarcinoma, 2 adenosqamoumas, 2 clear cell carcinoma and 1 endometrioid carcinoma. Among them 39 case treated by radical surgery (18 stage I, 21 stage II) and 35 case treated by radiotherapy (12 stage II and 23 stage III). Follow up from the time of diagnosis to December 1995. 4 cases was missing and 37 died of cancer in 5 years.

### Immunohistochemical Analysis

Paraffin-embedded tissues section was cut at 5 μm routinely dewaxed, rehydrated, 2 cycle of microwave (90C) over heating for 5 minutes in each cycle. The section was incubated with primary antibody (c-erbB-2 1:100, America oncogene SCI; PCNA 1:50, Denmark Dako company) for 60 minutes then overnight in 4℃ refrigerator. Biotinglated horse anti-rat IgG (1:200, America vector company) incubated for 3 min, washing with TBS (pH 7.6) then Avidinbiotin complex (1:100, America vector company) incubated for 60 min. The chromoge, diaminobenzidine was applied and allowed to react for 10 min.

Finally, the section was counterstained with hematoxylin. The negative control performed by substituting TBS for primary antibody.

Expression of c-erbB-2 was evaluated by tumor cell member showing brown staining and more than 25% of tumor cell staining was regarded as positive. PCNA staining positive was nuclear showing brown. In each case nucleus from 1000 tumor cells was counted and PCNA LI was calculated as the percentage of positive nuclei.

### Statistical Analysis

The results were evaluated statistically using $X^2$ or *t* text, survival rat was calculated by kaplan-meier's method and survival rates compare was use log-rank test.

## RESULTS

Of the 74 cervical adenocarcinoma patients, 34 (45.9%) was positive staining for c-erbB-2. The percentage of positive staining cell for PCNA varied from 0.1% to 91.4%, average 40.6%±20.1%.

Relationship of stain results and pathologic-clinical factors: In 39 cases treated by surgery, 14 was detected positive staining for c-erbB-2, 8 of the 14 cases had cervical lymph node metastasis on the other hand, in 25 cases with negative staining for c-erbB-2 only 6 had lymph node metastasis. It was statistically significant, P=0.041. In patients with invasion to ≥1/2 cervical myometrium disease, the positive staining for c-erbB-2 was more than that of invasion to <1/2 (9/11 Vs 5/28, P=0.0004). The average PCNA LI in patients with lymph node metastasis was 56.4%, in patients without lymph node metastasis was 38.5%. Patients with pelvic lymph node involved has a higher average PCNA LI, P=0.016. No relationship was founded between expression of c-erbB-2 or PCNA LI and FIGO stage, tumor size and histological grade.

The average PCNA LI was 47.7%±19.0% in 34 patients with positive staining for c-erbB-2, that was significantly higher than 34.6%±19.2% in 40 patients with negative staining for c-erbB-2, P=0.003.

Of the 34 patients with positive staining for c-erbB-2, 2 was missing and 22 died of cancer in 5 years, the 5-year survival rate was 32.4%; of the 40 patients with negative staining for c-erbB-2, 2 was missing and 15 died of cancer in 5 years, the 5-year survival rats was 58.9%. The 5-year survival rate of positive staining group was lower than that of negative staining group P=0.008. Of the 37 patients with PCNA LI ≥40%, 2 was missing and 24 died of cancer in 5 years, the 5-year survival rats was 28.7%; while 37 patients with PCNA LI<40%, 2 was missing and 13 died of cancer in 5 years, the 5-year survival rats was 64.4%, it was higher than that of PCNA LI≥40% group, P=0.005.

## DISCUSSION

The c-erbB-2/neu proto-oncogene codes a 185-kDa transmenber glycoprotein with tyrosine kinase activity. In human, this gene gets activation mainly by gene amplification and/or overexpression. Overexpression may convert the gene protein from normal receptor into an oncogene protein.[2,3] The relationship between c-erbB-2 and prognosis of breast cancer has been examined extensively, many studies considered that the expression of c-erbB-2 was associated with tumor recurrence and metastasis. The patients with overexpression of c-erbB-2 has a shout disease free and overall survival period.4,5 Our study show the 5-year survival rate for patients with positive staining for c-erbB-2 was significantly lower than that for patients with negative staining for c-erbB-2. Suggest that expression of c-erbB-2 was associated with poorer prognosis of patient with cervical adenocarcinoma. Recently observation found c-erbB-2 protein exclusively resides on microvilli and pseudopodia of cells. The involvement of the c-erbB-2 protein in cell motility is further supported by the detection of a 50-KDa c-erbB-2 protein biding motility factor. This factor causes fast cell spreading and translocation.6,7 In our study the lymph node metastasis and deep myometrium invasion in patients with positive staining for c-erbB-2 was more common than that of patents with negative stating. Indicate c-erbB-2 may play an important role in invasion and metastasis of cervical adenocarcinoma.

Proliferating cell nuclear antigen (PCNA) is a 36-KDa nuclear protein, act as an auxiliary factor of DNA polymerase δ. PCNA concentrations are correlate directly with the rates of cellular proliferation and DNA synthesis.[8,9] We use anti-PCNA monoclonal antibodies-PC10 to investigate PCNA label index in 74 case cervical adenocarcinoma. The PCNA LI was from 0.1% to 91.4% (average 40.6%±20.1%).

The PCNA LI for patients with lymph node metastasis was significantly higher than that for patients without lymph node involved. In addition the 5-year survival rate for patients with PCNA LI≥40% was significantly lower than that for patients with PCNA LI<40%. It suggested that tumor with high PCNA LI was more aggressive and the prognosis of patients was poor. We also founded that tumor with positive staining for c-erbB-2 was commonly with higher PECAN LI. Indicate c-erbB-2 protein may has a regulatory effect on cell proliferating.

## REFERENCES


1. Brand E, Berek JS, Hacker NF. Controversies in the management of cervical adenocarcinoma. Obstetrics Gynecology 1988; 71:261.
2. DiFiore PP, Pierce JH, Kraus MH, et al. C-erbB-2 is a protent oncogene when overexpressed in NIH/3Ts cell. Science 1987; 237:178.
3. Maguire HC, Greene MI. The neu/c-erbB-2 oncogene. Seminars Oncology 1989; 16:148.
4. Slamon DJ, Codolphin W, Jones La. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707.
5. Gullick WJ, Wright C. C-erbB-2 protienter over-expression in breast cancer is a risk fact in patient with involved and uninvolved nodes. Br J Cancer 1991; 63:434.
6. De Corte V, De Potter C, Vandenberghe D, et al. A 50 kd protein present in conditional medium of colo-16 cell simulates cell spreading and motility and actirates tyrosine phosphorylation of neu/Her-2 in human SK-BR-3 mammary cancer cell. J Cell Sci 1994; 107:405.
7. De Potter CR, Quatacker J. The P185 protein is localized on cell organelles involved cell motility. Clin Exp Metastasis 1993; 11:462.
8. Prelich G, Tan CK, Kostura M, et al. Functional identity of proliferating cell nuclear antigen and polymerase-δ auxiliary protein. Nature 1987; 326: 516.
9. Bravo R, Frank R, Blundell PA, et al. Cyclin/PCNA is the auxiliary protein of DNA polymerase-δ. Nature 1987; 326:512.