Expression of E-cd and nm23 genes and relationship with papillary thyroid carcinoma proliferation and metastasis☆

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Abstract

Objective. To investigate 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 proliferating cell nuclear antigen (PCNA) were detected by SP immunohistochemistry in seventy PTC specimens. *Results.* The positive rate of E-cd, nm23 and PCNA labeling index were 44.3%, 62.9% and (59.14 ± 14.57)% respectively. The expression of E-cd, nm23, PCNA were correlated with pathological grade, TNM stage and lymph node metastasis, but not with tumor size, gender and patients age. The coherent negative expression of E-cd and nm23 in PTC might indicated higher proliferation feature and higher potential of lymph node metastasis. *Conclusion.* 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. [Life Science Journal. 2007; 4(3): 13 – 16] (ISSN: 1097 – 8135).

Keywords: papillary thyroid carcinoma; epithelial cadherin; nm23; proliferating cell nuclear antigen; immunohistochemistry

1 Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm in thyroid tumor. PTC has no symptom in its early stage and it is liable to metastasize to cervical lymph node. So it is extremely significant to improve early diagnosis rate and detection on monitorring PTC's invasion and metastasis. Epithelial cadherin (E-cd), the epithelial cell-cell adhesion molecule, is a calcium dependent transmembrane glucoprotein, and it has been proven to be correlated with tumor invasion and metastasis[1]. The nm23 gene plays an 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]. The present expriment is to investigate the role of E-cd, nm23 and PCNA in carcinogenesis and development of PTC.

2 Materials and Methods

2.1 Samples data

Seventy formalin-fixed, paraffin-embedded PTC specimens (male: 18, female: 52, mean age 35 years old, range 13 – 70 years old), 25 specimens of thyroid adenomas, and 15 specimens of normal thyroid tissue were obtained from the archieve 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 grade I, 22 in grade II; 48 in stage I, 7 in stage II, 14 in stage III and 1 in stage IV. 29 of 70 PTC cases were with lymph node metastasis, and others were not.

2.2 Immunohistochemistry

Tissues were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Paraffin sections of thyroid samples (4 μm) were used for streptavidin peroxidase (SP) immunostaining. Sections were

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dewaxed, rehydrated and incubated with 0.3% hydrogen peroxide to quench endogenous peroxidase activity. Antigen retrieval was performed by microwave (750 W) pretreatment in 0.01 M sodium citrate buffer (PH 6.0) for 10 minutes. Subsequently, sections were incubated overnight at 4 ºC with monoclonal mouse antibody against E-cd (diluted to 1:30), nm23 (1:50), and PCNA (1:100). The bound antibody was detected by a streptavidin-biotin-peroxiease 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 include positive controls, and omission of the primary antibodies served as negative control.

2.3 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% as 0; 10% – 30% as 1; 31% – 60% as 2; > 60% as 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. One hundred nuclei 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 high proliferative index (HI, PI ≥ 50%) or low proliferative index (LI, PI < 50%).

2.4 Statistical analysis

Data are expressed as mean ± standard deviation. Comparison between the two groups was made by Student's *t* test. The discrete variables were compared using χ^2 test. Statistical texts were performed by a software package (SPSS 10.0). $P < 0.05$ was considered statistically significant.

3 Results

3.1 Expression of E-cd, nm23 and PCNA in different tissues

Thyoid adenoma tissues and normal thyroid tissues 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 (Figures 1A, 1B, 1C). The positive rates of E-cd, nm23, PCNA in normal thyroid tissue, thyroid adenomas and PTC were shown in Table 1.

Table 1. E-cd, nm23 expression and PCNA in different tissues

Tissues	Positive expression (%, *n/n*)		
	E-cd	nm23	PCNA
Normal	100 (15/15)	20.0 (3/15)	14.22 ± 7.76
Adenoma	72.0 (18/25)*	36.0 (9/25)*	27.88 ± 9.78*
PTC	44.3 (31/70)*#	62.9 (44/70)*#	59.14 ± 14.57*#

*: *vs.* normal tissues, $P < 0.05$; #: *vs.* adenoma tissues, $P < 0.05$.

3.2 Correlation of clinical data

In PTC, the expression of E-cd, nm23 and PCNA labeling index in grade I and in grade II were significantly different ($P < 0.05$); the expression of E-cd, nm23 and PCNA labeling index in stage I – II was different from that of stage III – IV ($P < 0.05$); the expression of E-cd, nm23 and PCNA labeling index in lymph node

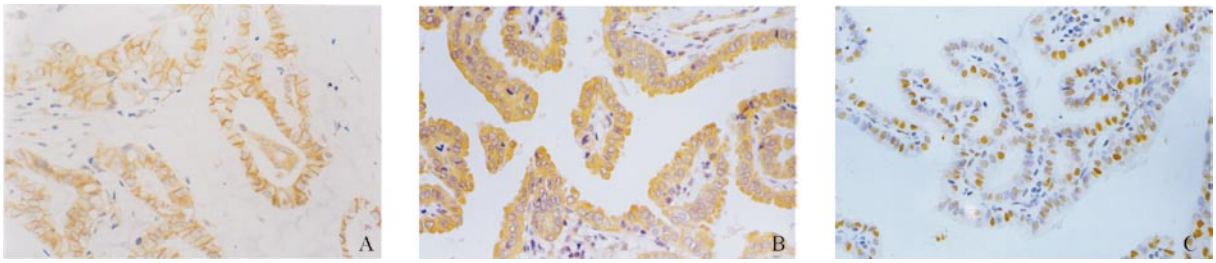

Figure 1. Genes expression in PTC. A: E-cd expression. Positive cells showed brown-yellow in cytomembrane (SP × 400). B: nm23 expression in PTC. Positive cells showed brown-yellow in cytoplasm (SP × 400); C: PCNA expression in PTC. Positive cells showed brown-yellow in nucleus (SP × 400).

metastatic group were also different from that of no metastasis group ($P < 0.05$). However, no difference could be found between the expression of E-cd, nm23, PCNA and tumor size, the gender and patient age ($P > 0.05$). Data were shown in Table 2.

Table 2. E-cd and nm 23 expression and PCNA labeling index in different groups

	E-cd	nm23	PCNA labeling index
Grade I	54.2	75	55.26 ± 13.15
Grade II	22.7	36.4	67.06 ± 14.36
Stage I – II	50.9	70.9	56.17 ± 13.21
Stage III – IV	20.0	33.3	70.00 ± 14.57
Lymph metastasis	27.6	31.0	71.13 ± 8.78
No lymph metastasis	56.1	85.4	50.66 ± 11.59

3.3 Relationship of co-expression of E-cd and nm23 with the proliferation and metastasis (Table 3 and Table 4)

Twelve percent cases, which both E-cd and nm23 were positive, were with lymph node metastasis. While 75.0% of those cases which were both negative, were with lymph node metastasis. It was significant of the difference ($P < 0.001$). Moreover, the both positive cases also has significantly higher proliferation activity than those both negative ($P < 0.01$).

Table 3. Relationship between co-expression of E-cd, nm23 and lymph node metastasis of PTC

Group	Metastasis	Without metastasis
E-cd (+) nm23 (+)	3	22
E-cd (-) nm23 (-)	15	5

$\chi^2 = 18.375$, $P < 0.001$.

Table 4. 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

$\chi^2 = 8.820$, $P < 0.01$.

4 Discussion

The initial step in the local invasion of malignant epithelial tumors is the detachment of cells from the original position on the epithelia layer. It's likely that this process involves loss of intercellular adhesiveness. E-cd is a calcium-dependent cell-cell adhesion molecular, critical to the functional integrity of the adhesion junction, and plays a role in the establishment and maintenance of epithelial cell morphology and differentiation. Reduced expression of E-cd has been found to be correlated with the progression aggressiveness and poor survival of several kinds of carcinoma[4,5]. The present results demonstrated that the expression of E-cd in PTC was significantly lower than that in benign thyroid adenoma. Furthermore, we found that in lymph node metastasis group, the positive rate of E-cd was significantly lower than that in no lymph node metastasis group. Inactivation of E-cd causes the disruption of cell-cell adhesion, then changes in intercellular adhesion might causes invasion and metastasis. Von[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 spread of thyroid carcinomas and might be a useful marker for the prognosis of these tumors. The present expriment got the similar results.

The nm23 gene has been identified as a suppressor gene to metastasis. The nm23 gene located on the long arm of chromosome 17, encoding the 18.5 KD proteins, and the gene product has been shown to be identical to human nucleoside diphosphate kinase (NDPK). A number of researches on human malignant tumors, showed the reduced expression of nm23 mRNA or its protein were correlated with the increased metastasis, which would lead to poorer prognosis[7,8]. In the current research, 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 metastasis group, the expression of nm23 was significantly lower than that in no lymph node metastasis group. Arai[9] found that nm23 was expressed in papillary thyroid carcinomas (14/15) and follicular thyroid carcinomas (21/22), but weakly or hardly expressed in metastated lymph nodes (6/23) and metastated bone marrow (1/4). The exact 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 36 KD acidic, non-histone 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[10]. Shimizu[11] found that mean proliferative index was significantly higher in follicular thyroid carcinoma (73%) than in microfollicular adenoma (19.6%). The present results confirmed his conclusion that PI could reflect the PTC clinical behavior.

Another important finding from this experiment 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 suggests that alteration of E-cd and nm23 might directly contribute to loss of differentiation and lymph node metastasis of PTC.

5 Conclusion

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 strategy.

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