The Expression of Tumor-Associated Genes in the Papillary Thyroid Carcinoma and the Relationship with the Proliferation and Metastasis of Tumor Cell

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Abstract **Objective** To study the expression of epithelial cadherin (E-cd) and nm23 in the papillary thyroid carcinoma (PTC) and the relationship with the proliferation and metastasis of tumor cell. **Methods** The expression of E-cd, nm23 and PCNA were detected by SP immunohistochemical technique in 70 paraffin-embedded tissue specimens of PTC. **Results** The positive rate of E-cd, nm23 and PCNA labeling index were 44.3%, 62.9% and 59.14%± 14.57% respectively. There were close relation between the expression of E-cd, nm23 and PCNA and pathological grade, TNM stage and lymph node metastasis, and no difference could be found between the expression of them and size of tumor, sex and age of patients. The coherent negative expression of E-cd and nm23 in PTC might indicate higher proliferation feature and higher potential of lymph node metastasis. **Conclusions** E-cd, nm23 and PCNA gene might play important roles in the carcinogenesis and development of PTC, and might be useful markers for evaluating the biological behavior of PTC.

Key words thyroid neoplasm; epithelial cadherin; nm23;

Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm in thyroid tumor. PTC has no symptoms in it's early stages and it is liable to metastasize to cervical reginal lymph nodes. So it is extremely significant to improve early diagnosis rate and explore a way to monitor PTC's invasion and metastasis. E-cadherin, the epithelial cell-cell adhesion molecule, is a transmembrane glucoprotein of calcium dependent, and it has tight relation to invasion and metastasis of tumor [1]. The nm23 gene plays a important role in transmitting signals, regulating cellular differentiation and hyperplasia and it has also relation to potential of metastasis of tumor[2]. Proliferating cell nuclear antigen (PCNA) is an auxiliary protein of DNA polymerase-delta and the expression of PCNA is related directly to the cell proliferative cycle[3]. At present, the expression of E-cadherin and nm23 in PTC has rarely been investigated. No study on the correlation between PCNA and the two proteins has been reported. In order to investigate the role of E-cadherin, nm23 and PCNA gene in carcinogenesis and development of PTC, the expression of those genes was analyzed by immunohistochemical SP technique in 70 cases of PTC, 25 cases of thyroid adenomas and 15 cases of normal thyroid tissue.

METHODS

Patients and tissue specimens

Formalin-fixed, paraffin-embedded specimens from 70 patients with PTC (18 men and 52 women, mean age 35 years, range 13-70 years) and 25 specimens of thyroid adenomas, 15 specimens of normal thyroid tissue were obtained from the archival material of the department of pathology, the first affiliated hospital, Zhengzhou University. In PTC, the pathological grade and TNM stage was based on the classification of thyroid carcinoma of UICC: 48 patients were in gradeI, 22 in grade II; 48 in stage I, 7 in stage II, 14 in stage III and 1 in stage IV respectively. Of 70 PTC, lymph node metastases occurred in 29 patients, and no lymph node metastases in 41 patients.

Immunohistochemistry

Tissues were fixed in 10 percent neutral buffered formalin, routinely processed, and embedded in paraffin. Four-micrometre thick paraffin sections of thyroid samples were used for SP (streptavidin peroxidase) immunostaining. Sections were dewaxed, rehydrated and incubated with 0.3% hydrogen peroxide to quench en-

dogenous peroxidase activity. Antigen retrieval was performed by microwave pre-treatment in 0.01M sodium citrate buffer (PH 6.0) for 10 min at 750 watt. Subsequently, sections were incubated overnight at 4℃ with monoclonal mouse antibody against E-cd (diluted at 1:30), nm23 (diluted at 1:50), and PCNA (diluted at 1:100) respectively. The bound antibody was detected by a streptavidin-biotin-peroxidase complex and visualized by 3,3'-dinminobenzidine tetrahydrochloride supplemented with 0.01% hydrogen peroxide. Finally, the slides were lightly counterstained with Mayer's hematoxylin. All series incluede positive controls, and omission of the primary antibodies served as negative control. All controls gave satisfactory results.

Evaluation of immunostaining

The expression of the antigens investigated was evaluated in a semiquantitative manner. For E-cd and nm23, a product score (percentage of positive cell×staining intensity) was calculated as follows: positive cell: <10% =0, 10%-30%=1, 31%-60%=2, >60%=3; staining intensity: 0, absent; 1, faint; 2, moderate; 3, strong staining. Level of immunoreaction were graded into three groups: (-), score 0; (+), score 1-4; (++), score >4. For PCNA, ten photomicrographs were taken at random in a uniformly stained field for each slide. The nuclei 100 epithelial cells were counted randomly in each photomicrograph, thus providing 1000 cells for each specimen. The percentage of positive nuclei was calculated and expressed as the proliferative index (PI)=positive cells/1000×100%. For purpose of statistical analysis, all cases were grouped either high proliferative index (HI, PI≥ 50%) or low proliferative index (LI, PI<50%).

Statistical analysis

Statistical texts were performed by a software package (SSCP 8.0). P<0.05 was considered statistically significant.

RESULTS

Expression of E-cd, nm23 and PCNA in normal, benign neoplastic thyroid tissue and PTC

Normal thyroid follicular cells included in benign neoplastic as well as control normal thyroid tissue showed clear immunoreactivity with E-cd. The signal obstained was clearly localized mainly at cell-cell contacts, the cytoplasm being only weakly reactive. However, immunoreactivity of E-cd in PTC was heterogeneous as positive areas co-existed side by side with negative areas. For nm23, all positive immunostaining exhibited cytoplasmic staining, while the anti-PCNA was reactive in all cases of thyroid tissue and was observed as nuclear staining. The positive rates of E-cd expression in normal thyroid tissue, thyroid adenomas and PTC were 100.0%(15/15), 72.0%(18/25) and 44.3%(31/70) respectively; The positive rates of nm23 in those were 20.0% (3/15), 36.0%(9/25) and 62.9%(44/70) respectively; PCNA labeling index in those were 14.22% ±7.76%, 27.88%±9.78% and 59.14%±14.57% respectively. The expression of the three proteins has significant difference among them (P <0.05).

Correlation with clinical variables

In PTC, the expression of E-cd, nm23 and PCNA labeling index in lymph node metastatic group were 27.6%, 31.0% and 71.13%±8.78%, but in no metastatic group were 56.1%, 85.4% and 50.66%±11.59%. There was significant difference between the two groups (P <0.01). However, no difference could be found between the expression of E-cd, nm23, PCNA and size of tumor, the sex and age of patients (P >0.05).

Relationship of co-expression of E-cd and nm23 with the proliferation and metastasis (Table 1 and Table 2)

The metastatic rate in the group, in which the expression of E-cadherin and nm23 were coherent positive, was 12.00%(3/25), but in the coherent negative group, the metastatic rate was 75.00%(15/20). There was significant difference between the two groups (P<0.001). Moreover, the group of former have significantly higher proliferation activity than that of latter (P <0.01).

Table 1 relationship between co-expression of E-cd, nm23 and lymph node metastasis of PTC

group	metastasis	No metastasis
E-cd (+) nm23 (+)	3	22
E-cd (-) nm23 (-)	15	5

X^2=18.375 P<0.001

Table 2 relationship between co-expression of E-cd, nm23 and proliferation activity of PTC

group	PCNA index	
	HI	LI
E-cd (+) nm23 (+)	12	13
E-cd (-) nm23 (-)	18	2

X^2=8.820 P<0.01

DISCUSSION

The initial step in the local invasion of malignant epithelial tumors is the detachment of cells from the original position within the epithelial sheet. It's likely that this process involves loss of intercellular adhesiveness. E-cd is a calcium-dependent cell-cell adhesion molecular, whose function is critical to the functional integrity of the adhesion junction and which plays a role in the establishment and maintenance of epithelial morphology and differentiation. Reduced expression of E-cd has been found to be correlated with the progression aggressiveness and poor survival in subsets of them[4,5]. In this study, we demonstrated that the expression of E-cd in PTC was significantly lower than that in benign thyroid tissue. Furthermore, we found that in lymph node metastatic group, the positive rate of E-cd was significantly lower than that in no metastatic group. Inactivation of E-cd causes the disruption of cell-cell adhesion, then changes in intercellular adhesion might causes invasion and metastasis. Von et al[6] studied the expression of E-cd in a larger series of thyroid tumors and found that E-cd expression seemed to be associated with the dedifferentiation progression and metastatic spread of thyroid carcinomas and might be a useful marker for the prognosis of these tumors. Their results were similar to ours.

The nm23 gene has been identified as a metastasis suppressor gene. The nm23 gene located on the long arm of chromosome 17, coding for the 18.5KD proteins, and the gene product has been shown to be identical to human nucleoside diphosphate kinase (NDPK). In a number of studies of human malignant tumors, an association between reduced expression of nm23 mRNA or its protein and an increasing metastatic ability resulting in poorer prognosis could be demonstrated. In the current study, we found that the positive rate of nm23 in normal thyroid tissue, thyroid adenoma and PTC was 20.0%, 36.0%, 62.9% respectively, and there was significant difference among them. While, in lymph node metastatic group, the expression of nm23 was significantly lower than that in no lymph node metastatic group. Arai et al[7] found that nm23 was expression in primary papillary and follicular thyroid carcinomas (14/15 and 21/22), but weakly or hardly expressed in metastatic lymph nodes and metastatic bone marrow (6/23 and 1/4). The precise mechanism by which nm23 modulates metastasis remains controversial. One hypothesis is that loss of nm23 protein causes defects in mitosis and/or protein synthesis due to disruption of microtubule spindle polymerization.

PCNA is a 36KD acidic, nonhistone auxiliary nuclear protein that plays a critical role in the initiation of DNA replication and cell proliferation. Studies indicated that PCNA could be useful biomarker for multistep carcinogenesis in a lot of human tumors. Shimizu et al[8] found that mean proliferative index(PI) was significantly higher for follicular thyroid carcinoma (73%) than microfollicular adenoma (19.6%). In our study, we could draw a similar conclusion, as PI could reflect the relative clinical behavior of PTC.

Another important finding resulting from our study was that the coherent negative expression of E-cd and nm23 in PTC might indicate higher proliferation feature and higher potential of lymph node metastasis. This finding suggest that alteration of E-cd and nm23 might directly contribute to loss of differentiation and lymph node metastasis of PTC.

In summary, E-cd, nm23 and PCNA gene might play important roles in the carcinogenesis and development of PTC. Moreover, Combining detection of the expression of E-cd, nm23 and PCNA might be useful to evaluate the biological behavior of PTC and helpful to get clinical treatment program.

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