

# Expression of aromatase and 17 $\beta$ -hydroxysteroid dehydrogenase types 1, 7 and 12 in breast cancer

## An immunocytochemical study

D. Song<sup>a</sup>, G. Liu<sup>a</sup>, V. Luu-The<sup>b</sup>, D. Zhao<sup>c</sup>, L. Wang<sup>a</sup>, H. Zhang<sup>c</sup>, G. Xueling<sup>a</sup>,  
S. Li<sup>b</sup>, L. Désy<sup>b</sup>, F. Labrie<sup>b</sup>, G. Pelletier<sup>b,\*</sup>

<sup>a</sup> First Teaching Hospital of Jilin University, ChangChun, China

<sup>b</sup> Molecular Endocrinology Laboratory, CHUL Research Center, Que., Canada

<sup>c</sup> Tumor Hospital of Jilin Province, ChangChun, China

### Abstract

It is known that there is a local biosynthesis of estradiol (E2) in breast carcinoma. The steroidogenic enzymes involved in E2 formation are aromatase which transforms testosterone into E2 and androstenedione into estrone (E1) and reductive 17 $\beta$ -hydroxysteroid dehydrogenases (17 $\beta$ -HSDs) which convert E1 into E2. Using immunocytochemistry, we have studied the expression of aromatase and the three reductive 17 $\beta$ -HSDs 17 $\beta$ -HSD types 1, 7 and 12 in 41 specimens of female human breast carcinoma and adjacent non-malignant tissues. These results were correlated with the estrogen receptor  $\alpha$  (ER $\alpha$ ) and  $\beta$  (ER $\beta$ ), progesterone receptor, androgen receptor, CDC47 and c-erb B-2 expressions and with the tumor stages. Aromatase was found in 58%, 17 $\beta$ -HSD type 7 in 47% and 17 $\beta$ -HSD type 12 in 83% of the breast cancer specimens. The 17 $\beta$ -HSD type 1 could be detected in only one tumor. A significant correlation was observed between the aromatase, 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12 expression, as well as between each of the two enzymes 17 $\beta$ -types 7 and 12 and the ER $\beta$  expression. The expression of 17 $\beta$ -HSD type 12 was significantly higher in breast carcinoma specimens than in normal tissue. There was also a significant association of CDC 47 expression with ER $\beta$ , AR and 17 $\beta$ -HSD type 12. The results indicate that aromatase, 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12, but not 17 $\beta$ -HSD type 1, are commonly expressed in human breast cancer. Moreover, the high expression of both 17 $\beta$ -HSD type 12 and ER $\beta$  in breast carcinoma cells may play a role in the development and/or progression of breast cancer.

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### 1. Introduction

It is well established that increased exposure to estradiol (E2) is an important risk factor for the genesis and evolution of breast cancer, most of which (approximately 95–97%) in their early stage being estrogen-sensitive [1]. However, the majority (two-thirds) of breast cancers occur during the postmenopausal period when the ovaries have ceased to be functional. Although serum E2 level in postmenopausal women is as low as approximately 1/10 of that of premenopausal

women, it is reported that intra-tumoral E2 level in postmenopausal patients is maintained at a level similar to that found in premenopausal patients [2]. Maintenance of such a high intra-tumoral E2 level in postmenopausal patients is considered to be attributable to the intratumoral biosynthesis of estrogens from androgens secreted from adrenal glands [2,3]. It has also been reported that the tissue concentrations of E2 are significantly higher than those found in the plasma or in normal areas in proximity of tumors, suggesting a specific tumoral biosynthesis [1,2].

There is substantial information that human breast cancer tissue contains all the enzymes responsible for the local biosynthesis of E2 from circulating precursors including P450 aromatase and 17 $\beta$ -hydroxysteroid dehydrogenases (17 $\beta$ -HSDs) and sulfatases [1]. Aromatase and reductive

\* Corresponding author at: Molecular Endocrinology Laboratory, CHUL Research Center, 2705 Laurier Boulevard, Que., G1V 4G2, Canada. Tel.: +1 418 654 2296; fax: +1 418 654 2761.

E-mail address: [georges.pelletier@crchul.ulaval.ca](mailto:georges.pelletier@crchul.ulaval.ca) (G. Pelletier).

17 $\beta$ -HSDs are the principal enzymes responsible for the last steps of E2 formation: Aromatase transforms androgens into E2 and androstenedione to the weak estrogen estrone (E1), while reductive 17 $\beta$ -HSDs convert E1 into E2.

17 $\beta$ -HSD types 1, 7 and 12 are all involved in the conversion of E1 to E2. 17 $\beta$ -HSD type 1, originally cloned from human placenta [4], is a member of the short-chain alcohol dehydrogenase superfamily. Variable amounts of the type 1 enzyme have been shown to be expressed in human breast epithelial cell lines [5,6]. It has been reported that 17 $\beta$ -HSD type 1 was not expressed in normal breast tissue specimens from postmenopausal women [6].

Human 17 $\beta$ -HSD type 7, a membrane-associated reductive enzyme which produces active estrogens while inactivating active androgens, most probably plays a crucial role in estrogen-sensitive cells and tissues [7,8]. By RT-PCR, the enzyme has been found to be expressed in a variety of tissues, including human mammary gland [9].

Human 17 $\beta$ -HSD type 12 is the most recent member of this enzyme family. It is ubiquitously expressed with the highest levels in liver, muscle and kidney. Interestingly, it was classified as 17 $\beta$ -HSD based only on sequenced similarity lacking any information about substrate specificities [8]. Recently, we have shown that type 12 17 $\beta$ -HSD can convert E1 to E2 [10].

Aromatase expression has been observed in a large proportion of breast cancer cases [11], while the expression of 17 $\beta$ -HSD type 1 has been found in lower proportion of breast cancers [6,11]. So far, the expression of both 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12 has not been reported in breast cancer.

In order to obtain more information about the exact sites of action of enzymes involved in E2 synthesis and then a better knowledge of the role of these enzymes in breast cancer, we developed specific antibodies against human 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12 and we immunolocalized aromatase, 17 $\beta$ -HSD types 1, 7 and 12 in 41 specimens of female human breast carcinoma and adjacent non-malignant tissues. We also correlated those results with estrogen receptor  $\alpha$  (ER- $\alpha$ ), ER- $\beta$ , progesterone receptor (PR), androgen receptor (AR), CDC47 and c-erbB-2 expression.

## 2. Materials and methods

### 2.1. Patients

This study was approved by the institutional review board at First Teaching Hospital of Jilin University and Tumor Hospital of Jilin Province, ChangChun, China. All the patients agreed to participate in this research project. Forty-one women with primary breast cancer were enrolled in this study. All patients underwent modified radical mastectomy at the First Hospital of Jilin University and Tumor Hospital of Jilin Province during the year 2004. Before surgery, they had received no treatment. Patient characteristics are summa-

Table 1  
Patient characteristics

	Cases (%)
Age	
<50 years	21 (51.2)
$\geq$ 50 years	20 (48.8)
Tumor stage <sup>a</sup>	
I	3 (7.3)
II	35 (85.3)
III	3 (7.3)
Histological type	
Infiltrating ductal	36 (87.8)
Infiltrating lobular	3 (7.3)
Others <sup>b</sup>	2 (4.9)

<sup>a</sup> Tumors were classified according to the WHO classification [12].

<sup>b</sup> One atypical and one typical medullary carcinoma.

rized in Table 1. The median age of the patients was 48 years (range: 36–73).

The samples of breast tumors and adjacent non-neoplastic tissues taken out at more than 5 cm from the tumors were collected at surgery. They were fixed in 4% paraformaldehyde in 0.2 M phosphate buffer (pH 7.4) for 24 h. The tissues were then dehydrated through increasing concentrations of ethanol, cleared in toluene, and embedded in paraffin. For histopathologic diagnosis, sections were stained with hematoxylin-eosin. Eighty eight percent of the cases were infiltrating ductal carcinoma, 7% lobular carcinoma and the rest other histologic types (Table 1). In adjacent non-neoplastic tissues, the mammary gland structures had a normal appearance without any signs of inflammation.

### 2.2. Immunocytochemistry

#### 2.2.1. Hormone receptors, CDC47 and c-erbB-2

Paraffin sections (4  $\mu$ m) were deparaffinized, hydrated, and then treated with 3% H<sub>2</sub>O<sub>2</sub> in PBS (pH 7.6) for 15 min. These steps were followed by heating the sections in a microwave oven for antigen retrieval using citrate buffer (pH 5.5), as previously described [13,14]. The sections were then incubated overnight at 4 °C with ER $\alpha$ , ER $\beta$ , PR, AR, CDC47 [15], and C-erbB-2 antibodies at the dilution indicated in Table 2. Control sections were incubated with antibod-

Table 2  
List of primary antibodies

Antibody	Dilution	Source	Catalog no.
ER $\beta$	1:200	Abcam (Cambridge, MA)	GTX-70174
ER $\alpha$	1:2000	Santa Cruz Biotechnology, Santa Cruz (CA)	SC-543
PR	1:500	Santa Cruz Biotechnology (SC)	SC-7208
AR	1:1000	Santa Cruz Biotechnology (SC)	SC-816
CDC47	1:500	Medicorp (Montréal, Canada)	MS-862-P1
c-erbB-2	1:500	DaKo Diagnostics Canada (Mississauga, Canada)	A 0485

ies pre-absorbed with an excess of corresponding antigens ( $10^{-6}$  M).

### 2.3. Steroidogenic enzymes

For localization of the steroidogenic enzymes, the antigen retrieval technique was not applied. Deparaffinized sections were incubated overnight at  $4^{\circ}$  with the antisera to  $17\beta$ -HSD types 1, 7 and 12 and aromatase diluted 1: 500. The antiserum against human  $17\beta$ -HSD type 1 has been described previously [4,16]. For preparation of antibodies to  $17\beta$ -HSD types 7 and 12, we selected the following peptide sequences: P261 to L342 for the type 7 and H217 to N312 for the type 12. These peptide sequences were overproduced in *E. coli* BL-1 using PET23a expression vector (EMD Biosciences, San Diego, CA). The purified proteins were subsequently diluted in phosphate saline buffer containing 50% complete Freund's adjuvant (concentration: 3 mg/ml) and injected sc with 1 ml at multiple sites of New Zealand rabbits. The animals were treated twice with the same amounts of proteins in 50% of incomplete Freund's adjuvant at 1-month intervals. Antisera were analyzed by immunoblot using HEK-293 cells non-transfected and stably transfected with  $17\beta$ -HSD type 7 or  $17\beta$ -HSD type 12, as negative and positive controls, respectively. The proteins were separated by 12% SDS-PAGE, and transferred onto a nitrocellulose for analysis with the protein A purified antibody to  $17\beta$ -HSD type 7 (1.18 mg/ml) and type 12 (0.40 mg/ml). Anti-rabbit IgG horse antibody conjugated with horseradish peroxidase (Amersham, Biosciences, Inc., Baie d'Urfé) was used as secondary antibody (dilution: 1:15,000) and the resulting immunocomplexes were then visualized using enhanced chemiluminescence kit (Perkin Elmer Life Science) and exposed on a X-OMAT blue film

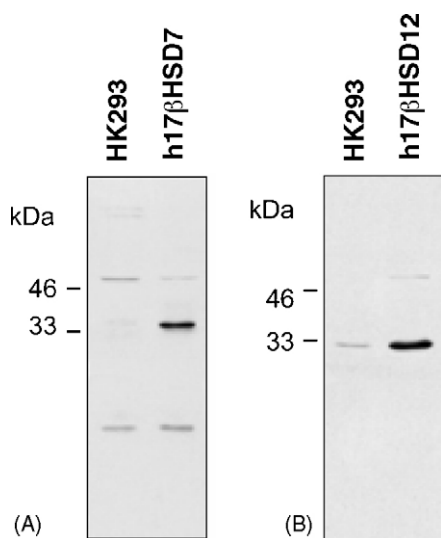


Fig. 1. Western blot analysis of proteins from untransfected or transfected HEK293 cells stably expressing human  $17\beta$ -HSD type 7 (Mw: 37 kDa) (A) and 12 (Mw: 33 kDa) (B). Each antiserum specifically reacts with the corresponding overexpressed enzyme.

for 30 s (Fig. 1). The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC; Ottawa, ONT, Canada) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC; Rockville, MD, USA). The study was performed in accordance with the CCAC Guide for Care and Use of Experiments Animals. The rabbit antiserum to equine P450 aromatase was kindly provided by Dr G.E. Seralini (Caen, France) [17].

Control experiments were performed on adjacent sections by substituting pre-immune rabbit serum (1:500) or the antiserum preabsorbed with an excess of the corresponding human enzyme ( $10^{-6}$  M). To check the crossreactivity of the antibodies to equine aromatase, we performed immunoadsorption with purified human aromatase. All the primary antibodies were detected following incubation at room temperature for 4 h with peroxidase-labeled goat antirabbit  $\gamma$ -globulin (HyClone Laboratories, Inc., Logan, UT) diluted at 1: 200 [14].

### 2.4. Scoring of immunoreactivity

The data were generated from independent observations by two of the authors (D. Song and G. Pelletier). Differences were resolved by joint examination of the slides, and the final reconciled values were used in all statistical analyses. For  $ER\alpha$ ,  $ER\beta$ , PR, AR and CDC47, we counted the number of immunostained nuclei from 300 cells in three randomly chosen fields of each tumor and adjacent non-malignant tissue. In the latter case, only epithelial cells were analyzed. We then calculated the mean percentage of labelled nuclei (labelling index). To quantify enzyme expression, we similarly evaluated the percentage of malignant and non-malignant labelled cells from 300 cells present in three randomly chosen fields. The intensity of malignant and non-malignant epithelial labelling was not considered due to variations in the background staining between sections. A tissue was considered as positive when more than 10% of cells were immunolabelled.

Only cases showing unequivocal staining of membranes were regarded as positive for c-erbB-2 over expression. A score was determined in accordance with the criteria used in the approval trials for trastuzumab [18]. In brief, only cases with more than 50% strong complete membrane staining cases were considered positive for analysis.

### 2.5. Statistical analysis

Associations between categorical variables were assessed by Chi-square ( $\chi^2$ ) tests or Fisher's exact tests. Associations between continuous variables were analyzed using Spearman correlation coefficients. The differences of continuous variables or ranked variables between two groups were compared using T-tests and Kruskal-Wallis test (Chi-Square Approximation), respectively. All analyses were performed using SAS software (Version 6.12).

Table 3  
Summary of individual results

Case no.	Age	Tissue	Stage	Type	c-erbB-2	ER $\alpha$	ER $\beta$	PR	AR	CDC47	17 $\beta$ -HSD type 7	17 $\beta$ -HSD type 12	Arom.
1	58	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	– –	+ +	+ –	+ +	+ +	+ +
2	51	Tumor N-malignant	III	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ +	– +	+ –	+ +
3	59	Tumor N-malignant	II	Inf. duct.	+ –	– +	+ +	– +	+ +	+ –	+ +	+ +	+ +
4	37	Tumor N-malignant	II	Inf. duct.	– –	+ +	– –	– +	+ +	+ –	+ +	+ –	+ +
5	73	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ –	– +	+ –	+ –
6	45	Tumor N-malignant	II	Inf. lob.	+ –	+ +	+ +	+ +	+ +	+ +	– +	– –	+ +
7	44	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ –	– –	+ –	+ +
8	40	Tumor N-malignant	I	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ –	+ –	+ –	+ –
9	45	Tumor N-malignant	II	Inf. duct.	+ –	+ +	+ +	+ +	+ +	+ –	+ +	+ +	+ +
10	47	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
11	45	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ –	+ –	+ –	+ +	+ –	+ –
12	61	Tumor N-malignant	II	Inf. duct.	+ –	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ –
13	45	Tumor N-malignant	II	Inf. duct.	– –	– +	+ +	– +	+ +	+ +	+ +	+ +	+ +
14	50	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ +	+ –	+ –	– +	+ –	– –
15	47	Tumor N-malignant	II	Inf. duct.	– –	+ –	+ –	+ –	+ +	+ –	– –	+ –	– –
16	63	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ –	+ –	+ –	+ –	– –	– –
17	45	Tumor N-malignant	II	Inf. duct.	+ –	+ +	+ –	– +	+ +	+ –	+ +	+ –	+ +
18	47	Tumor N-malignant	II	Inf. duct.	– –	+ –	+ +	+ –	+ +	+ –	– +	+ –	– –
19	60	Tumor N-malignant	III	Other	– –	+ +	– –	– –	+ +	+ –	– +	– –	– –
20	53	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	+ +	+ +	+ –	+ +	+ +	– –
21	59	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ +	– +	+ +	+ +	– +	+ +	+ +
22	50	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ –	+ +	+ +	+ –	– +	+ +	+ –
23	43	Tumor N-malignant	II	Inf. duct.	– –	+ +	+ –	+ +	+ +	+ –	– +	– –	– –
24	47	Tumor N-malignant	II	Inf. duct.	– –	– –	– –	– +	– –	– –	– –	– –	– –
25	47	Tumor N-malignant	I	Inf. duct.	– –	– –	– –	– –	+ +	+ +	– –	– –	– +

Table 3 (Continued)

Case no.	Age	Tissue	Stage	Type	c-erbB-2	ER $\alpha$	ER $\beta$	PR	AR	CDC47	17 $\beta$ -HSD type 7	17 $\beta$ -HSD type 12	Arom.
26	36	Tumor	II	Inf. duct.	–	–	+	–	+	+	–	+	–
		N-malignant			–	+	–	+	+	–	+	–	–
27	40	Tumor	II	Other	–	–	–	–	+	+	–	+	+
		N-malignant			–	+	–	+	+	+	–	–	+
28	62	Tumor	II	Inf. duct.	–	+	+	+	+	+	–	+	–
		N-malignant			–	+	–	–	+	–	–	–	–
29	37	Tumor	II	Inf. lob	–	+	+	+	+	+	+	+	+
		N-malignant			–	+	+	–	+	+	–	+	+
30	54	Tumor	II	Inf. duct.	–	+	+	+	+	+	–	+	–
		N-malignant			–	+	–	–	+	–	–	–	–
31	57	Tumor	II	Inf. lob	+	–	+	–	+	+	+	+	+
		N-malignant			–	–	–	–	+	–	–	–	–
32	47	Tumor	II	Inf. duct.	–	+	+	+	+	+	–	+	–
		N-malignant			–	+	–	+	–	+	–	–	–
33	56	Tumor	II	Inf. duct.	–	–	+	–	+	+	+	+	+
		N-malignant			–	+	+	–	+	–	+	–	–
34	59	Tumor	II	Inf. duct.	–	+	–	–	+	+	–	–	+
		N-malignant			–	+	–	+	+	+	–	–	+
35	66	Tumor	II	Inf. duct.	–	+	+	–	+	+	+	+	–
		N-malignant			–	–	–	–	–	–	–	–	–
36	35	Tumor	II	Inf. duct.	–	–	+	–	–	+	+	+	–
		N-malignant			–	+	+	–	+	–	+	+	–
37	66	Tumor	II	Inf. duct.	–	–	–	–	–	–	+	–	–
		N-malignant			–	–	–	–	–	–	–	–	–
38	51	Tumor	II	Inf. duct.	+	–	+	–	–	–	+	+	+
		N-malignant			–	+	+	–	+	–	+	+	+
39	46	Tumor	II	Inf. duct.	–	–	+	–	+	+	+	+	–
		N-malignant			–	+	+	–	+	–	+	+	–
40	51	Tumor	II	Inf. duct.	+	+	+	–	+	+	+	+	+
		N-malignant			–	+	+	–	+	–	+	+	–
41	49	Tumor	II	Inf. duct.	+	+	+	+	+	+	+	+	+
		N-malignant			–	+	+	–	+	+	+	+	–

Inf. duct. = infiltrating ductal.

Inf. lob. = infiltrating lobular.

A tissue is + when more than 10% of cells are immunolabelled. The intensity of staining was not considered. Only one tumor (patient # 25) showed a positive reaction for 17 $\beta$ -HSD type 1.

### 3. Results

#### 3.1. Steroidogenic enzymes

As shown in Fig. 1, the Western blot analysis demonstrated that the antisera to 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12 specifically reacted with the corresponding overexpressed enzyme. Control experiments were performed following immunoabsorption of each antiserum with an excess of the corresponding enzyme. No staining could be detected (data not shown).

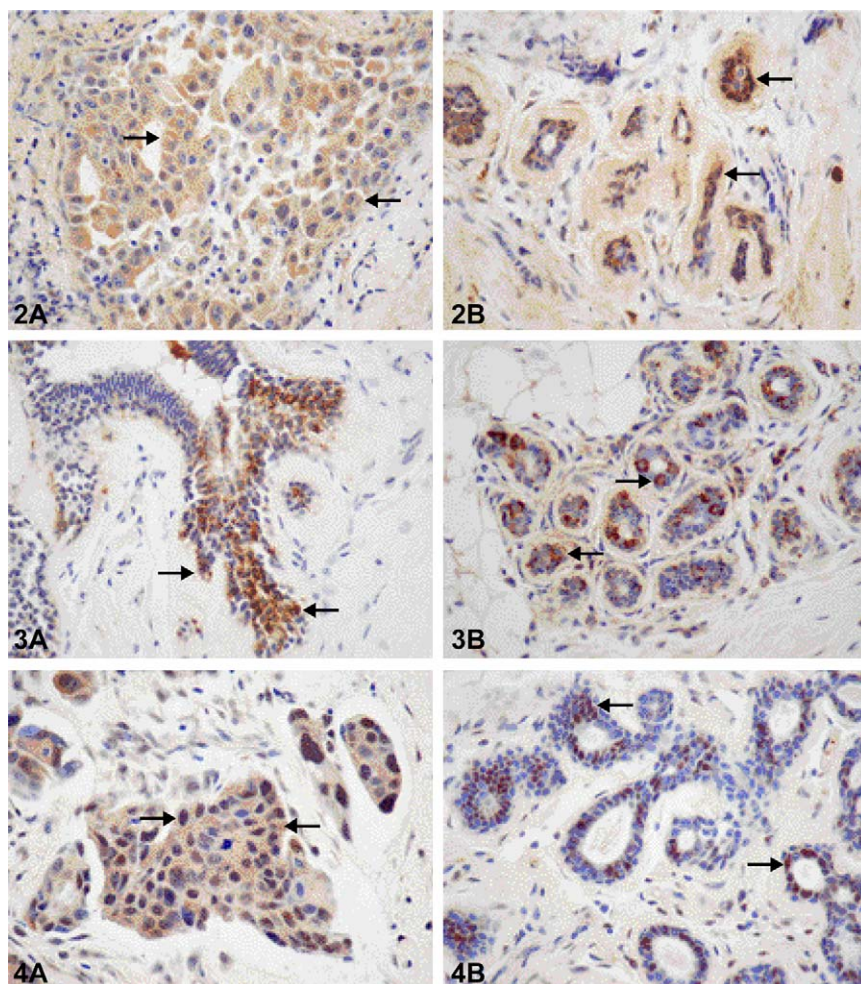
Immunostaining for aromatase was seen in the cytoplasm of malignant cells in 58% of patients. The surrounding interstitial cells were generally devoid of any specific staining (Tables 3 and 4, Fig. 2A). In non-malignant adjacent tissues,

Table 4

Comparison of the expression of steroid receptors, CDC47 and steroidogenic enzymes between cancer and normal adjacent tissues

	Cancer		Normal tissue		<i>P</i>
	Number of cases		Number of cases		
Aromatase	24	(58%)	17	(42%)	N.S.
17 $\beta$ -HSD7	20	(49%)	24	(58%)	N.S.
17 $\beta$ -HSD12	34	(83%)	15	(37%)	<0.001
ER $\alpha$	29	(71%)	34	(83%)	N.S.
ER $\beta$	34	(83%)	25	(60%)	<0.05
PR	21	(51%)	23	(56%)	N.S.
AR	37	(90%)	35	(85%)	N.S.
CDC47	39	(95%)	16	(39%)	<0.001

A tissue was considered as positive when more than 10% of cells were immunolabelled.



Figs. 2–4. Sections through infiltrating ductal carcinoma (2A, 3A and 4A) and adjacent non-malignant breast tissue (2B, 3B and 4B). X 700. (2) Immunostaining for aromatase. Positive reaction is observed in the cytoplasm of carcinoma cells (arrows). In normal ductal epithelial cells (arrows), immunolabelling is detected in the cytoplasm and a few nuclei. (3) Immunostaining for 17 $\beta$ -HSD type 7. Labelling is seen in the cytoplasm of most carcinoma and normal ductal epithelial cells (arrows). (4) 17 $\beta$ -HSD type 12 immunostaining. In both carcinoma and normal ductal epithelial cells (arrows), staining is present in the cytoplasm and nucleus.

aromatase staining was seen in the cytoplasm and occasionally in nuclei of epithelial cells in both acini and ducts in 42% of cases (Fig. 2B). 17 $\beta$ -HSD type 7 immunoreactivity was also detected in the cytoplasm of tumor cells (Fig. 3A). Twenty cases (47%) were considered as positive. In non-malignant adjacent tissues, specific staining was observed in the cytoplasm of epithelial cells in acini and ducts and a very few stromal cells in 58% of cases (Fig. 3B). Immunoreactive 17 $\beta$ -HSD type 12 could be localized in both nuclei and cytoplasm of malignant cells in several cancer specimens (Fig. 4A). In fact, 34 cases (83%) were considered as being positive for this enzyme. In non-malignant tissues, epithelial cells also exhibited nuclear and cytoplasmic labelling (Fig. 4B). The percentage of positive carcinoma was significantly higher than that observed in non-malignant tissues (37%). 17 $\beta$ -HSD type 1 immunoreactivity could be detected in only one tumor out of 41. In this case, the labelling was present in the cytoplasm of malignant cells. In non-malignant

tissues, no staining could be detected in any of the 41 patients.

### 3.2. Steroid receptors, CDC47 and *c-erbB-2*

Steroid hormone receptors (ER $\alpha$ , ER $\beta$ , PR and AR) as well as CDC47 immunoreactivity was almost exclusively found in nuclei of epithelial cells in both tumoral and non-malignant adjacent tissues. Out of 41 tumors, 29 (71%) were positive for ER $\alpha$ , 34 (83%) for ER $\beta$ , 21 (51%) for PR, 37 (90%) for AR, and 39 (95%) for CDC47. In non-malignant specimens, ER $\alpha$  was positive in 83%, ER $\beta$  in 60%, PR in 56% and AR in 85% of cases (Tables 3 and 4). The expression of ER- $\beta$  was significantly higher ( $p < 0.05$ ) in carcinoma than in non-malignant tissue. For all the other steroid receptors, the percentage of positive tumors were not statistically different from that of positive non-malignant breast tissues. On the other hand, as expected, the percentage of tumors considered

as positive for hCDC47 was significantly higher than that of non-tumoral adjacent tissues (95% vs. 39%;  $p < 0.001$ ). Immunostaining for c-erbB-2 was considered as positive in nine cases of breast cancer (22%). In non-malignant tissues, no significant labelling for c-erbB-2 was found in any of the 41 specimens examined. No staining was detected when the antisera were immunoabsorbed with the corresponding antigen (data not shown).

### 3.3. Correlation between the different parameters evaluated in tumors

Sixty-six percent of carcinoma have been shown to express 17 $\beta$ -HSD type 7, 17 $\beta$ -HSD type 12 and aromatase. A significant correlation was observed between the expression of the three enzymes aromatase, 17 $\beta$ -HSD types 7 and 12 ( $p = 0.05$ ) as well as between the two enzymes 17 $\beta$ -HSD types 7 and 12 expression and ER $\beta$  expression ( $p = 0.02$ ). There was also a significant association of CDC47 expression with ER $\beta$ , AR and 17 $\beta$ -HSD type 12 expression ( $p = 0.01$ ). No other correlations were observed among steroid receptors, 17 $\beta$ -HSDs, aromatase, CDC47, c-erbB-2, histological type, tumor stage and age.

## 4. Discussion

The enzymes aromatase and reductive 17 $\beta$ -HSDs are involved in the local biosynthesis of E2 in a variety of peripheral tissues [19,20]. Aromatase catalyzes the conversion of androstenedione to E1 and the conversion of testosterone to E2, while reductive 17 $\beta$ -HSDs, including the types 1, 7 and 12, convert E1 to E2.

In the present study, we found significant expression (>10% labelled cells) of 17 $\beta$ -HSD type 12 in 83% of breast cancer cases, while 17 $\beta$ -HSD type 7 and aromatase immunoreactivities were observed in 47% and 58% of cases, respectively. Activity of aromatase measured in adipose cells have been found to be higher in breast quadrants associated with the tumors as compared with the other non-involved quadrants [3]. It is possible that, in the present study, the tissue located at proximity of the tumors might have a higher expression compared to more distant parts of the breast. This point remains to be clarified. Both aromatase and 17 $\beta$ -HSD type 7 have been localized in the cytoplasm of normal ductal epithelial cells and carcinoma cells. Moreover, aromatase was occasionally detected in nuclei of normal ductal epithelial cells. 17 $\beta$ -HSD type 12 was observed in both the cytoplasm and nuclei in these cells. By immunocytochemistry, aromatase has already been reported to be expressed in the cytoplasm of breast cancer cells [11]. This is the first report on the subcellular localization of both 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12. The function of aromatase and 17 $\beta$ -HSD type 12 in the nuclear compartment remains to be fully explored. We have previously observed that another steroidogenic enzyme 3 $\beta$ -HSD was also expressed in both the

cytoplasm and nuclei of guinea pig adrenocortical cells [21]. Surprisingly, 17 $\beta$ -HSD type 1 was found to be expressed in only one out of the 41 cases. The antibody to 17 $\beta$ -HSD type 1 used in the present study has already been successfully used to localize by immunocytochemistry the enzyme in placenta [16]. Previous reports indicated a variation in the levels of expression of 17 $\beta$ -HSD type 1. Sasano et al. [11] reported that 17 $\beta$ -HSD type 1 immunoreactivity was detected in carcinoma cells in 23 of 41 cases (56%). By in situ hybridization, Oduwole et al. [6] found in a study of 125 breast cancer specimens that only 16% of specimens expressed 17 $\beta$ -HSD type 1. The same authors also observed no expression of 17 $\beta$ -HSD type 1 mRNA in normal breast tissue from postmenopausal women [6].

This is the first report on the expression of 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12 in normal breast tissues and breast carcinoma. Interestingly, it was found that 66% of carcinoma co-expressed aromatase, 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12. Significant association was also found between the expression of the three enzymes in breast carcinoma, but not in non-malignant tissue. The present results then suggest that the combined action of aromatase and reductive 17 $\beta$ -HSD (types 7 and 12) might contribute to the local production of E2 in breast cancer.

Reports of any correlations between ER and aromatase activity in human breast cancer have been inconsistent [2,11]. Although intratumor aromatase activity did not significantly correlate with steroid receptors, there was a trend for ER+ tumors to express aromatase [22]. In the present study, no correlation was observed between aromatase and steroid receptors in carcinoma. However, a significant correlation between 17 $\beta$ -HSDs type 12 and ER $\beta$  but not ER $\alpha$  has been demonstrated. The involvement of ER $\beta$  in the development and progression of breast carcinoma cells is currently unknown. Since both ER $\alpha$  and ER $\beta$  are expressed in the majority of breast tumors, it is possible that signalling can be mediated by ER $\alpha$ /ER $\beta$  heterodimers. However, the presence and significance of ER $\alpha$ /ER $\beta$  heterodimers remain to be established. There are studies indicating that ER $\beta$  expression is significantly associated with poor prognostic features including lymph node positive tumors [23] and epidermal growth factor receptor positivity [24]. Others showed no correlation of ER $\beta$  expression with histopathological features such as tumor size, grade, and node status [25–29]. Recently, the presence of ER $\beta$  was found to be associated with a favourable response to tamoxifen therapy [30].

CDC47 which is a protein implicated in DNA replication in S phase has been used to study the index cell proliferation in breast cancer sections [15]. All malignant lesions contained significantly more labelled nuclei than their adjacent normal counterparts. Estimation of the proliferative status in breast cancer tissues appears as an important factor in patient management and prognosis [31]. In the present study, we found significant correlation between ER $\beta$  and 17 $\beta$ -HSD type 12 and CDC47 status. Since ER $\beta$  and 17 $\beta$ -HSD type 12 are more

frequently significantly expressed in cancer cells than in non-malignant adjacent tissues, it might be hypothesized that the overexpression of ER $\beta$  and 17 $\beta$ -HSD type 12 might lead to increased estrogen-dependent proliferation and progression of breast cancer.

It is well documented that inhibitors of aromatase activity have beneficial effects in breast cancer patients [32,33]. Since there is evidence that reductive 17 $\beta$ -HSDs might also be involved in estrogen biosynthesis in breast carcinoma, it appears of great interest to develop inhibitors for each reductive enzyme, especially 17 $\beta$ -HSD types 7 and 12. We are now moving towards an area of more refined therapeutic approaches based on the complete hormonal receptor and steroidogenic enzyme profile of each patient.

In summary, we report that aromatase, 17 $\beta$ -HSD type 7 and 17 $\beta$ -HSD type 12, but not 17 $\beta$ -HSD type 1, are commonly expressed in human breast cancer. The increased co-expression of 17 $\beta$ -HSD type 12 and ER $\beta$  in breast carcinoma cells compared with their expression in adjacent non-malignant tissue may play an important role in the development and/or progression of breast cancer.

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