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5α-Reductases in Human Breast Carcinoma: Possible Modulator of *in Situ* Androgenic Actions*

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ABSTRACT

The expression of 5α-reductase types 1 and 2 was examined in human breast carcinoma using immunohistochemistry and RT-PCR. Immunoreactivity for 5α-reductase isozymes was also correlated with various clinicopathological parameters to examine possible local regulatory mechanisms of sex steroids, including progesterone and androgens, in human breast carcinoma tissues. Immunoreactivity for 5α-reductase type 1 was detected in the cytoplasm and possibly in the nuclear membrane of tumor cells in 35 of 60 invasive ductal carcinomas (58%), and type 2 signal was detected in 9 of these 60 cases (15%). The results from RT-PCR (n = 8) were consistent with those from immunohistochemistry. A significant positive correlation was detected between 5α-reductase type 1 immunoreactivity and androgen and progesterone receptor A or B labeling indexes, and immunoreactivities of 5α-reductase type 2, 17β-hydroxysteroid dehydrogenase type 5, or 3β-hydroxysteroid dehydrogenase, which recognizes both types I and II. An inverse correlation was detected between 5α-reductase type 1 immunoreactivity and tumor size, histological grade, or Ki-67 labeling index. 5α-Reductase type 2 immunoreactivity was significantly correlated with 17β-hydroxysteroid dehydrogenase type 5 immunoreactivity, but not with other parameters. This study suggests that 5α-reductase type 1 is mainly expressed in human breast carcinoma, which may play an important role in the *in situ* production and actions of the potent androgen, 5α-dihydrotestosterone, including inhibition of cancer cell proliferation, in hormone-dependent human breast carcinoma. (*J Clin Endocrinol Metab* **86:** 2250–2257, 2001)

BREAST CARCINOMA is one of the most common malignancies in women world-wide. It is well known that human breast tissue is a target for sex steroids, which play important roles in the development of hormone-dependent breast carcinomas (1, 2). Among the various sex steroids, estrogens contribute immensely to the growth of breast carcinoma through binding with the estrogen receptor (ER) (1, 2). In contrast, various previous *in vitro* studies have demonstrated that progesterone and androgens inhibit the proliferation of breast carcinoma cells via progesterone (PR) and androgen (AR) receptors, respectively (3–6). PR and AR have been shown to be expressed in the majority of breast carcinoma tissues (7, 8) and are considered to be important mediators of hormonal additive therapy, including progesterone and androgen, in human breast tumors (9, 10). However, the detailed mechanism of progesterone and androgen actions in human breast carcinoma tissues is largely unknown compared with that of estrogen actions.

5α-Reductases catalyze the conversion of testosterone to the bioactive and potent androgen, 5α-dihydrotestosterone (DHT), and metabolize progesterone to 5α-dihydroprogesterone (5αDHP) (11). Therefore, 5α-reductase expression is an important regulator of the local actions of androgens and progesterone. Two isoforms of 5α-reductase have been cloned and characterized in mammals. The type 1 5α-reductase is located on the distal short arm of chromosome 5 (11) and is mainly expressed in the liver and skin (12). Type 2 5α-reductase is located in band p23 of chromosome 2 (13) and is expressed in the liver, prostate, seminal vesicle, and epididymis (12). Recently, several selective inhibitors of 5α-reductase isozymes (*e.g.* MK-386 for type 1 and finasteride for type 2) have been developed and used in the treatment of androgen-dependent disorders, including skin and prostate pathologies (14). Therefore, it is very important to examine the expression of 5α-reductase isozymes to obtain a better understanding of the role of 5α-reductase and its regulation in various human tissues and their disorders. Enzymatic activity for 5α-reductase has been demonstrated in both breast cancer cell lines (15) and breast cancer tissues themselves (16). 5α-Reductase isozymes are considered to play important roles as local regulators of sex steroid actions in human breast carcinoma, similar to P450 aromatase, which is regulator of estrogen actions. However, the expression of 5α-reductase isozymes has not been examined in breast carcinoma, and its biological significance remains unknown. Therefore, in this study we examined the expression of 5α-reductase type 1 and type 2 in human breast carcinoma tissues using immunohistochemistry and RT-PCR. In addition, we statistically correlated the immunoreactivity of 5α-reductase isozymes with various clinicopathological parameters in 60 cases of human breast carcinoma to study the

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possible role of 5α-reductases in hormone-dependent breast cancer.

Materials and Methods

Patients and Tissues

Sixty specimens of invasive ductal carcinoma of the breast were obtained from women who underwent mastectomy from 1984–1987 at the Department of Surgery, Tohoku University Hospital (Sendai, Japan). Their mean age was 50.9 yr (range, 27–78). All patients examined in this study received neither irradiation nor chemotherapy before surgery. The clinical data, including patient age, menopausal status, stage according to International Union Against Cancer TNM classification (1987), tumor size, and lymph node status, were retrieved from the charts of the patients. The histological grade of each specimen was evaluated by three of the authors (T.S., N.A., and T.M.), based on the modified method of Bloom and Richardson (17) according to Elston and Ellis (18). The mean follow-up time was 107 months (range, 15–155). Disease-free survival data were available for all patients. All specimens were fixed with 10% formalin and embedded in paraffin wax at the Department of Pathology, Tohoku University Hospital.

Antibodies

5α-Reductase type 1 and type 2 antibodies were rabbit polyclonal antibodies against a synthesized peptide corresponding to amino acids 232–256 for 5α-reductase type 1 (12) and amino acids 227–251 for 5α-reductase type 2 (12), respectively. These antibodies were provided by Dr. D. W. Russell (University of Texas Southwestern Medical Center, Dallas, TX). The polyclonal antibody for 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5) was raised in rabbits against a synthetic peptide corresponding to amino acids 297–320 for 17βHSD5 (19) and was provided by Dr. V. Luu-The (Laval University Hospital Center, Quebec, Canada). The characterization of these three antibodies was confirmed by Western blotting (12, 19), and use of antibodies for 5α-reductase type 2 (20) and 17βHSD type 5 (19) on immunohistochemistry has been previously reported. Polyclonal antibody for 3βHSD was provided by Dr. J. I. Mason (University of Edinburgh, Edinburgh, UK). It was raised in rabbits against purified human placental 3βHSD (21), and the immunoreactivity has also been demonstrated in human adrenals (22), indicating that this antibody detects both 3βHSD types I and II (23). Monoclonal antibodies for AR (AR441), two isoforms of PR [PR-A (hPRa7) (24) and PR-B (hPRa2) (25)], ER (ER1D5), and Ki-67 (MIB1) were purchased from DAKO Corp. (Carpinteria, CA) NeoMarkers (Fremont, CA), NeoMarkers, Immunotech (Marseilles, France), and Immunotech, respectively. Rabbit polyclonal antibody for HER-2/*neu* (A0485) was obtained from DAKO Corp.

Immunohistochemistry

Immunohistochemical analysis was performed employing the streptavidin-biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan) and has been previously described in detail (26). For immunostaining of AR, PR-A, PR-B, ER, Ki-67, and HER-2/*neu*, the slides were heated in an autoclave at 120 C for 5 min in citric acid buffer (2 mmol/L citric acid and 9 mmol/L trisodium citrate dehydrate, pH 6.0) after deparaffinization for antigen retrieval. The dilutions of primary antibodies used in our study were as follows: 5α-reductase type 1, 1:1000; 5α-reductase type 2, 1:1000; AR, 1:100; PR-A, 1:150; PR-B, 1:150; ER, 1:2; 17βHSD5, 1:1000; 3βHSD, 1:1000; Ki-67, 1:50; and HER-2/*neu*, 1:200. The antigen-antibody complex was visualized with 3,3′-diaminobenzidine solution [1 mmol/L 3,3′-diaminobenzidine, 50 mmol/L Tris-HCl buffer (pH 7.6), and 0.006% H_2O_2] and counterstained with hematoxylin. Tissue sections of liver and prostate were used as positive controls for 5α-reductase type 1 and type 2, respectively (12), and nonneoplastic breast tissue was used as a positive control for 17βHSD type 5 (19). As a negative control, normal rabbit or mouse IgG was used instead of the primary antibodies, and no specific immunoreactivity was detected in these sections.

RT-PCR

RT-PCR analysis was performed on eight specimens of invasive ductal carcinoma. Total ribonucleic acid (RNA) was extracted from frozen breast carcinoma tissue samples using an RNeasy Total RNA isolation kit (QIAGEN, Hilden, Germany). RNA concentrations were determined spectrophotometrically. Total RNA (5 μg) was denatured at 70 C for 10 min and was reverse transcribed in the presence of 25 ng/μl oligo(deoxythymidine)$_{12–18}$ primer (Life Technologies, Inc., Tokyo, Japan), 2.5 mmol/L $MgCl_2$, 0.5 mmol/L deoxy-NTPs, 10 mmol/L dithiothreitol, and 10 U ribonuclease H^- reverse transcriptase (SuperScript II RT, Life Technologies, Inc., Tokyo, Japan) for 20 min at 23 C, 60 min at 42 C, and 15 min at 70 C. Subsequently, 1 μL of the resulting complementary DNA (cDNA) was used as a template for PCR. The primer sequences used in this study (27–30) are listed in Table 1. PCR amplifications were performed in a final volume of 50 μL in the presence of 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 0.2 mmol/L deoxy-NTPs, 2 mmol/L $MgCl_2$, 0.8 μmol/L of each primer, and 2.5 U *Taq* DNA polymerase (Life Technologies, Inc.). An initial denaturing step of 95 C for 2 min was followed by 40 cycles (AR and 5α-reductase type 1 and type 2) and 30 cycles (β-actin), respectively, of 95 C for 1 min; 1-min annealing at 50 C (5α-reductase type 1), 60 C (5α-reductase type 2), 56 C (AR), and 58 C (β-actin); and a final extension for 2 min at 72 C. All PCR cycling was performed on a PTC-200 Peltier Thermal Cycler DNA Engine (MJ Research, Inc., Watertown, MA). After PCR, the products were resolved on a 2% agarose ethidium bromide gel. Images were captured with Polaroid (Hertfordshire, UK) film under UV transillumination. In initial experiments, after amplification PCR products were purified and subjected to direct sequencing (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 310 Genetic Analyzer, Perkin-Elmer Corp. PE Applied Biosystems, Foster City, CA) to verify amplification of the correct sequences. As a positive control, frozen tissues of liver were used for 5α-reductase type 1 and AR, and those of prostate were used for 5α-reductase type 2. Negative control experiments lacked the cDNA substrate to check for the possibility of exogenous contaminant DNA. No amplified products were observed under these conditions.

Scoring of immunoreactivity

For statistical analyses of 5α-reductase type 1 and type 2, 17βHSD type 5, 3βHSD, and HER-2/*neu* immunoreactivity, the carcinomas were classified into two groups (+, positive carcinoma cells; and −, no im-

TABLE 1. Primer sequences used in RT-PCR analysis

cDNA	Sequence	Position in cDNA	Size (bp)	Ref. no.
5α-Reductase type 1	Forward 5′-TGGGAGGAGGAAAGCCTATG Reverse 5′-GCCACACCACTCCATGATTTC	347–366 654–634	308	Delos *et al.* (27)
5α-Reductase type 2	Forward 5′-CATACGGTTTAGCTTGGGTGT Reverse 5′-GCTTTCCGAGATTTGGGGTAG	456–476 750–770	315	Andersson *et al.* (28)[a]
AR	Forward 5′-GTCAAAAGCGAAATGGGCCCC Reverse 5′-CTTCTGGGTTGTCTCCTCAGT	Spans exons 2–3	420	Zhu *et al.* (29)
β-Actin	Forward 5′-GATTCCTATGTGGGCGACGAG Reverse 5′-CCATCTCTTGCTCGAAGTCC	192–212 704–723	532	Willey *et al.* (30)

[a]Oligonucleotide primers for 5α-reductase type 2 were designed using the previously published cDNA sequence for human 5α-reductase type 2 by Andersson *et al.* (28).

munoreactivity) by three of the authors (T.S., T.M., and N.A.) independently. Cases with discordant results among the observers were reevaluated. Scoring of AR, PR-A, PR-B, ER, and Ki-67 in carcinoma cells was performed on high power fields (×400) using a standard light microscope. In each case, more than 500 carcinoma cells were counted independently by these same three authors, and the percentage of immunoreactivity, *i.e.* labeling index (LI), was determined. In the present study interobserver differences were less than 5%, and the mean of the three values was obtained.

Statistical analysis

Values for patient age, tumor size, and LIs for AR, PR-A, PR-B, ER, and Ki-67 were presented as the mean ± 95% confidence interval (95% CI), and associations between the immunoreactivity of 5α-reductases and these parameters were evaluated using a Bonferroni test. Statistical differences between immunoreactivity for 5α-reductases and menopausal status, stage, lymph node status, histological grade, and immunoreactivity for 17βHSD5, 3βHSD, and HER-2/*neu* were evaluated in a cross-table using the χ^2 test. $P < 0.05$ was considered significant. Overall and disease-free survival analyses were calculated according to the Kaplan-Meier test. The statistical significance of differences in the survival analyses was calculated using the log-rank test.

Results

Immunohistochemistry

Immunoreactivity for 5α-reductase type 1 and type 2 was detected in the cytoplasm and possibly in the nuclear membrane of carcinoma cells (Fig. 1, A and B). The number of positive cases and percentages were 35 of 60 cases (58.3%) for 5α-reductase type 1, and 9 of 60 cases (15.0%) for 5α-reductase type 2, respectively. Immunoreactivity for 5α-reductase type 1 and type 2 was focally detected in morphologically normal glandular epithelia adjacent to the carcinoma. To examine the possible effects of long-term storage of the samples on immunoreactivity of 5α-reductases, we performed immunohistochemistry for 5α-reductase types 1 and 2 in 12 invasive ductal carcinomas collected within 1 yr. Immunoreactivity for 5α-reductase type 1 and type 2 was detected in 8 of 12 (66.7%) and 3 of 12 (25.0%), respectively.

Immunoreactivity for AR (Fig. 1C), PR-A, PR-B, and ER was detected in the nuclei of carcinoma cells, and the number of positive cases and percentages were 49 of 60 (81.7%), 33 of

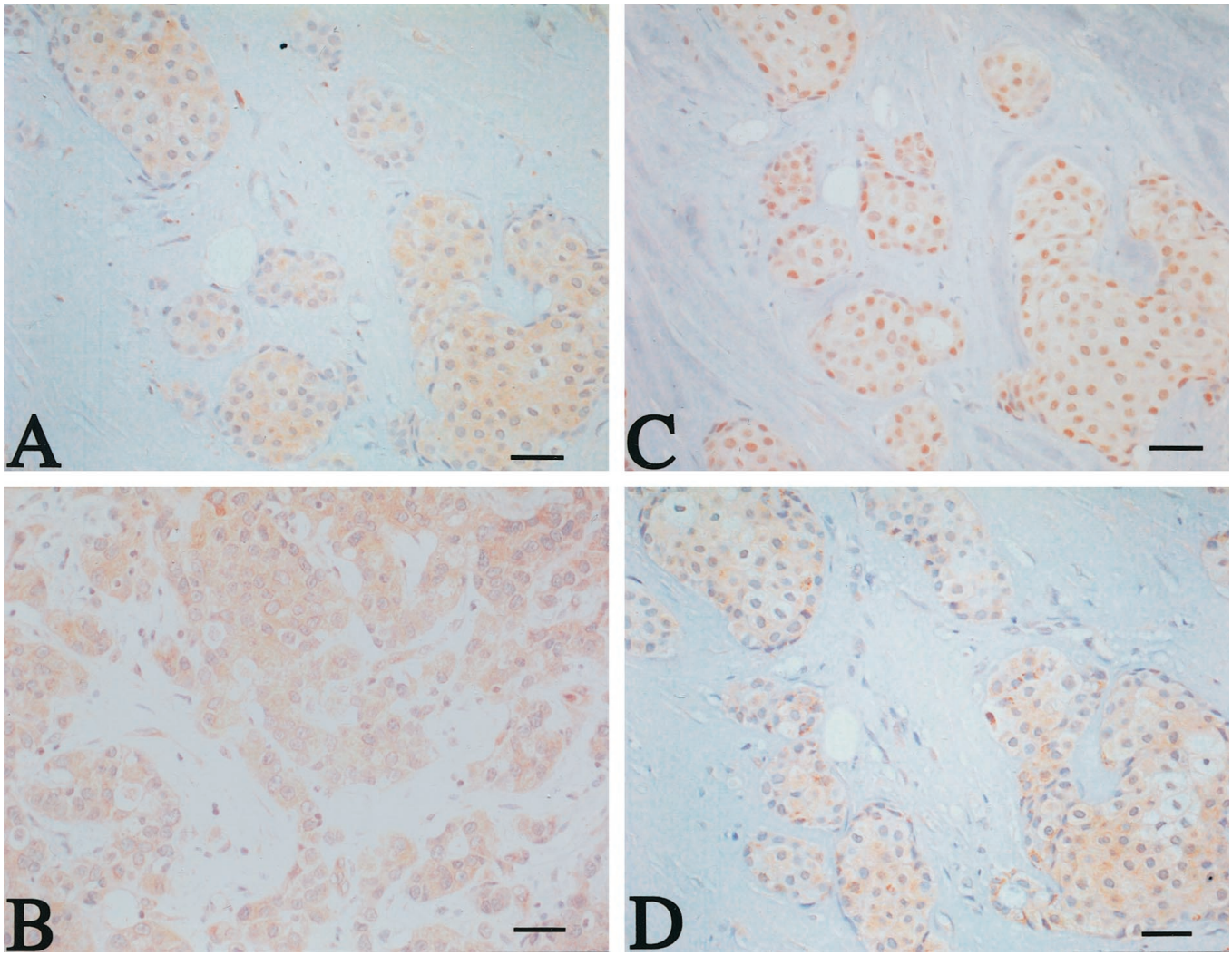

FIG. 1. Immunohistochemistry for 5α-reductase type 1 (A) and type 2 (B), AR (C), and 17βHSD type 5 (D) in invasive ductal carcinoma. Immunoreactivity of 5α-reductase type 1 (A) and type 2 (B), and 17βHSD type 5 (D) was detected in the cytoplasm of carcinoma cells, whereas that of AR (C) was detected in the nucleus of carcinoma cells. The same field as A, C, and D. Original magnification, ×140. *Bar*, 50 μm.

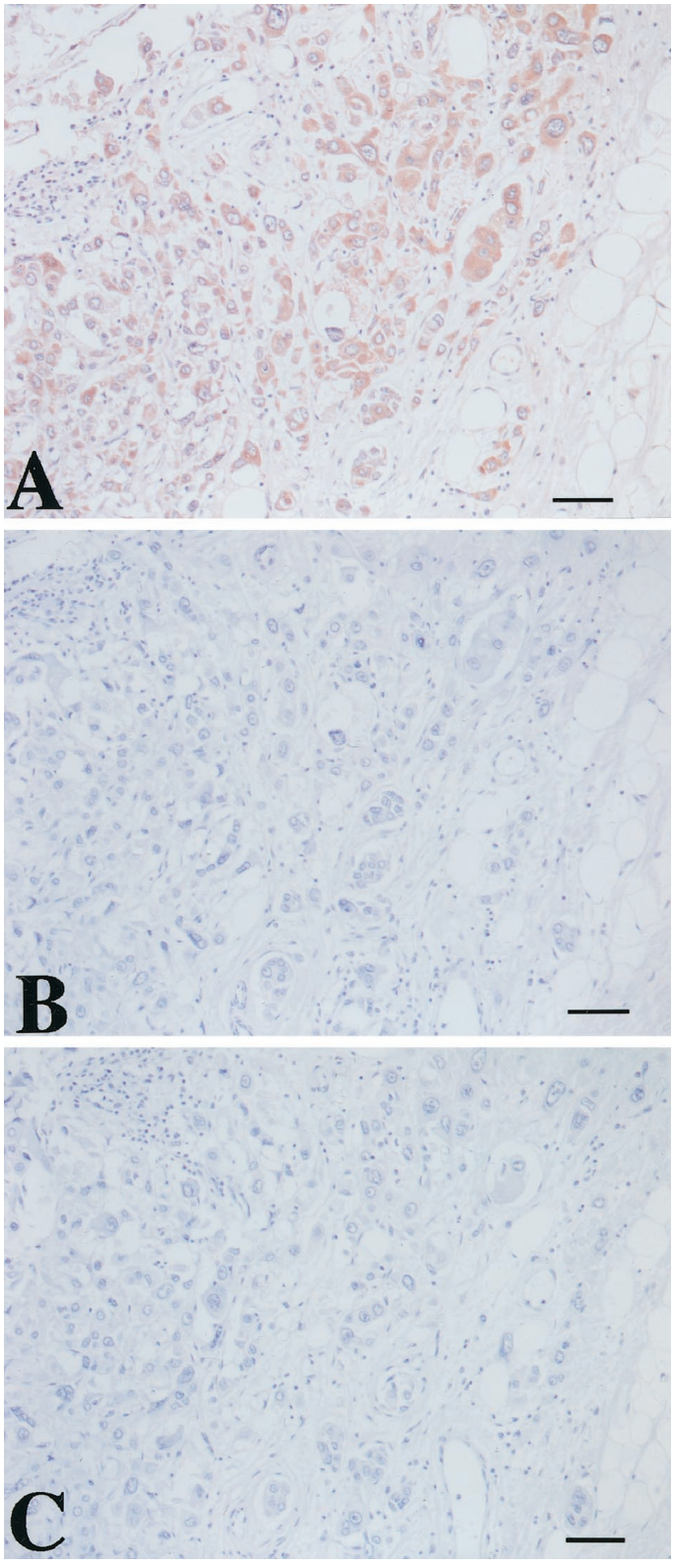

FIG. 2. Immunohistochemistry for 3βHSD in invasive ductal carcinoma. Immunoreactivity for 3βHSD (A) was detected in the cytoplasm of carcinoma cells. Immunoreactivity for 5α-reductase type 1 (B) and type 2 (C) was not detected in this case. Same field. Original magnification, ×140. *Bar*, 50 μm.

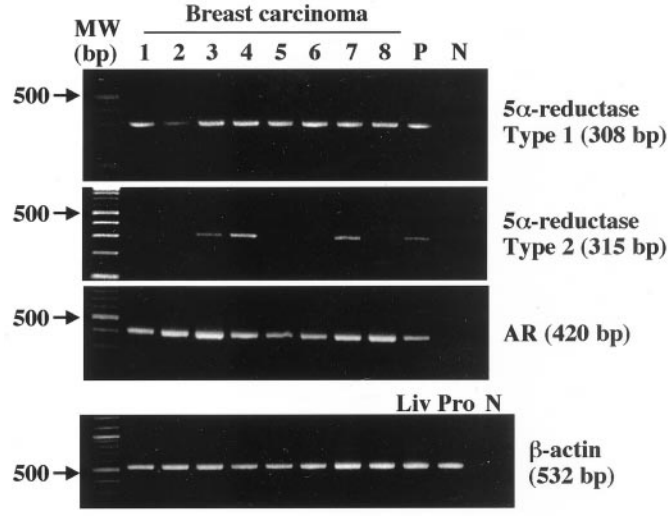

FIG. 3. RT-PCR analysis for 5α-reductase type 1 and type 2 and AR in eight invasive ductal carcinomas. mRNA expression for 5α-reductase type 1 and 2, AR, and β-actin were detected as a specific single band (308 bp for 5α-reductase type 1, 315 bp for 5α-reductase type 2, 420 bp for AR, and 532 bp for β-actin). mRNA expression was detected in eight of eight cases for 5α-reductase type 1, three (cases 3, 4, and 7) of eight cases for 5α-reductase type 2, and eight of eight cases for AR. P, Positive controls (liver for 5α-reductase type 1 and AR, and prostate for 5α-reductase type 2); Liv, liver; Pro, prostate; N, negative control (no cDNA substrate).

60 (55%), 38 of 60 (63.3%), and 51 of 60 (85.0%), respectively. Immunoreactivity for 17βHSD type 5 was detected in cytoplasms of carcinoma cells (Fig. 1D) in 37 of 60 cases (61.7%) examined. Immunoreactivity for 17βHSD5 was also detected in the cytoplasms of morphologically normal glandular epithelial cells and some stromal cells adjacent to the carcinoma, as reported previously (19). 3βHSD immunoreactivity was detected in the cytoplasms of carcinoma cells in 32 cases (53.3%; Fig. 2). Ki-67 immunoreactivity was detected in the nuclei of carcinoma cells as well as some stromal cells in all cases examined. HER-2/*neu* immunoreactivity was detected in membranes of carcinoma cells in 27 of 60 cases (45%) in this study.

RT-PCR

As shown in Fig. 3, messenger RNA (mRNA) expression for 5α-reductase type 1 and type 2 and AR was detected as a specific single band (308 bp for 5α-reductase type 1, 315 bp for 5α-reductase type 2, and 420 bp for AR) in eight of three (100%), three of eight (37.5%), and eight of eight (100%) cases, respectively. The results of RT-PCR analyses in each case were consistent with those of immunohistochemistry.

Correlation between 5α-reductases and clinicopathological parameters

The results of correlation between 5α-reductases and clinicopathological parameters are summarized in Table 2. As shown in Table 2A, a significant inverse correlation was detected between 5α-reductase type 1 immunoreactivity and tumor size ($P = 0.043$) or histological grade of the carcinoma tissue ($P = 0.013$). There was, however, no significant correlation between 5α-reductase type 1 immunoreactivity and

TABLE 2A. Correlation between 5α-reductase type 1 immunoreactivity and clinicopathological parameters in human breast carcinomas

	5α-Reductase type 1 immunoreactivity		*P* value
	+ (n = 35)	− (n = 25)	
Age* (yr)	49.1 ± 4.1	53.6 ± 3.7	NS
Menopausal status			
Premenopausal	23 (38.3)	11 (18.3)	
Postmenopausal	12 (20.0)	14 (23.3)	NS
Stage			
I	10 (17.0)	3 (5.1)	
II	17 (28.8)	18 (30.5)	
III	6 (10.2)	3 (5.1)	
IV	1 (1.7)	1 (1.7)	NS
Tumor size (mm)	24.1 ± 4.6	31.4 ± 6.1	0.043
Lymph node status			
n, +	19 (31.7)	16 (26.7)	
n, −	16 (26.7)	9 (15.0)	NS
Histological grade			
I	10 (16.7)	2 (3.3)	
II	14 (23.3)	6 (10.0)	
III	11 (18.3)	17 (28.3)	0.013

Data for age and tumor size are presented as the mean ± 95% confidence interval. All other values represent the number of cases and their respective percentages (in *parentheses*).

TABLE 2B. Correlation between 5α-reductase type 2 immunoreactivity and clinicopathological parameters in human breast carcinomas

	5α-Reductase type 2 immunoreactivity		*P* value
	+ (n = 9)	− (n = 51)	
Age* (yr)	51.1 ± 9.1	50.9 ± 2.9	NS
Menopausal status			
Premenopausal	4 (6.7)	30 (50.0)	
Postmenopausal	5 (8.3)	21 (35.0)	NS
Stage			
I	3 (5.1)	10 (16.9)	
II	6 (10.2)	29 (49.2)	
III	0 (0.0)	9 (15.3)	
IV	0 (0.0)	2 (3.4)	NS
Tumor size (mm)	21.4 ± 4.7	28.3 ± 4.0	NS
Lymph node status			
n, −	3 (5.0)	19 (31.7)	
n, +	6 (10.0)	32 (53.3)	NS
Histological grade			
I	3 (5.0)	9 (15.0)	
II	2 (3.3)	18 (30.0)	
III	4 (6.7)	24 (40.0)	NS

Data for age and tumor size are presented as the mean ± 95% confidence interval. All other values represent the number of cases and their respective percentages (in *parentheses*).

patient age, menopausal status, stage, or lymph node status. 5α-Reductase type 2 immunoreactivity was not significantly correlated with any clinicopathological parameters examined in this study (Table 2B).

Correlation between 5α-reductases and steroid receptors and enzymes

The results of correlation between 5α-reductase type 1 and immunohistochemical parameters are summarized in Table 3A. There was a strong correlation between 5α-reductase type 1 immunoreactivity and AR LI ($P = 0.0026$). A significant positive correlation was also detected between 5α-reductase type 1 immunoreactivity and 5α-reductase type 2 immunoreactivity ($P = 0.030$), PR-A LI ($P = 0.039$), PR-B LI ($P = 0.042$), 17βHSD type 5 immunoreactivity ($P = 0.0034$), and 3βHSD immunoreactivity ($P = 0.023$). There was a significant inverse correlation between 5α-reductase type 1 immunoreactivity and Ki-67 LI ($P = 0.020$). The results of correlation between 5α-reductase type 2 and these parameters

TABLE 3A. Correlation between 5α-reductase type 1 immunoreactivity and immunohistochemical parameters in human breast carcinomas

	5α-reductase type 1 immunoreactivity		*P* value
	+ (n = 35)	− (n = 25)	
5α-Reductase type 2 immunoreactivity			
+	8 (13.3)	1 (1.7)	
−	27 (45.0)	24 (40.0)	0.030
AR LI	44.3 ± 10.9	22.1 ± 9.2	0.0026
PR-A LI	24.2 ± 12.6	9.3 ± 7.7	0.039
PR-B LI	33.8 ± 14.4	16.1 ± 10.2	0.042
ER LI	45.4 ± 11.8	29.0 ± 18.5	NS
17βHSD type 5 immunoreactivity			
+	27 (45.0)	10 (16.7)	
−	8 (13.3)	15 (25.0)	0.0034
3β-HSD immunoreactivity			
+	23 (38.3)	9 (13.3)	
−	12 (16.3)	16 (11.7)	0.023
Ki-67 LI	26.4 ± 4.8	37.5 ± 8.7	0.020
HER-2/*neu* immunoreactivity			
+	14 (23.3)	13 (21.7)	
−	21 (35.0)	12 (20.0)	NS

Data for age and tumor size are presented as the mean ± 95% confidence interval. All other values represent the number of cases and their respective percentages (in *parentheses*).

TABLE 3B. Correlation between 5α-reductase type 2 immunoreactivity and immunohistochemical parameters in human breast carcinomas

	5α-Reductase type 2 immunoreactivity		*P* value
	+ (n = 9)	− (n = 51)	
AR LI	44.1 ± 25.7	33.6 ± 7.7	NS
PR-A LI	17.4 ± 23.8	17.4 ± 8.2	NS
PR-B LI	27.4 ± 27.7	25.4 ± 9.4	NS
ER LI	31.9 ± 27.4	39.7 ± 8.5	NS
17βHSD type 5 immunoreactivity			
+	9 (15.0)	28 (46.7)	
−	0 (0.0)	23 (38.3)	0.010
3β-HSD immunoreactivity			
+	7 (11.7)	25 (41.7)	
−	2 (3.3)	26 (43.3)	NS
Ki-67 LI	29.0 ± 16.6	31.4 ± 4.9	NS
HER-2/*neu* immunoreactivity			
+	3 (5.0)	24 (40.0)	
−	6 (10.0)	27 (45.0)	NS

Data for age and tumor size are presented as the mean ± 95% confidence interval. All other values represent the number of cases and their respective percentages (in *parentheses*).

above are summarized in Table 3B. 5α-Reductase type 2 immunoreactivity was significantly correlated with 17βHSD type 5 immunoreactivity ($P = 0.010$), but other significant correlations were not detected in this study.

Correlation between 5α-reductases and prognosis

No significant correlation was detected between 5α-reductase type 1 or type 2 immunoreactivity and overall or disease-free survival in the 60 invasive ductal carcinomas investigated in this study.

Discussion

In this study immunoreactivity for 5α-reductase type 1 was detected in carcinoma cells in 35 of 60 human breast carcinomas (58.3%), whereas that of 5α-reductase type 2 was detected in carcinoma cells in 9 of 60 cases (15.0%) examined. Activity of 5α-reductase has previously been demonstrated in various human breast carcinoma cell lines (15, 31), and Wiebe *et al.* (16) reported that 5α-reductase activity was 4–8 times elevated in breast cancer tissues compared with that in nontumorous breast tissues. Results from our present study are consistent with these previous reports and suggest that type 1 5α-reductase is mainly expressed in carcinoma cells of human breast carcinoma tissues. Immunoreactivity for 5α-reductase types 1 and 2 was detected in the cytoplasm and possibly in the nuclear membrane in this study. These findings are consistent with previous reports that describe both of these antigens as being integral membrane proteins localized to the endoplasmic reticulum and the contiguous nuclear membrane (11). However, further investigations, including ultrastructural immunohistochemistry, are required to study the precise subcellular localization of 5α-reductase type 1 and type 2 in human breast carcinoma cells.

In this study 5α-reductase type 1 immunoreactivity was significantly correlated with AR LI ($P = 0.0026$), which suggests that 5α-reductase type 1 plays an important role in the regulation of local androgenic actions. Previously, Isola (8) reported that 79% of breast carcinomas expressed AR, suggesting that androgenic actions are present in human breast carcinoma tissues. Physiological concentrations of potent androgens such as DHT were very low in normal woman (32–34) and in breast cancer patients (35). However, Recchione *et al.* (36) demonstrated that DHT concentrations were significantly higher (3-fold increase; $P = 0.0001$) in breast cancer tissues than in plasma and suggested the possible local production of DHT in human breast carcinoma tissues. Recently, 17βHSD type 5, which specifically catalyzes the reduction of androstenedione to testosterone, was cloned by Dufort *et al.* (37). 17βHSD type 5 is expressed in various peripheral tissues, including the breast (19), and El-Alfy (38) proposed possible *in situ* production of DHT from the inactive adrenal precursor dehydroepiandrosterone (DHEA) by 3βHSD (conversion from DHEA to androstenedione), 17βHSD type 5 (reduction of androstenedione to testosterone), and 5α-reductases (metabolism of testosterone to DHT) in various peripheral tissues. Previous studies have reported the presence of 3βHSD activity in breast carcinoma tissues (39) and that of 17βHSD activity in breast carcinoma cell lines (31) in addition to 5α-reductase activity in human breast carcinoma tissues (16). An immunohistochemical study of 3βHSD in breast carcinoma cells has been previously reported by Sasano *et al.* (40), but that of 17βHSD type 5 or 5α-reductases has not been examined. In this study immunoreactivity for 3βHSD and 17βHSD type 5 was detected in carcinoma cells in 32 (53.3%) and 37 (61.7%) of 60 invasive ductal carcinomas, respectively, and immunoreactivity for 5α-reductase type 1 was correlated with that of 3βHSD ($P = 0.023$) or 17βHSD type 5 ($P = 0.0034$) immunoreactivity. Therefore, the results of our study suggest that DHT is locally produced by 3βHSD, 17βHSD type 5, and 5α-reductase type 1 in carcinoma cells and acts on these cells locally via AR in human breast carcinoma tissues.

There was a significant inverse correlation between 5α-reductase type 1 immunoreactivity and Ki-67 LI ($P = 0.020$) or tumor size ($P = 0.043$) in this study. Monoclonal antibody Ki-67 has been demonstrated to recognize cells in all phases of the cell cycle except the G_0 (resting) phase (41), and Ki-67 LI is well recognized to be a useful factor to evaluate the proliferative activity of various neoplastic tissues, including breast carcinoma (42). Previous *in vitro* studies have demonstrated that DHT inhibited the basal and estrogen-induced cell proliferation in human breast cancer cell lines through actions on AR, and this effect occurred at physiological concentrations (4, 6). Our present findings are in good agreement with these previous *in vitro* studies mentioned above and suggest that 5α-reductase type 1 locally induces DHT production and results in a relatively low proliferation rate in human breast carcinoma. 5α-Reductase type 1 immunoreactivity was also inversely correlated with histological grade ($P = 0.013$). Therefore, breast carcinomas positive for 5α-reductase type 1 are considered to be relatively well differentiated and may maintain some hormonal regulatory mechanisms. Administration of androgens has been reported to result in the regression of some cases of breast carcinoma, but also to cause androgenic side-effects (5). Therefore, induction of 5α-reductase type 1 may be effective, with fewer side-effects, in the treatment of breast carcinomas as one possible endocrine therapy.

Recent studies have demonstrated that the bioactive estrogen, estradiol, was produced locally in breast carcinoma tissues. P450 aromatase converts androstenedione to estrone and testosterone to estradiol. Enzymatic activity of aromatase has been demonstrated in human breast carcinoma tissues (43), and aromatase immunolocalization has been reported in stromal cells of breast carcinoma tissues (40). Immunoreactivity for 17βHSD type 1, which catalyzes the conversion of estrone to estradiol, has been reported in carcinoma cells in breast carcinoma tissues (44–46) and has been correlated with ER LI (46). AR-positive breast carcinomas are frequently positive for ER (8), and thus, it is reasonable to observe a strong correlation between AR and ER LIs ($r = 0.400$; $P = 0.0015$) in this study. Therefore, 17βHSD type 5 and 5α-reductases may act to increase DHT production through competition with aromatase of estrogen production in hormone-dependent breast carcinomas (Fig. 4).

Previous studies have reported that progesterone exerts a direct antiproliferative effect (3, 5) and abolishes the stimulatory effects of estradiol on the growth of breast cancer cells

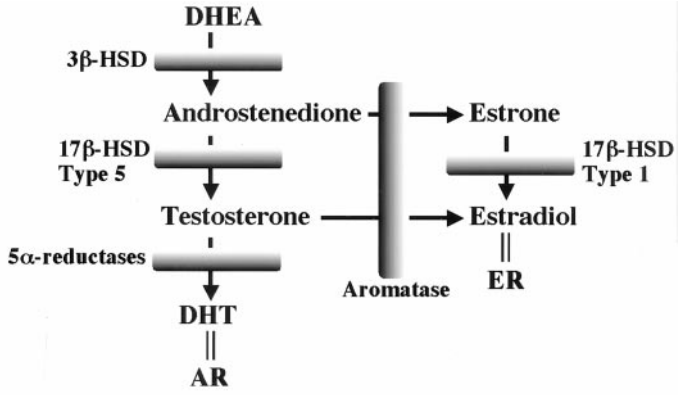

FIG. 4. Flow chart illustrating the possible cascade of local production of androgens and estrogens in human breast carcinoma. DHT is locally produced from the inactive adrenal precursor DHEA by 3βHSD (conversion from DHEA to androstenedione), 17βHSD type 5 (reduction of androstenedione to testosterone), and 5α-reductases (metabolism of testosterone to DHT) and acts on the carcinoma cells through the AR. On the other hand, estradiol is locally produced by aromatase (conversion from androstenedione to estrone and from testosterone to estradiol) and 17βHSD type 1 (conversion of estrone to estradiol) and binds to ER in the carcinoma cells.

(5). Progesterone actions are mediated via two isoforms of PR, PR-A (81–83 kDa) and PR-B (116–120 kDa). PR is present in approximately 60% of breast carcinoma tissues (7), and the presence of PR is an important indicator of the responsiveness to endocrine agents in human breast carcinoma (10). Recently, Wiebe *et al.* (16) demonstrated that progesterone metabolites to 5αDHP and 3α-hydroxyprogesterone (3α-HP) by 5α-reductase and 3α-hydroxysteroid oxidoreductase, respectively, and the ratio of 5αDHP/3α-HP was nearly 30-fold higher in tumorous than in nontumorous breast tissues using enzymatic assay. They also reported that 5αDHP stimulated, whereas 3α-HP inhibited, the proliferation and detachment of breast cell lines *in vitro*. These previous findings above suggest that 5α-reductase may decrease the biological actions of progesterone, including the antiproliferative effect. However, in this study, 5α-reductase type 1 immunoreactivity was correlated with PR-A and PR-B LIs ($P = 0.039$ and $P = 0.042$, respectively), and inversely correlated with Ki-67 LI ($P = 0.020$) or tumor size ($P = 0.043$). Therefore, evidence of regulation of progesterone action by 5α-reductases could not be verified. This may partly be due to the concentration of progesterone being lower than that of DHT, which may be locally produced in human breast carcinoma tissues. However, studies are required to confirm these speculations.

In summary, we demonstrated that 5α-reductase type 1 is mainly expressed in human breast carcinomas by immunohistochemistry and RT-PCR. Immunoreactivity for 5α-reductase type 1 was significantly correlated with AR, PR-A or PR-B LI, and 5α-reductase type 2, 17βHSD type 5, or 3βHSD immunoreactivity and was inversely correlated with tumor size, histological grade, or Ki-67 LI. Our present data suggest that 5α-reductase type 1 plays an important role in the regulation of *in situ* DHT production and actions, including the inhibition of cancer cell proliferation, in hormone-dependent breast carcinomas.

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