Estrogen Receptors (α and β) and 17β-Hydroxysteroid Dehydrogenase Type 1 and 2 in Thyroid Disorders: Possible *In Situ* Estrogen Synthesis and Actions

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Both epidemiological and experimental findings suggest the possible roles of sex steroids in the pathogenesis and/or development of various human thyroid disorders. In this study, we evaluated the expression of estrogen receptors (ER) α and β in normal thyroid glands (N = 25; female: n = 13, male: n = 10, unknown: n = 2) ranging in age from fetus to adult. Furthermore, using immunohistochemistry, we investigated the expression of ERα and β in 206 cases of thyroid disorders, including 24 adenomatous goiters, 23 follicular adenomas, and 159 thyroid carcinomas. In addition, we also studied the mRNA expression of ERα and β and 17β-hydroxysteroid dehydrogenase Type 1 and 2, enzymes involved in the interconversion between estrone and estradiol, using reverse transcription polymerase chain reaction (RT-PCR), in 48 of these 206 cases (10 adenomatous goiters, 10 follicular adenomas, and 28 papillary thyroid carcinomas) in which fresh frozen tissues were available for examination to further elucidate the possible involvement of intracrine estrogen metabolism and/or actions in thyroid disorders. ERα labeling index, or percentage of cells immunopositive for ERα, was significantly higher in adenomatous goiter (14.2 ± 6.4), follicular adenoma (13.4 ± 5.1), and thyroid carcinoma (16.4 ± 2.1) than in normal thyroid gland (0; P < .05). Few follicular cells were positive for ERα in normal thyroid glands. In papillary carcinoma, ERα labeling index was significantly higher in premenopausal women (28.1 ± 4.5) than in postmenopausal women (14.2 ± 2.9) and in men of various ages (7.6 ± 2.7; P < .05). In other histological types of thyroid carcinoma, no significant correlations were detected. ERβ immunoreactivity was detected in both follicular and C-cells of normal thyroid glands, including those in developing fetal thyroid glands. In addition, ERβ immunoreactivity was detected in the nuclei of various thyroid lesions. But no significant correlations were detected between ERβ labeling index and clinicopathological findings including age, menopausal status, gender, and/or histological type of thyroid lesions. 17β-hydroxysteroid dehydrogenase Type 1 expression was detected in 31/48 (64.0%) of the cases examined, whereas Type 2 was detected only in 3/46 (6.3%) of all the cases examined. These results demonstrated that estrogens may influence the development, physiology, and pathology of human thyroid glands, and these effects, especially through ERα, may become more pronounced in neoplasms, particularly in papillary carcinoma arising in premenopausal women.

KEY WORDS: Estrogen receptors, Human thyroid lesions, 17β-hydroxysteroid dehydrogenase, Immunohistochemistry, Labeling index, Reverse transcription polymerase chain reaction.

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Biological effects of estrogens are generally mediated through an initial interaction with the estrogen receptor (ER), a member of the superfamily of nuclear receptors. Identification of ER is an initial step in understanding the estrogenic effects on various

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tumors. ER has been identified, for instance, in a wide range of human neoplasms, including carcinoma arising in colon (1), lung (2), pancreas (3), and other organs classically considered not to be targets for estrogens. However, their biological significance in these tumors has not been fully elucidated. Thyroid tumors are well known to occur with approximately three times more frequency in women than in men (4). Given the higher incidence and relatively better prognosis of thyroid carcinomas diagnosed in women (5), especially in premenopausal women, it is reasonable to speculate that these thyroid lesions may be influenced by sex hormones, especially estrogens, in their pathogenesis and/or development.

In addition to the classical ERα isoform, a second isoform of ER or ERβ, has been recently identified in humans (6). ERβ has been demonstrated to be widely distributed in various human tissues (7, 8), including many organs of the human fetus (9, 10) in contrast to ERα. Several investigators have demonstrated the presence of ERα in thyroid tissues from human thyroid tumors and normal thyroid glands (11–13), but not that of ERβ. In addition, Valle *et al.* (11) reported an overexpression of aromatase, the enzyme involved in conversion of androgens to estrogens, in several human thyroid lesions. However, results of these studies on ERα in normal and pathologic human thyroids have been inconsistent and could not necessarily account for the marked prevalence of thyroid lesions among female subjects. 17β-Hydroxysteroid dehydrogenase catalyzes the reversible interconversion between estrone and estradiol. However, 17β reduction and oxidation of estrogens is catalyzed by different 17β-hydroxysteroid dehydrogenase isozymes. 17β-hydroxysteroid dehydrogenase Type 1 mainly catalyzes the conversion of inactive estrogen, estrone, to the biologically active estrogen, estradiol (14–16), whereas 17β-hydroxysteroid dehydrogenase Type 2 predominantly catalyzes the conversion of estradiol to estrone (17). 17β-hydroxysteroid dehydrogenase Type 1 and 2 both are known to regulate the *in situ* levels of estradiol, and subsequently to modulate estrogenic actions in estrogen target tissues. Examination of 17β-hydroxysteroid dehydrogenase Type 1 and 2 in human thyroid lesions, in addition to aromatase and ER isoforms, therefore, become very important in obtaining a better understanding of the local or intracrine regulation of estrogenic actions in human thyroid lesions. Therefore, in this study, we first examined the expression of ERα and β in normal thyroid glands obtained from fetal, pediatric, and adult patients. We then studied the expression of ERα and β, and 17β-hydroxysteroid dehydrogenase Types 1 and 2 in adenomatous goiters (n = 24), follicular adenomas (n = 23), and thyroid carcinomas (n = 159), using immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) to elucidate the possible roles of estrogens in the development, physiology, and pathology of the human thyroid gland.

MATERIALS AND METHODS

Tissue Preparation

Human fetal tissues (10–21 weeks gestational age) were obtained after elective termination in normal pregnant women at Tohoku University Hospital and Nagaike Maternal Clinic (Sendai, Japan). Informed consent was obtained from these pregnant women before elective termination. The ages of the fetuses were estimated by the last menstrual date, body weight, and/or crown-rump length. Human fetal tissues of 24, 27, 37, and 39 weeks of age were obtained at the time of autopsy at Tohoku University Hospital. The ethics committee of Tohoku University School of Medicine approved this research protocol. The time elapsed from demise to the removal of the tissues ranged from 30 minutes to 1 hour in the case of elective termination, and from 2 to 6 hours in autopsy cases. After careful evaluation, these specimens did not appear to have any significant histopathological abnormalities. The specimens for immunohistochemistry were fixed in 10% neutral formalin for 18 hours at 4° C and then embedded in paraffin.

Patients and Tissues

Paraffin-embedded blocks from 206 patients who underwent thyroidectomy from 1990 to 2000 at Tohoku University Hospital, Sendai City Hospital, and Itou Hospital (Tokyo, Japan) were used for immunohistochemical studies. The mean age of the patients was 52.8 $\pm$ 17.5 years old (range, 12–86 y). Twenty-four cases of adenomatous goiter (18 females, 6 males; 53.5 $\pm$ 1.0 y), 23 cases of follicular adenoma (17 females, 6 males; 48.1 $\pm$ 14.9 y), and 159 cases of carcinoma (116 females, 43 males; 53.8 $\pm$ 17.5 y) were retrieved from the surgical pathology files at all three hospitals mentioned above. Carcinoma cases that we examined included 100 papillary (79 females, 21 males; 53.5 $\pm$ 17.6 y), 14 follicular (8 females, 6 males; 47.6 $\pm$ 17.5 y), 25 anaplastic (15 females, 10 males; 65.2 $\pm$ 9.1 y), and 20 medullary (14 females, 6 males; 45.7 $\pm$ 18.6 y) carcinomas (Table 1). All patients examined in this study received neither irradiation nor chemotherapy before surgery. The World Health Organization classification of thyroid lesions and the general rules for the description of thyroid cancer in Japan (18) were used as the basis for histological classification of thyroid tumors investigated in this study.

TABLE 1. Histological Diagnosis of Paraffin-Embedded Thyroid Tissues

	Women		Men	Total
	Premenopausal	Postmenopausal		
Normal	3	3	5	11
Adenomatous goiter	7	11	6	24
Follicular adenoma	10	7	6	23
Carcinoma	47	69	43	159
Papillary	35	44	21	100
Follicular	5	3	6	14
Anaplastic	0	15	10	25
Medullary	7	7	6	20
Total	67	91	59	217

Fresh thyroid tissues were available for total RNA extraction and RT-PCR examination in 48 patients with thyroid disease from 1997 to 2000 at the three institutions above. Thyroid tissues were immediately frozen in liquid nitrogen and stored at −80° C until use for the RT-PCR study. The mean age of the patients was 53.3 ± 15.5 years (range, 20–86 y). These 48 cases included 28 papillary carcinomas (21 females, 7 males; 55.8 ± 16.2 y), 10 follicular adenomas (8 females, 2 males; 47.2 ± 14.6 y), and 10 adenomatous goiters (9 females, 1 males; 52.7 ± 13.9 y). All of these cases were histologically confirmed.

Antibodies

The polyclonal antibody for ERβ was raised in rabbit against synthesized peptides of the C-terminal region of ERβ (CSPAEDSKSKEGSQN-PQSQ). This antibody was purified on affinity columns bound with the synthetic peptide. The characterization of this antibody was confirmed by Western blotting (19), and use of the ERβ antibody for immunohistochemistry has been previously reported. The monoclonal antibody for ERα (ER1D5) was purchased from Immunotech (Marseille, France).

Immunohistochemistry

In this study, immunohistochemical analyses were performed using the streptavidin-biotin amplification method and a Histofine Kit (Nichirei, Tokyo, Japan) for ERα, and Envision+ (DAKO, Carpinteria, CA) for ERβ. For antigen retrieval, slides were deparaffinized and heated in an autoclave at 120° C for 5 minutes in a citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0) for the immunostaining of ERα and ERβ. The dilutions of primary antibodies used in this study were as follows: ERα 1/100, and ERβ 1/100. The antigen–antibody complex was visualized with 3.3-diaminobenzidine (3,3'-diaminobenzidine) solution M 3,3'-diaminobenzidine, 50 mM Tris-HCl buffer [pH 7.6], and 0.006% H_2O_2, and counterstained with hematoxylin. Breast cancer was used as a positive control for ERα, and normal breast was also used as a positive control for ERβ. Normal rabbit or mouse IgG was used instead of the primary antibodies as a negative control. No specific immunoreactivity was detected in these sections.

RT-PCR Analysis

Total RNA was extracted by homogenizing tissue specimens in guanidium thiocyanate, followed by ultracentrifugation in cesium chloride. Total RNA was spectrophotometrically quantified at 260 nm. An RT-PCR kit (SUPERSCRIPT Preamplification system, Gibco-BRL, Grand Island, NY) was employed in the synthesis and amplification of cDNA. cDNAs were synthesized from 5 μg of total RNA using random hexamer primer. Reverse transcription was carried out for 60 minutes at 42° C with SUPERSCRIPT II reverse transcriptase. After an initial 1-minute denaturation step at 95° C, 40 cycles of PCR were carried out on a Light Cycler PCR machine (Roche Diagnostics GmbH, Mannheim, Germany) under the following condition: 0 seconds of denaturation at 95° C, 15 seconds of annealing at 62° C for ERα or at 60° C for ERβ and GAPDH, followed by a 15-second extension at 72° C. The primer sequences used in this study are listed in Table 2 (21–24). After PCR, the products were resolved on a 2% agarose ethidium bromide gel, and images were captured with Polaroid film under ultraviolet transillumination. In initial experiments, amplified PCR products were purified and subjected to direct sequencing (ABI PRISM Biosystems, Foster City, CA; and ABI Prism 310 Genetic Analyzer) to verify amplification of the correct sequences. As a positive control, T-47D breast cancer cells were used for ERα and ERβ; frozen placental tissue was used as a positive control for 17β-HSD Type 1 and 2. In the present study, we tested for the presence of exogenous and/or non-gene-specific contaminant DNA by synthesizing cDNA in the absence of reverse transcriptase and also by performing a standard PCR without cDNA substrate. We observed no amplification of gene-specific products under either of these conditions.

Scoring of Immunoreactivity

Scoring of ERα and ERβ in tumor cells was performed on high-power field (400×) using a standard light microscope. In each case, the fields for examination were simultaneously determined by two of the authors (WK and TS) using a double-headed light microscope. More than 1000 tumor cells were counted independently by the two authors above. Nuclear immunoreactivity was considered positive regardless of immunointensity. The

TABLE 2. Primer Sequences Used in RT-PCR Analysis

cDNA	Sequence	Position in cDNA	Size (bp)	Reference Number
ERα	Forward 5′AAGAGCTGCCAGGCCTGCC	702–720	167	21
	Reverse 5′TTGGCAGCTCTCATGTCTCC	850–869		
ERβ	Forward 5′ GCATGGAACATCTGCTCAAC	1513–1532	229	22
	Reverse 5′ ACGCTTCAGCTTGTGACCTC	1721–1740		
17β-HSD type 1	Forward 5′CTCTGGGCTGCCCCAACAC	598–615	352	23
	Reverse 5′GGACGTGCTGGTGTGTAAC	264–282		
17β-HSD type 2	Forward 5′CTTTGTGACCTCCACAGTTC	667–686	418	23
	Reverse 5′GGTGTCATGCTTCCTCATGT	269–288		
GAPDH	Forward 5′TGAACGGGAAGCTCACTGG	731–750	307	24
	Reverse 5′ TCCACCACCCTGTTGCTGTA	1018–1038		

percentage of positive immunoreactivity, that is, labeling index, was determined. When interobserver differences were >5%, the cases were simultaneously re-evaluated using a double-headed light microscope. When interobserver differences were <5%, the mean values of these two observed data were determined as the labeling index for that case. Interobserver differences in our study were <1%.

Statistical Analysis

The gender and menopausal status of the patients in this study were tentatively classified into three groups (premenopausal women, postmenopausal women, and men of various ages). Values for each category and the labeling index for ERα and ERβ are presented as mean ± standard error of means (SEM). Associations between the labeling index for ERα and ERβ and each histological category were evaluated using an ANOVA test. A *P*-value of <.05 was considered significant.

RESULTS

Immunohistochemistry

Results are summarized in Tables 3 and 4.

ERα

ERα immunoreactivity was detected in the nuclei of invasive ductal carcinoma employed as positive control of immunostain. Very few follicular cells were positive for ERα in the normal thyroid gland of both women and men. ERα immunoreactivity was not detected in the nuclei of follicular cells in developing fetal thyroid glands. However, ERα immunoreactivity was detected in the nuclei of parenchymal cells in thyroid disorders (Fig. 1, A–D). The numbers of ERα-positive cases were as follows: 9/24 (37.5%: 6/18 [33.3%] females, 3/6 [50%] males) in adenomatous goiters, 6/23 (26.1%: 4/17 [23.5%] females, 2/6 [33.3%] males) in follicular adenomas, and 74/159 (46.5%: 58/116 [50.0%] females, 16/43 [37.2%] males) in carcinomas. ERα labeling index or the percentage of ERα-positive cells was as follows: 14.2 ± 6.4 in adenomatous goiter, 13.4 ± 5.1 in follicular adenoma, 17.8 ± 2.7 in papillary carcinoma, 13.3 ± 6.3 in follicular carcinoma, 18.8 ± 5.7 in anaplastic carcinoma, and 5.5 ± 3.8 in medullary carcinoma.

Results of the correlation between ERα labeling index and clinicopathological parameters are summarized in Table 3. ERα labeling index was significantly higher in adenomatous goiter (14.2 ± 6.4), follicular adenoma (13.4 ± 5.1), and thyroid carcinoma (16.4 ± 2.1%) than in normal thyroid gland (0; $P < .05$). In papillary carcinoma, ERα labeling index was significantly higher in premenopausal women (28.1 ± 4.5) than in postmenopausal women (14.2 ± 2.9) and men of various ages (7.6 ± 2.7; $P < .05$). With respect to anaplastic carcinoma, ERα labeling index tends to be higher in men of various ages (34.7 ± 10.1) than in postmenopausal women (8.2 ± 5.4), but the differences did not reach statistical significance. No significant correlations were detected in other histological types. Clinical

TABLE 3. Mean ± SEM of Labeling Indexes of ERα immunoreactivity

	Women		Men	Total
	Premenopausal	Postmenopausal		
Normal	0	0	0	0
Adenomatous goiter	8.2 ± 6.9	10.5 ± 6.3	23.2 ± 13.0	14.2 ± 6.4
Follicular adenoma	6.8 ± 4.8	23.5 ± 4.1	11.8 ± 10.8	13.4 ± 5.1
Carcinoma				
Papillary	28.1 ± 4.5	14.2 ± 2.9	7.6 ± 2.7	17.8 ± 2.7
Follicular	20.3 ± 10.5	14.3 ± 14.3	21.8 ± 10.4	13.3 ± 6.3
Anaplastic	—	8.2 ± 5.4	34.7 ± 10.1	18.8 ± 5.7
Medullary	6.3 ± 5.5	10.2 ± 9.4	0	5.5 ± 3.8

TABLE 4. Mean ± SEM of LIs of ERβ immunoreactivity

	Women		Men	Total
	Premenopausal	Postmenopausal		
Normal	12.5 ± 2.6	13.0 ± 3.9	21.6 ± 3.0	22.4 ± 2.5
Adenomatous goiter	17.2 ± 14.5	12.2 ± 7.4	16.2 ± 9.2	15.2 ± 7.3
Follicular adenoma	29.0 ± 5.2	23.5 ± 10.8	34.0 ± 19.0	29.2 ± 8.2
Carcinoma				
Papillary	15.4 ± 4.5	17.2 ± 3.2	19.6 ± 6.2	17.0 ± 2.9
Follicular	12.5 ± 11.2	23.3 ± 13.6	13.6 ± 6.2	15.5 ± 5.3
Anaplastic	—	38.4 ± 7.1	42.0 ± 9.5	39.9 ± 5.6
Medullary	7.2 ± 2.7	40.5 ± 16.2	24.3 ± 13.7	24.0 ± 7.9

follow-up data of these patients with thyroid carcinoma also were not available for present study.

ERβ

ERβ nuclear immunoreactivity was detected widely in both follicular or parenchymal and C-cells of normal thyroid glands, including those in the developing fetal thyroid glands (Fig. 1, E–F). In addition, ERβ immunoreactivity was detected in the nuclei of various thyroid lesions. The number of ERβ-positive cases or percentages of ERβ-positive cases were as follows: 9/24 (37.5%: 6/18 [33.3%] females, 3/6 [50%] males) in adenomatous goiters, 6/23 (26.1%: 4/17 [23.5%] females, 2/6 [33.3%] males) in follicular adenomas and 74/159 (46.5%: 70/116 [60.3%] females, 25/43 [58.1%] males) in carcinomas. ERβ labeling index was as follows: 17.7 ± 4.6 in normal thyroid gland, 15.2 ± 7.3 in adenomatous goiter, 29.2 ± 8.2 in follicular adenoma, 17.0 ± 2.9 in papillary carcinoma, 15.5 ± 5.3 in follicular carcinoma, 39.9 ± 5.6 in anaplastic carcinoma, and 24.0 ± 7.9 in medullary carcinoma.

Results of the correlation between ERβ labeling index and clinicopathological parameters of patients were summarized in Table 4. ERβ labeling index tended to be higher in anaplastic carcinoma (39.9 ± 5.6) than in other histological types, but the differences did not reach statistical significance. There were no significant correlations between ERβ labeling index and patient age, menopausal status, gender, benign and malignant tumors, histological type and follow-up data (data not shown).

RT-PCR

Messenger RNA (mRNA) expression for ERα, ERβ, and 17β-hydroxysteroid dehydrogenase Type 1 and 2 was detected as a signal gene-specific band (168 bp for ERα, 228 bp for ERβ, 352 bp for 17β-hydroxysteroid dehydrogenase Type 1, and 418 bp for 17β-hydroxysteroid dehydrogenase Type 2; Fig. 2) in 48/48 (100%), 48/48 (100%), 31/48 (64.6%), and 3/48 (6.3%) specimens examined, respectively. Results of RT-PCR analysis were consistent with those of immunohistochemistry in each case.

No significant correlations were detected between 17β-hydroxysteroid dehydrogenase Type 1 and the labeling index for ERα and β, or between 17β-hydroxysteroid dehydrogenase Type 2 and the labeling index for ERα and β.

DISCUSSION

In our present study, ERα immunoreactivity was not detected in the normal thyroid gland, but ERβ immunoreactivity was detected in the nuclei of follicular epithelial cells and C-cells throughout the development of the normal thyroid gland. The presence of ERβ previously has been demonstrated in the normal rat thyroid gland by immunohistochemistry (24). In addition, ERβ has very recently been demonstrated in adult normal human thyroid gland by immunohistochemistry (8). Results from our present study are also consistent with those from previous studies. In our study, ERβ immunoreactivity was detected throughout the development of the human thyroid gland from 11 gestational weeks. In normal pregnancy, large quantities of estrogens are produced by placental syncytiotrophoblasts, which subsequently move into both the fetal and maternal circulation. The effects of estrogens on thyroid development have not been well studied, but results of our present study suggest that estrogens may also be involved in the development of fetal thyroid glands. These effects are most likely to be predominantly mediated via ERβ.

ERα immunoreactivity was detected in the nuclei of thyroid parenchymal cells of various thyroid lesions. The presence of ERα has previously been examined in human thyroid neoplasms by a number of investigators (11–13, 25–27). However, results from these studies were inconsistent, possibly because of the different methods employed. In our present study, we employed immunohistochemistry in combination with labeling index, one of the most reliable methods for detecting ERα in clinical specimens, and also RT-PCR. ERα labeling index was significantly higher in adenomatous goiters, follicular adenomas and thyroid carcinomas than in normal thyroid glands. In the papillary carcinoma,

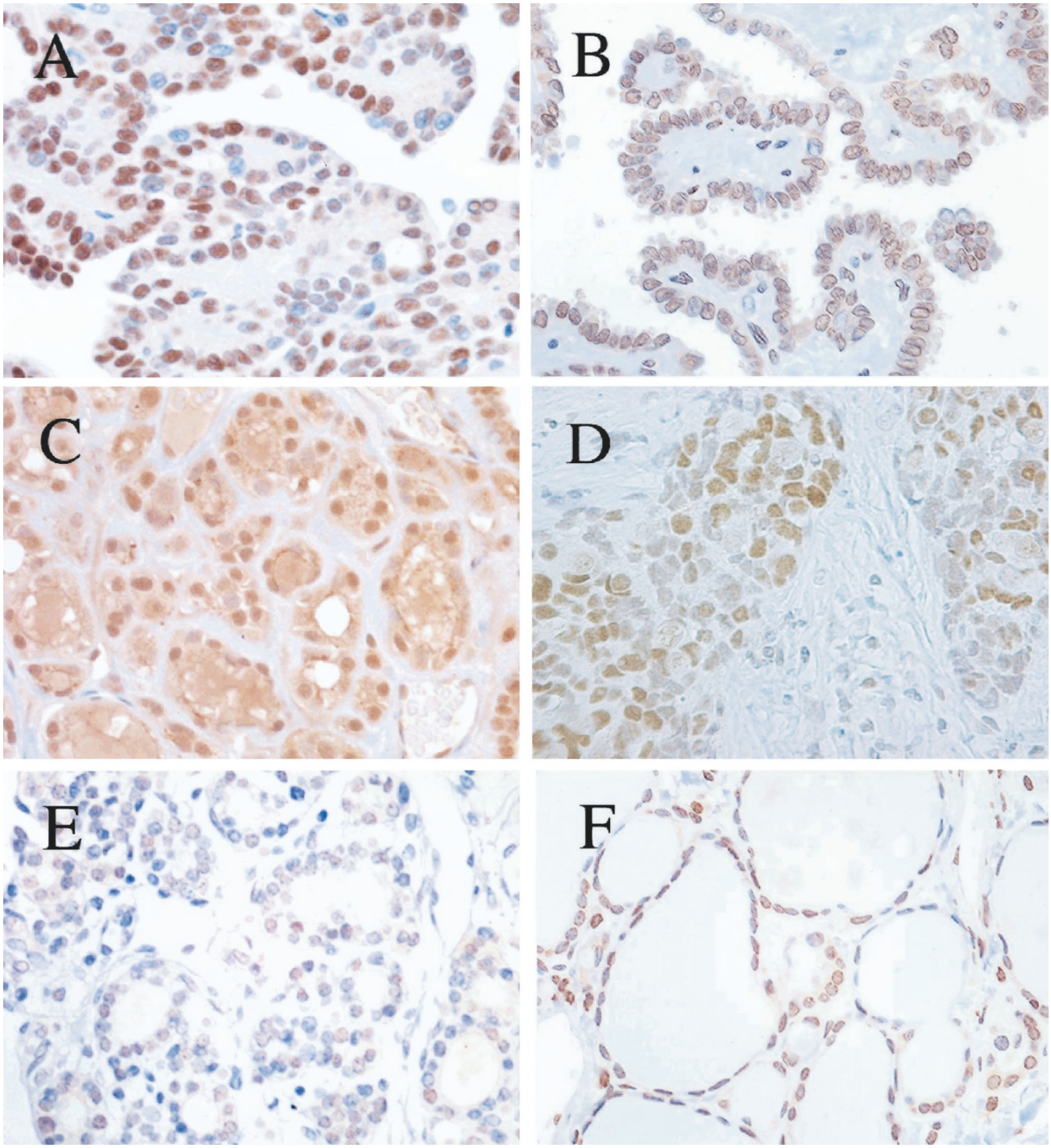

FIGURE 1. Immunohistochemistry for ERα in (**A, B**) papillary carcinoma, (**C**) adenomatous goiter obtained from premenopausal women, respectively, and (**D**) anaplastic carcinoma obtained from postmenopausal women. Immunohistochemistry for ERβ in (**E**) the fetal thyroid gland (14 gestational weeks) and (**F**) in the adult thyroid gland.

ERα labeling index was significantly higher in postpuberty/premenopausal women than in postmenopausal women and men of various ages. A statistically significant association between ERα and the age of the patients was not detected in other types of thyroid lesions. Estradiol has been demonstrated to stimulate cell proliferation of FRTL-5 rat cells, a well-established cell line derived from thyroid papillary carcinoma (28), and the human thyroid papillary carcinoma cell line, HTC-TSHr (29). These results all suggest that higher estrogen concentrations in the serum may be responsible for the relatively high incidence of thyroid papillary carcinoma in premenopausal women. In addition, a higher level of ERα labeling index may also be involved in the activation of

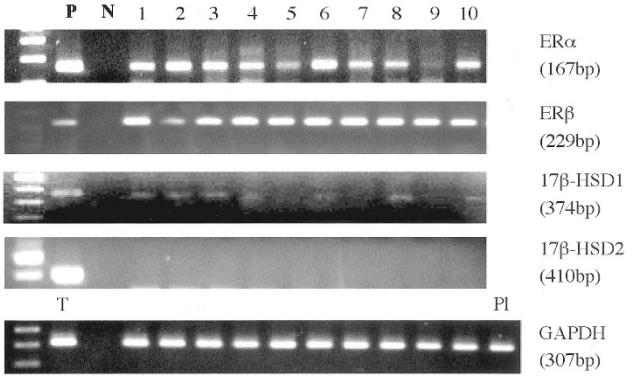

FIGURE 2. RT-PCR analysis for ERα, ERβ, and 17β-HSD Type 1 and Type 2 in human thyroid tumors. mRNA expression for ERα, ERβ, 17β-HSD Type 1, Type 2, and GAPDH were detected as a single gene-specific band (168 bp for ERα, 228 bp for ERβ, 352 bp for 17β-HSD Type 1, 418 bp for 17β-HSD Type 2 and 307 bp for GAPDH). P, Positive controls (T-47D for ERα and ERβ, placenta for 17β-HSD Type 1 and Type 2); T, T-47D; Pl, placenta; N, negative control (no cDNA substrate). All thyroid lesion specimens were obtained from (pre-) or (post-) menopausal women with the exception of 6 and 10, which were obtained from male patients. 1–2, adenomatous goiter (post-); 3–4, papillary carcinoma (pre-); 5, adenomatous goiter (pre-); 6, papillary carcinoma; 7, follicular adenoma (pre-); 8, adenomatous goiter (pre-); 9, papillary carcinoma (post-); 10, papillary carcinoma.

tumorigenesis of papillary carcinoma in premenopausal women, but it awaits further investigations for clarification.

The status of ERβ has not been well studied in human thyroid disorders. In our present study, ERβ nuclear immunoreactivity was detected relatively widely in thyroid neoplastic parenchymal cells. There were, however, no significant correlations between ERβ immunoreactivity and any clinicopathological parameters examined, including patient age, menopausal status, gender, histological type, and fetal developmental stage. In addition, ERβ is widely distributed in follicular cells and C-cells of the normal thyroid gland throughout development. These results above suggest that estrogenic effects via ERβ play important roles in the maintenance of homeostasis of thyroid follicles, and possibly of thyroid hormone biosynthesis.

P450 aromatase converts androstenedione to estrone and testosterone to estradiol. Recent studies demonstrated that bioactive estrogen, estradiol, is produced locally in breast carcinoma (30). Estrogen also has been reported to be produced locally in human thyroid tumors (11). In addition, aromatase immunoreactivity has been demonstrated to be expressed in the cytoplasm of follicular epithelial cells (11). In our present study, the expression of 17β-hydroxysteroid dehydrogenase Types 1 and 2 was demonstrated by RT-PCR. Especially, 17β-hydroxysteroid dehydrogenase Type 1 was detected in 31/48 (64.0%) of the cases examined. Expression of ER, aromatase, and 17β-hydroxysteroid dehydrogenase Type 1 has been demonstrated in human thyroid lesions as in human breast carcinoma (30). This mechanism can effectively produce estrogens *in situ* from circulating androgens and exert their effects locally. Further investigations are required to fully elucidate the roles and mechanisms of estrogenic actions with respect to pathogenesis and/or development of human thyroid disorder.

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