

Relation between Estrogen Receptor and Malignancy of Thyroid Cancer

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The relationship between the histological grade of dedifferentiation of thyroid cancer and estrogen receptors (ER) was examined immunohistochemically. Thyroid cancers were from postmenopausal females of almost the same mean age (69-73 years old) and within the same period of time (1974-1983). ER immunoreactivity located in the nucleus of the epithelium was found in all 6 well differentiated papillary cancers, and 5 of them (83.3%) showed ER-immunoreactive (ER-IR) cells amounting to 20 or more per visual field ($\times 100$) under a light microscope. Of the 6 cases of poorly differentiated papillary cancer, 5 (83.3%) had 1-19 ER-IR cells per visual field. ER-IR cells were negative in 5 out of 6 cases (83.3%) of anaplastic cancers. Thus, the number of ER-IR cells tended to decrease with the degree of atypism of thyroid cancer ($P < 0.001$).

Key words: Estrogen receptor — Thyroid cancer — Malignancy — Papillary cancer — Anaplastic cancer

The relationship between clinical behavior and histologic characteristics of thyroid cancer including anaplastic transformation from preexisting differentiated tumors has been well recognized.¹⁻³ Regarding anaplastic transformation from preexisting thyroid differentiated tumors such as papillary cancer, the roles of sex hormone, radiation and other factors have been discussed. Through clinicopathological study, the development of poorly differentiated cancer of a nonglandular component from preexisting differentiated tumors was proposed by Sakamoto *et al.*,^{4,5} and thus thyroid cancer derived from follicular cells can be divided into well differentiated, poorly differentiated and anaplastic cancers. The prognosis is best for well differentiated cancer, worse for poorly differentiated cancer and worst for anaplastic cancer.

Recently immunohistochemical studies have been done by using a specific monoclonal antibody against human estrogen receptors (ER),⁶⁻⁸ and ER were also observed in thyroid cancer tissue by the dextran-coated charcoal (DCC) assay.⁹

This paper deals with the relationship between the grade of dedifferentiation of thyroid papillary cancer and the immunoreactivity of the estrogen receptors (ER) in tumor tissue. To identify this relation, the cases examined were restricted to postmenopausal females of almost the same mean age, and within the same period of time.

A total of 18 cases of thyroid cancer were evaluated. These cases consisted of 6 cases of well differentiated papillary cancer (WDPC), 6 cases of poorly differentiated papillary cancer (PDPC) and 6 cases of anaplastic

cancer. All were surgically resected between 1974 and 1983 (within the 10-year period) at our department, and all were postmenopausal females 60 to 83 years of age (70.9 ± 6.9 yr). Only primary surgical cases were used. Tumors were surgically resected, fixed in 10% formalin and embedded in paraffin. One or two representative paraffin blocks were selected from each case for light microscopy and immunohistochemistry. Only primary thyroid cancer lesions were used.

Immunohistochemistry: Immunohistochemical study of ER on paraffin sections was performed by using a modified avidin-biotin-peroxidase (ABC) method after Andersen *et al.*¹⁰ The selected blocks were cut into sections of 4 μ m thick, conventionally deparaffinized and rehydrated. The specimens were washed in Tris-PBS (phosphate-buffered saline) and trypsinized with 0.1% trypsin in 0.1% CaCl_2 for 15 min, followed by washing in Tris-PBS. The sections were incubated with normal goat serum diluted 1:10 for 10 min and, without washing, covered with primary monoclonal antibody and incubated at 4°C for 16 h. After being washed in Tris-PBS, the sections were covered with biotin-conjugated goat anti-rat IgG diluted 1:10 for 60 min, washed, and then incubated with Vectastain avidin-biotin complex (Vector Lab., USA) for 60 min. The sections were washed again in Tris-PBS, and stained with 3,3'-diaminobenzidine tetrahydrochloride containing 0.001% H_2O_2 for 45 min. Rat monoclonal antibody to ER was purchased from Abbott Laboratories (USA). Positive ER immunoreactivity was confined to the nuclei of the epithelial cells. The specificity of immunoreactivity was confirmed by replacing the primary antibody with normal rat serum.

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As a positive control, one case of breast cancer with a high concentration of ER in the DCC assay was used to prepare paraffin sections for immunostaining. Breast cancer cells provided with the ER kit from Abbott Lab. were also used as a positive control.

The characteristic feature of the histology of poorly differentiated papillary cancer is the presence of solid, trabecular and/or scirrhous patterns in the papillary cancer tissue.

The presence of brown staining granules in the nuclei of cells is the sign of ER-immunoreactive (ER-IR) cells. The number of these ER-IR cells was compared among the three grades of differentiation of thyroid cancers. These groups all consisted of postmenopausal females of almost the same average age of 69–73 years. As the number of ER-IR cells was smaller than in breast cancer, ER-IR cells in one visual field with a magnification of 100 (10×10) were counted under a light microscope. ER-IR

Table I. Results of ER Immunohistochemistry in Thyroid Cancers

Well differentiated papillary cancer			Poorly differentiated papillary cancer			Anaplastic cancer		
Case	Age ^{a)}	ER cells ^{b)}	Case	Age	ER cells	Case	Age	ER cells
1. T.S.	68	1-4	1. H.Y.	60	1-2	1. Y.S.	69	0
2. O.T.	68	100+ ^{c)}	2. F.S.	71	1-4	2. K.M.	67	0
3. Y.K.	75	20-30	3. A.Y.	77	1-3	3. M.K.	80	0
4. S.T.	64	100+	4. N.S.	77	1-4	4. T.S.	82	0
5. S.M.	66	100+	5. K.S.	61	10-19	5. O.K.	67	5-9
6. M.K.	83	50-90	6. D.M.	68	0	6. N.S.	73	0
Mean age	70.7±7.1			69.0±7.5			73.0±6.6	

a) Years old. b) ER-positive cells in one visual field (×100).
c) Equal to or greater than 100.

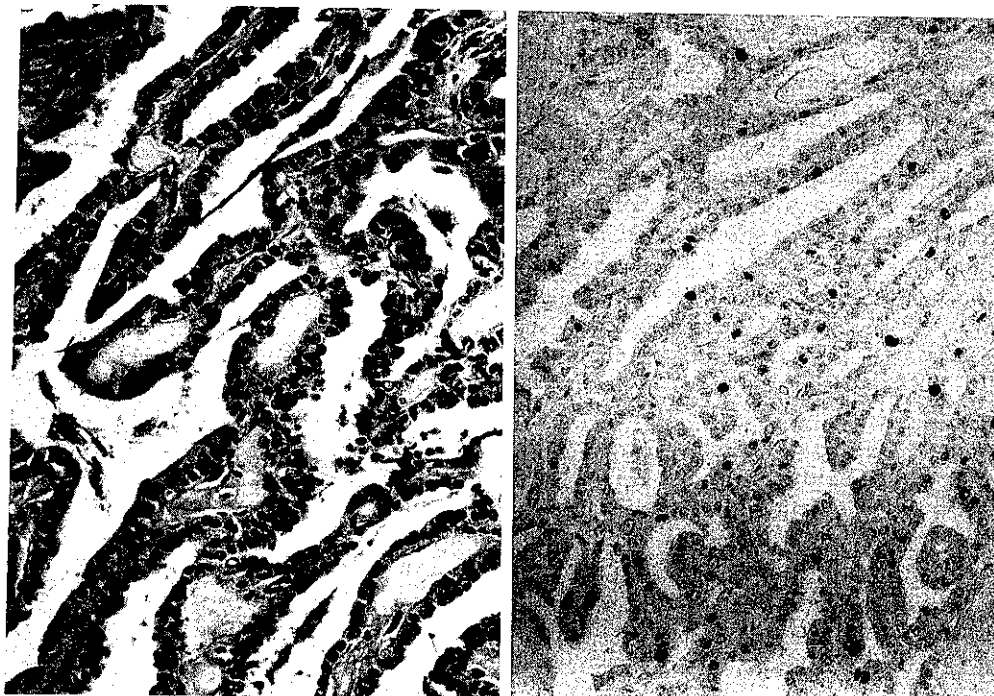


Fig. 1. ER-IR cells in Case 5 of WDPC. Strongly positive ER-IR cells are found in the papillary structure. (left, HE stain; right, ER immunohistochemical stain, ×100)

cells were counted in several fields of the best-stained lesions in each case. If only one ER-IR cell was found in several visual fields was, the number was taken as 0.

ER-IR-positive cells were found in all WDPC as shown in Table I. The mean age of WDPC patients was 70.7 ± 7.1 years. Among 6 WDPC cases, 5 cases (83.3%) showed more than 20 ER-IR-positive cells in one visual field, and 3 cases (50%) had more than 100 ER-IR cells. Positive staining for ER was restricted to the nuclei of papillary cancer cells and the intensity of ER immunoreactivity in this type was strong (Fig. 1). Almost all of the normal thyroid epithelial cells in these cancer cases, including PDPC and anaplastic cancer, did not show ER immunoreactivity. Out of 6 cases of PDPC, 5 (83.3%) had a few ER-IR cells (less than 19 per visual field). The intensity of ER immunoreactivity was somewhat weak compared with that in WDPC cells (Fig. 2). The mean age of this group was 69.0 ± 7.5 years. In 1 of 6 cases of anaplastic cancer, 5 to 9 immunoreactive cells were observed. In the other 5 cases (83.3%), ER-IR cells were negative. The mean age of these anaplastic cancer cases was 73.0 ± 6.6 years old.

The numbers of ER-immunoreactive cells in these 3 histological types were classified into 3 groups of zero, 1–19 and 20 or more per visual field as shown in Table II.

The frequency of ER-IR cells was highest (83.3%) in the 20 or more group among WDPC, highest (83.3%) in the 1–19 cell group among PDPC and highest (83.3%) in the 0 cell group among anaplastic cancers. There appeared to be a trend that the number of ER-IR cells decreased with the degree of dedifferentiation of thyroid cancer. This trend was statistically significant ($P < 0.001$) by the exact probability method.

Sex, age and histological type of tumor are important factors in the prognosis of thyroid cancer patients.^{3–5, 11, 12} As to the relationship between histologic types of thyroid cancer and prognosis, well differentiated, poorly differentiated and anaplastic cancers^{4, 5}) show a poorer prognosis in that order, and the female-to-male ratio also decreased in the same order. Recently, a specific monoclonal antibody to human ER heralded a new era in the histological analysis of ER.^{6, 7} This monoclonal antibody was useful for ER immunochemistry not only on frozen sections but also on formalin-fixed paraffin sections. The results obtained using paraffin sections were highly correlated with those obtained by DCC assay.^{10, 13–15} The existence of ER in thyroid cancer tissue has also been confirmed by Clark *et al.* using DCC assay.⁹

From these data, it is suspected that there is a relation between thyroid cancer and sex hormonal environment.

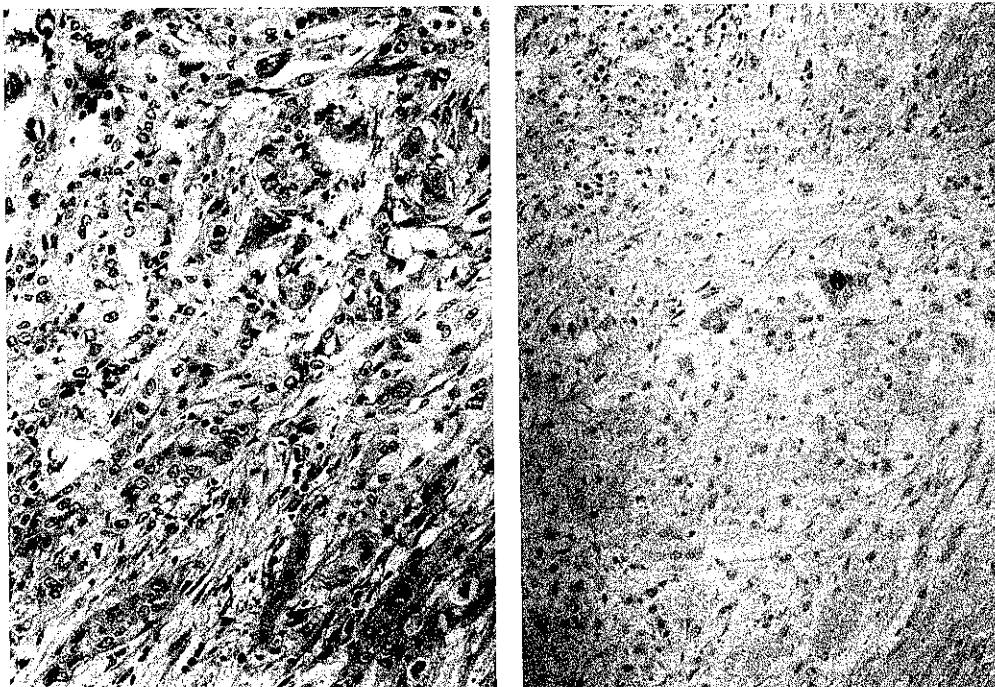


Fig. 2. ER-IR cells in Case 3 of PDPC. Only one ER-IR cell in a nonglandular component is seen. The intensity of ER immunoreactivity is somewhat weak compared with that in Fig. 1. (left, HE stain; right, ER immunochemical stain, $\times 100$)

Table II. Comparison of ER Immunoreactivity between Three Grades of Differentiation of Papillary Cancers

Grade of differentiation	Immunoreactive cells in one visual field			Total
	0	1-19	20+ ^{a)}	
Well differentiated papillary cancer	0	1 (16.7%)	5 (83.3%)	6
Poorly differentiated papillary cancer	1 (16.7%)	5 (83.3%)	0	6
Anaplastic cancer	5 (83.3%)	1 (16.7%)	0	6
Total	6	7	5	18

a) Equal to or greater than 20.

To examine the relationship between the prognosis and ER of thyroid cancers, three types of thyroid cancers, WDPC, PDPC, and anaplastic cancer were studied. Thyroid cancer cases from menopausal females of almost the same mean age (69-73 years old) in the same period of time (1974-1983) have been evaluated to exclude differences due to sex and menopausal condition. Under these conditions, immunoreactivity of ER was present in

WDPC and was located in the nuclei. There was a trend for ER-IR cells to decrease with increase of dedifferentiation of thyroid cancer, which may suggest that weak ER-immunoreactivity implies a poor prognosis.

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