Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-1287-2
April 2014, Vol. 13, No. 4, P149–P152

Expression and significance of ER and PR in differentiated thyroid carcinoma

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Received: 8 January 2014 / Revised: 10 February 2014 / Accepted: 12 March 2014

Abstract ***Objective:*** The aim of the study was to investigate the relationship between expressions of estrogen receptor (ER), progesterone receptor (PR) and gender, age, tumor size, lymph node metastasis, capsular invasion and histological type of differentiated thyroid carcinoma (DTC). ***Methods:*** Seventy cases of DTC who received surgery in our hospital (No. 401 Hospital of People's Liberation Army, Qingdao, China) between January 2008 and December 2011 were selected. Among them, 61 cases were papillary carcinoma and 9 cases were follicular carcinoma. Twenty cases were normal thyroid tissue adjacent to the tumor which was used as control. Immunohistochemical SP method was employed to detect the expression of ER and PR. ***Results:*** The positive rates of ER and PR in tumor tissues of DTC patients were 21.4% (15/70) and 31.4% (22/70), respectively, and no expression of ER or PR were founded in normal thyroid tissue ($P < 0.01$). The expressions of ER and PR were related to the lymph node metastasis and capsular invasion ($\chi^2 = 16.913$ and 6.327, $P < 0.05$; $\chi^2 = 7.516$ and 12.727, $P < 0.05$). No relationship was observed between the expressions of ER/PR and gender, age, and histological type ($P > 0.05$) of DTC patients. ***Conclusion:*** The expression levels of ER and PR in the tumor tissue of DTC patients were higher than those in the normal thyroid tissue nearby the tumor. Therefore, ER and PR expression might be clinical markers for DTC and its prognosis.

Key words differentiated thyroid carcinoma (DTC); estrogen receptor (ER); progesterone receptor (PR)

Epidemiological studies have shown that a significant gender difference exists in the incidence of thyroid cancer, women are about three times more likely than men to have the thyroid cancer [1]. Differentiated thyroid carcinoma (DTC) is a common malignant tumor in the neck. Because both estrogen receptor (ER) and progesterone receptor (PR) levels are higher in the bodies of adult women than men, estrogen, progesterone and their specific receptors – ER and PR may take part in the development of DTC [2]. However, it is not yet clear whether ER and PR directly affect DTC formation or not. In the current study, we detected the ER and PR expression levels in the thyroid cancer tissue of DTC patients to investigate their effects on the development and metastasis of thyroid cancer and provide a new theoretical basis for the clinical diagnosis, treatment and prognosis evaluation.

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Materials and methods

General data

Between January 2008 and December 2011, 70 thyroid tumor specimens obtained after thyroidectomy from patients with DTC in the No. 401 Hospital of Chinese People's Liberation Army, Qingdao, China. DTC in these patients was diagnosed by postoperative pathological examinations. Among them, 61 patients had papillary carcinoma and 9 patients had follicular carcinoma. The mean age was (42 ± 6.7) years (range, 22 to 76 years), 21 were males and 49 were females. Subgroup division was carried out according to "the management guidelines for patients with thyroid nodules and differentiated thyroid cancer (2012)". Thirty patients were more than or equal to 45 years old, 40 patients were less than 45 years old, and the tumor diameter was more than 1 cm in 18 cases and less than or equal to 1 cm in 52 cases. Sixteen patients had lymph node metastases and 54 patients did not have lymph node metastasis, and capsular invasion occurred in 16 cases but did not occur in 54 cases. In addition, 20 specimens were harvested from the normal thyroid tissue adjacent to the tumor and used as the experimental

control.

Methods

Expression of ER and PR in thyroid cancer

Immunohistochemical SP assay was carried out to detect the thyroid specimens harvested from selected patients with thyroid carcinoma. All specimens were fixed in 10% neutral buffered formalin, dehydrated routinely, and embedded. The 4 μm thick serial sections were cut and stained using HE and immunohistochemical SP methods. A known positive section was used as the positive control, a negative section was used as the negative control, and PBS was chosen as the blank control of the primary antibody.

Immunohistochemical SP staining process was mentioned below. (1) Placed sections in a microwave oven at 68 ℃ for 20 min. (2) Performed the deparaffinization with xylene and rehydration in a graded series of alcohol. (3) Incubated sections in 3% H_2O_2 at 37 ℃ for 10 min and rinsed sections with PBS three times for 5 min each. (4) Boiled sections in 0.01 M citrate buffer (pH 6.0) at 95 ℃ for 15–20 min and cooled sections at room temperature for more than 20 min, rinsed the staining jar with cold water to accelerate the cooling, and rinsed sections with PBS (same as above). (5) Applied normal goat serum blocking solution at 37 ℃ for 10 min. (6) Applied primary antibody (1:100) at 4 ℃ for overnight. Rinsed sections with PBS (same as above) (added equal volume of PBS to the negative control). Applied biotin-labeled secondary antibody, incubated for 30 min at 37 ℃, and rinsed sections with PBS. (7) Applied horseradish peroxidase-labeled streptavidin working solution, incubated for 30 min at 37℃, and rinsed sections with PBS. (8) Proceeded with chromogen of final developmental DAB. Washed sections with running water, performed hematoxylin counterstaining, dehydration, vitrification, and mounting, and visualized the staining using a light microscope.

Evaluation of results

Cells with brown granules distributed in the cell membrane, cytoplasm or nucleus were defined as positive cells. Semi-quantitative criteria of the positive cells: five high-power fields (up, down, left, right, and middle) were observed for each slide. The "–" represented no positive cell, "+" represented 1%–20% of positive cells, "++" represented 20%–50% of positive cells, and "+++" represented more than 50% of positive cells. Two experienced pathologists evaluated the pathology slides under double-blind conditions.

Statistical analysis

SPSS 19.0 was used for statistical analysis. Measurement data were represented as mean ± standard deviation (SD) and count data were represented as percentages. The χ^2 test and t test were used for statistical analysis, and a significance level of 0.05 was adopted.

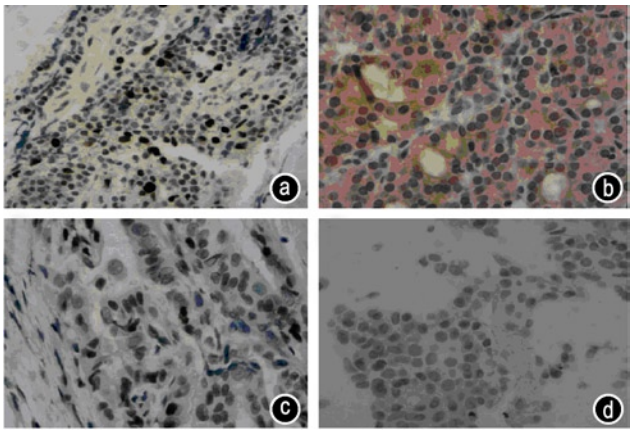

Fig. 1 Positive ER and PR expressions in the tumor tissue of DTC. (a) Positive ER expression in the papillary thyroid carcinoma tissue; (b) Positive ER expression in the follicular thyroid carcinoma tissue; (c) Positive PR expression in the papillary thyroid carcinoma tissue; (d) Positive PR expression in the follicular thyroid carcinoma tissue (SP staining × 200)

Results

Expression of ER and PR in the tumor tissue of DTC and their positive rates in the normal thyroid tissue adjacent to the tumor

ER and PR were mainly found in the cytoplasm and a few of them were found in the nucleus (Fig. 1). In 70 cases of differentiated thyroid carcinoma, positive rates of ER and PR were 21.4% (15/70) and 31.4% (22/70) respectively. No ER or PR expression was observed in the normal tissue nearby the tumor.

Relationships between the ER and PR expression and the lymph node metastasis and capsular invasion

Both rates of ER and PR expression were 62.5% in DTC cases with lymph node metastasis, while the rates of ER and PR expression were 9.2% and 22.2% respectively in cases without lymph node metastasis, and the differences were statistically significant (χ^2 = 16.913 and 7.516, P < 0.05). The rates of ER and PR expression were 16.9% and 23.7% respectively in DTC cases with capsular invasion, while the rates of ER and RP expression were 45.4% and 72.7% respectively in cases without capsular invasion, and the differences were statistically significant (χ^2 = 6.327 and 12.727, P < 0.05; Table 1).

Relationships between the ER and PR expression and the gender and histological type

The rates of ER and PR expressions were 19.0% and 28.5% respectively in male DTC patients, while the rates

Table 1 Relationships between the positive rates of ER and PR and the clinicopathological characteristics of DTC patients

Characteristic	Cases	Positive ER	Positive rate (%)	Positive PR	Positive rate (%)
Gender					
Male	21	4	19.0	6	28.5
Female	49	11	22.4	16	32.6
Age (years)					
≥ 45	30	6	20.0	9	30.0
< 45	40	9	22.5	13	32.5
Tumor diameter (cm)					
> 1	18	7	38.8	8	44.4
≤ 1	52	8	15.4	14	26.9
Lymph node metastasis					
Yes	16	10	62.5	10	62.5
No	54	5	9.2	12	22.2
Capsular invasion					
Yes	59	10	16.9	14	23.7
No	11	5	45.4	8	72.7
Histological type					
Papillary carcinoma	61	12	19.6	19	31.1
Follicular carcinoma	9	3	33.3	3	33.3

were 22.4% and 32.6% respectively in female DTC patients, there were no significant differences (χ^2 = 0.942 and 0.116, P > 0.05). The rates of ER and PR expressions were 19.6% and 31.1% respectively in cases with papillary carcinoma, while both rates were 33.3% respectively in cases with follicular carcinoma, and there were no significant differences (χ^2 = 1.653 and 0.913, P > 0.05; Table 1).

Relationships between the ER and PR expression and the tumor size and patient age

The rates of ER and PR expressions were 38.8% and 44.4% respectively in DTC cases with more than 1 cm of tumor diameter, while the rates were 15.4% and 26.9% respectively in cases with less than or equal to 1 cm of tumor diameter, and the differences were not statistically significant (χ^2 = 0.084 and 0.063, P < 0.05). The rates of ER and PR expressions were 20.0% and 30.0% respectively in DTC patients more than or equal to 45 years old, while the rates were 22.5% and 32.5% respectively in patients less than 45 years old, and the differences were not statistically significant (χ^2 = 0.063 and 0.049, P > 0.05; Table 1).

Discussion

Thyroid carcinoma is one of the common malignant tumors in women, and DTC is the most common in the thyroid carcinomas. DTC often occurs in young premenopausal women, especially in women aged 20–45 years. At present, the pathogenesis of DTC has not yet clear and considered to be correlated with factors such as diet (high iodine or iodine-deficient diet), radiation exposure history, increased secretions of estrogen and progesterone, genetics, etc., or it might be caused by the malignant transformation of benign tumors including nodular goiter and thyroid adenoma [3].

Estrogen and progesterone indirectly affect the thyroid mainly by influencing the pituitary TSH secretion via a mechanism of feedback inhibition. However, expressions of the receptors of these two hormones (ER and PR) in the thyroid tissue of DTC and their roles in the pathogenesis of DTC are not yet clear. Results of the current study showed that ER and PR expression could be detected in tumor tissue of DTC but could not be observed in the normal thyroid tissue. It was reported that ER expression in the thyroid cancer tissue is significantly higher than that in the normal thyroid tissue [4].

Mechanisms of estrogen and progesterone participating in DTC pathogenesis via ER and PR are as follows: (1) Estrogen and progesterone bind to their receptors, activate the mitogen-activated protein kinase (MAPK) signaling pathway, and enhance mitosis. (2) Estrogen and progesterone transmit signals to the nucleus via ER and PR, upregulate cyclin D1 expression, and enhance proliferation of the thyroid tumor tissue. (3) Estrogen and progesterone activate Ras-Raf-MAPK signaling pathway [5]. Results of the current study presented that the positive rates of ER and PR expressions were significantly higher in the DTC thyroid cancer tissue more than 1 cm in diameter than that less than or equal to 1 cm in diameter, namely ER and PR expression levels were correlated with tumor size, and whether there's lymph node metastasis and capsular invasion. However, ER and PR expression levels were not related to the histological type, gender and age. It suggests that estrogen and progesterone take part in the DTC

pathogenesis via ER and PR. Estrogen and progesterone bind to ER and PR, activate downstream signaling pathways, simulate proliferation and growth of thyroid cancer cells to reduce G0/G1 phase cells and increase S phase cells, results in excessive proliferation of tumor cells and tumor development. In addition, ER and PR expression levels and positive rates increase continuously along with the increase of tumor tissue volume.

Many studies have been carried out using immunohistochemical methods, there has been no unified opinion in the relationship between the ER and PR and the patient age, gender, tumor type, etc [6]. Results of the current study showed that there is no statistically significant relationship between the ER and PR expressions and the difference in patient gender. Besides the genetic and environmental factors, the mechanism causing different incidences of DTC in men and women may be related to the subtypes of the receptors, hormone levels and different sensitivities of the receptors to the hormones.

It is reported that ERβ expression could upregulated in the tissues with lymph node metastasis and tumor invasion, which suggests that ERβ may be related to the invasiveness of the thyroid carcinoma [7–8]. Whereas, some scholars reported that the expression of ER or PR might not influenced by sex, age, tumor diameter or extent of tumor invasiveness [9]. The current study showed that no significant relationships between the ER and PR expressions and the lymph node metastasis and capsular invasion, which might be due to the application of monoclonal antibodies for ER subunits in the immunohistochemical staining.

It is reported that the order of positive rates of ER and PR in the different histological types of thyroid carcinoma is as follows: papillary carcinoma > follicular carcinoma > medullary carcinoma > undifferentiated carcinoma, namely positive rates of ER and RP are relatively high in highly differentiated thyroid carcinoma tissues. In the current study, there were no significant relationships between the ER and PR expressions and the histological type of DTC. However, the expression rates of ER and PR in the papillary carcinoma were lower than those in the follicular carcinoma. It may be caused by the differences in sample size, antigen retrieval method and sensitivity and specificity of various experimental systems and different ways in identifying the positive rates of ER and PR. Based on the ER and PR expression in tumor tissue of DTC and their relationships with the proliferation of thyroid tissue in DTC patients, their antagonists may become new targets of clinical treatment of DTC patients [10].

Summing up, detection of ER and PR expression levels in DTC tissues might provide a theoretical reference for the diagnosis, treatment and prognosis evaluation of DTC patients in the clinical practice.

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