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RESEARCH

Expression of 11β-hydroxysteroid Dehydrogenase Type 1 in Breast Cancer and Adjacent Non-Malignant Tissue. An Immunocytochemical Study

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Abstract Intratumoral biosynthesis of hormone steroids is thought to play a role in the pathogenesis and development of human breast cancer. There is evidence that glucocorticoids may inhibit the development and progression of breast cancer. 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 is the enzyme which converts inactive cortisone to active cortisol. In order to study the expression of 11β-HSD type 1 in breast cancer and non-cancerous breast tissue, we have developed specific antibodies to 11β-HSD type 1 and proceeded to localization of the enzyme in 84 specimens of breast carcinoma and adjacent non-malignant tissues by immnohistochemistry. The results were correlated with the expression of androgen receptor, estrogen receptor, progesterone receptor, glucocorticoid receptor and CDC47, a cell division marker, as well as the tumor stage, tumor size, nodal status and menopausal status. The expression of 11β-HSD type 1 in 64% of breast cancer specimens appeared significantly lower than that observed in normal adjacent tissues (97% of cases being positive). There was no significant correlation between 11β-HSD type 1 expression and the clinicopathological parameters studied. The decrease in 11β-HSD type 1 expression in breast cancer as compared to that observed in the adjacent normal tissues may play a role in the development and/or progression of the cancer by modifying the intratumoral levels of glucocorticoids.

Keywords 11β-hydroxysteroid dehydrogenase type 1 · Breast cancer · Glucocorticoid receptors · Immunocytochemistry, Cortisol

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Introduction

Sex steroid hormones as well glucocorticoids exert important roles in the development and functions of the mammary gland [14]. Glucocorticoids have been shown to induce differentiation of mammary epithelial cells in primary culture [3] and to be essential for the initiation and maintenance of lactation [14]. The responsiveness of human breast cancer cells to steroids has been extensively investigated in vitro. Thus, glucocorticoid-dependent inhibition of cell growth has been demonstrated in different breast cancer cell lines [6, 13, 15]. By ligand-binding assays, glucocorticoid receptors (GR) have been found to be present in about 33–50% of human breast cancer [1, 22]. Recently, it has been shown that activation of glucocorticoid receptor by dexamethasone inhibited estrogen-dependent breast cancer growth in vitro and in xenograft model [5].

In glucocorticoid target tissues, intracellular glucocorticoid levels are modulated by two 11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes, type 1 and type 2, which catalyze the interconversion of glucocorticoids from their inactive (cortisone) to their active form (cortisol) [2, 11]. 11β-HSD type 1 is the isoenzyme which converts cortisone to

cortisol while 17β-HSD type 2 converts cortisol into cortisone in several tissues [18, 21]. Gene deletion experiments in mice suggest that the role of 17β-HSD type 2 is both to maintain circulating glucocorticoid levels and to provide higher local concentration of glucocorticoids in the liver [10]. On the other hand, inhibition of the activity of 11β-HSD type 2, the isoform which converts cortisol to the inactive steroid cortisone, has been shown to enhance the antiproliferative effect of glucocorticoids [8]. Interestingly, Feigelson et al. [4] have recently identified 11β-HSD type 1 from a genome-wide linkage analysis as a possible candidate gene for breast cancer risk in postmenopausal women.

To better understand the role of 11β-HSD type 1 in breast cancer development and/or evolution, we developed antibodies to 11β-HSD type 1 and immunolocalized the enzymes in 84 specimens of breast cancer and adjacent non-malignant tissues. We also correlated the results with various clinical and histopathological parameters.

Table 1 Clinicopathological parameters

	n
Age	
< 50 years	49
≥ 50 years	35
Menopausal status	
Premenopause	49
Postmenopause	32
Unknown	3
Tumor stage	
I	16
II	50
III	18
Nodal status	
0	56
1–3	14
> 3	14
Tumor size (cm)	
< 3	47
≥ 3	37

Materials and Methods

Patients

This study was approved by the institutional review board at First Teaching Hospital of Jilin University, ChangChun, China. All the patients agreed to participate in this research project. Eighty-four women with primary breast cancer were enrolled in this study. All the patients underwent total mastectomy, axillary dissection and reconstruction at the First Hospital of Jilin University during the year 2009. Before surgery, they had received no treatment. Patient characteristics are summarized in Table 1. The median age of the patients was 52 years (range: 34–71).

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 24–48 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. All the tumors were infiltrating ductal carcinoma (Table 1). In adjacent non-neoplastic tissues, the mammary gland structures had a normal appearance without any signs of inflammation.

Immunocytochemistry

1. Steroid hormone receptors and CDC47

Estrogen receptor α (ERα), progesterone receptor (PR), androgen receptor (AR) and CDC47, a cell division marker, were immunolocalized as previously described [20]. Briefly, the sections were deparaffinized, hydrated and then treated with 3% H_2O_2 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). The sections were then incubated overnight at 4°C with ERα, AR, PR, GR and CDC47 rabbit antibodies at the dilution indicated in Table 2. Control sections were incubated with antibodies pre-absorbed with an excess of the corresponding antigens (10^{-6} M). The antibodies were detected as previously described [20]. Thereafter, the sections were counterstained with hematoxylin.

2. 11βHSD type 1

To develop antibodies to 11-HSD1 type 1, we selected the peptide sequence located at amino acid position 42–225 of the human 11-HSD1 type 1. This peptide sequence was overproduced in E. coli BL-21 using PET23a expression vector (EMD Biosciences, San Diego, CA). The purified protein was 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 on four 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 11-HSD type 1, as negative and positive controls, respectively. The proteins were separated by 12% SDS-PAGE and transferred onto a nitrocellulose gel for analysis with the protein A-purified antibody to 11-HSD type 1 type 1(diluted: 1:1,000).

Table 2 List of primary antibodies

Antibody	Dilution	Source	Catalog no.
ERα	1:1000	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	SC-543
AR	1:1000	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	SC-816
PR	1:50	Medicorp (Montreal, Canada)	MS-192
GR	1:400	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	SC-1004
CDC47	1:500	Medicorp (Montreal, Canada)	MS-862

Horse anti-rabbit IgG antibody conjugated with horseradish peroxidase (Amersham Biosciences, Inc., Baie d'Urfé, Montréal) was used as secondary antibody (dilution: 1: 10,000) and the resulting immunocomplexes were then visualized using enhanced chemiluminescence kit (Perkin Elmer Life Science) and exposed on a X-OMAT blue film for 20 s. As shown in Fig. 1, the antibodies react only with the overexpressed protein. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAS; Ottawa, Ontario, Canada) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC; Rockville, MD). The study was performed in accordance with the CCAC Guide for Care and Use of Experiment Animals.

The localization of 11β-HSD type 1 was performed as previously described [20]. The antiserum to 11β-HSD type 1 was used to a dilution of 1:500. Control experiments were performed on adjacent sections by substituting pre-immune rabbit serum (1:500) or the antiserum preabsorbed with an excess of the enzyme (10^{-6}M).

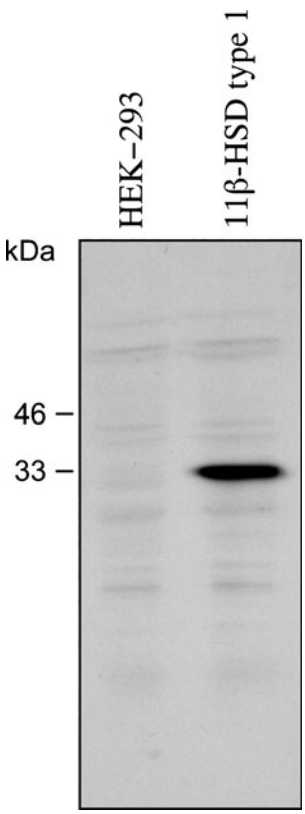

Fig. 1 Western blot analysis of protein from untransfected or transfected HK293 cells stably expressing human 11β-HSD type 1 (MW: 32 kDA). The antiserum specifically reacts with the enzyme

Scoring of Immunoreactivity

The data were generated from independent observations by three of the authors (G. Zhao, L. Lu and G. Pelletier). Differences were resolved by joint examination of the slides, and the final reconciled values were used in all statistical analyses. For AR, ERα, PR, GR and CDC47, we counted the number of immunostained nuclei from 300–400 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 labeled nuclei. To quantify 11β-HSD type 1 expression, we similarly evaluated the percentage of malignant and non-malignant labeled cells from 300–400 cells present in three randomly chosen fields. The intensity labeling 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 immunostained. Associations between categorical variables assessed by Chi-square (X^2) tests or Fisher's extract tests while 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 Kruskall-Wallis test (Chi-square Approximation), respectively. All analyses were performed using SAS software (Version 6.12).

Results

11β-HSD Type 1 Expression

As shown in Fig. 1, Western Blot analysis clearly demonstrated that antibodies to 11β-HSD type 1 specifically react with the overexpressed enzyme. Immunostaining for 11β-HSD type 1 could be detected in the cytoplasm of tumor cells in 54 cases out of the 84 cases of breast cancer (64%) (Table 3, Fig. 2). Usually, the cytoplasmic staining expression appeared weak. The surrounding interstitial cells were generally devoid of any specific staining. In non-

malignant adjacent tissues, strong staining was seen in epithelial cells bordering the lumen (luminal cells) in both acini and ducts in 97% of cases (Table 3, Fig. 3). Labeled stromal cells were occasionally observed. No staining could be detected when the antiserum immunoabsorbed with the antigen (11β-HSD type 1) or pre-immune serum was used (not shown). The expression of the enzyme was significantly lower ($p<0.001$) in tumors than in normal adjacent tissues.

Steroid Receptors and CDC47

Steroid hormone receptors (ERα, AR, PR and GR) as well as CDC47 immunoreactivity was almost exclusively found in nuclei of both cancer cells and epithelial cells from non-malignant adjacent tissues. In the 84 tumors examined, 71% were positive for ERα, 83% for AR, 79% for PR., and 73% for GR. In non-malignant specimens, ERα was positive in 68%, AR in 62%, PR in 64% and GR in 95% of cases (Table 3). The percentage of tumors considered as positive for AR and PR was significantly higher ($p<0.01$ and 0.05, respectively) than the percentage of positive normal adjacent tissues (Table 3). On the other hand, the percentage of tumors positive for GR was significantly lower ($p<0.001$) than that of positive normal adjacent tissues. As expected, the percentage of tumors considered as positive for CDC47 was significantly higher than that of non-tumoral adjacent tissues (77% vs. 15%; $p<0.001$). No staining was detected when the antisera were immunoabsorbed with the corresponding antigen (data not shown).

Correlation Between the Different Parameters Studied

In tumors, positive correlations were only observed between AR and ERα ($R=0.49$, $p<0.001$), ERα and CDC47 ($R=0.22$, $p<0.05$) and ERα and GR ($R=0.32$, $p<0.01$), while no negative correlation was found between the different parameters studied.

Table 3 Comparison of the expression of steroid receptors, CDC47 and 11β-HSD type 1 between cancer and normal adjacent tissues in 84 patients

	Cancer		Adjacent normal tissue		*P*-value
	n	%	*n*	%	
11β-HSD type 1	54	64	77	97	< 0.001
CDC-47	65	77	13	15	< 0.001
ERα	60	71	57	68	N.S.
AR	70	83	52	62	< 0.01
PR	66	79	54	64	< 0.05
GR	61	73	80	95	< 0.001

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

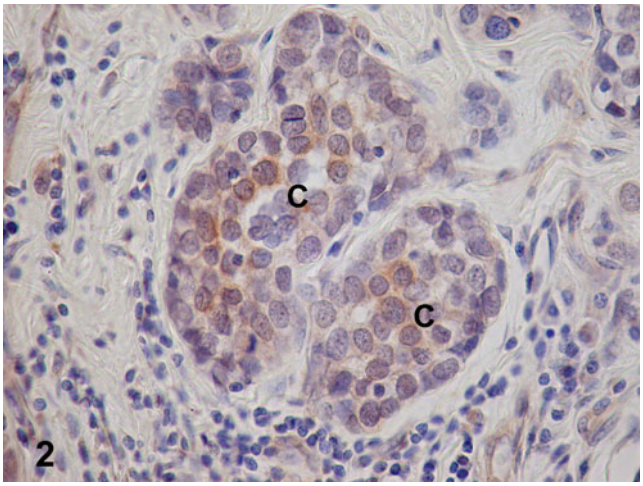

Fig. 2 Micrographs illustrating immunostaining for 11β-HSD type 1. X 500. Infiltrating ductal carcinoma. Most of the cancer cells (C) are weakly stained

Discussion

The role of glucocorticoids in the development and progression of breast cancer is still elusive. In several human breast cancer cell lines, glucocorticoids could inhibit cell growth [6, 13, 15]. Recently, it has been shown that activation of GR inhibited human breast cancer cells MCF-7 growth in cell culture and in vivo by a mechanism involving activation of estrogen sulfotranferase [5]. In a model of breast cancer, the potent glucocorticoid dexamethasone has been shown to potentiate the anti-tumor

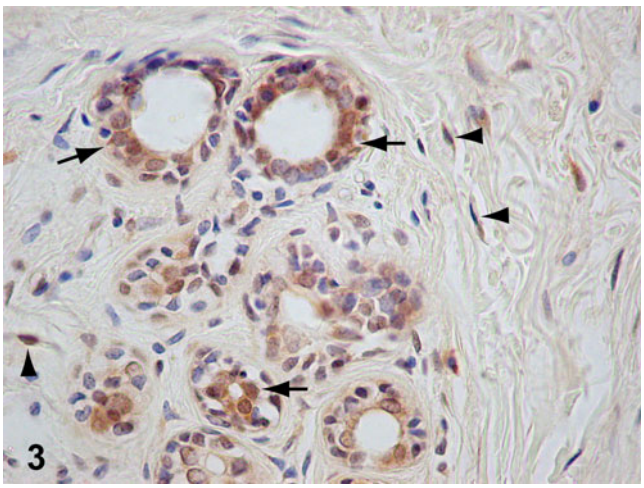

Fig. 3 Micrographs illustrating immunostaining for 11β-HSD type 1. X 500. Non malignant tissue. Strong immunolabelling is observed in epithelial cells (arrows) in acini as well as a few stromal (arrowheads)

activity of adriamycin and then could be used as a chemosensitizer agent [23].

Moreover, clinical studies have shown that glucocorticoids have beneficial effects in breast cancer as monotherapy [7, 9]. On the other hand, it has been reported that in breast cancer xenografts, glucocorticoids could decrease response to Paclitaxel through inhibition of tumor cell apoptosis [16].

On the basis that intratumoral production of estrogens may play a role in the pathogenesis of breast cancer [17, 19], we hypothesized that local synthesis and/or metabolism of cortisol might contribute to the development and progression of breast cancer. In the present study, we observed that 11β-HSD type 1 was detected in 64% of breast cancer, while it was highly expressed in almost all the adjacent normal tissue specimens (97%). Moreover, the staining was generally much weaker in cancer cells than in adjacent normal cells. Since malignant and non-malignant tissues were from the same patients, it might be suggested that, during the carcinogenesis process, expression of 11β-HSD type 1 has been considerably reduced or lost. The decrease in expression of 11β-HSD type 1 found in carcinoma cells could induce a low production of cortisol which has been shown to exert negative influence in breast cancer cells [13, 15]. In agreement with this hypothesis, it has been reported that overexpression of 11β-HSD type 2, which inactivates cortisol, led to an increase in MCF-7 breast cancer cellular growth [8].

Recently, 11β-HSD type 1 was identified from a genome-wide linkage analysis as a possible candidate gene for breast cancer development in post-menopausal women [4]. This finding reinforces the hypothesis that the enzyme might play a role in pathogenesis in breast cancer.

Also of interest was the observation that the expression of GR was significantly lower in cancer (73% of cases) than in adjacent normal tissues (95% of cases). By binding assays, it has been reported that 33–50% of breast cancer expressed GR [22]. In those previous studies, there was no identification of the cells which expressed GR. Low expression of GR might be responsible for reducing the negative influence of cortisol on breast cancer cell growth. On the other hand, Lien et al. [12], using immunohistochemistry, have found that GR was expressed in myoepithelial cells but not in luminal epithelial cells and strongly expressed in metaplastic carcinoma. They also reported a lack of GR expression in cancer cells of non-metaplastic carcinoma [12]. The discrepancies between the results presented herein and those reported by Lien et al. [12] might be tentatively explained by difference in the antibodies used. Clearly, more studies are required to clarify the involvement of GR in breast cancer pathogenesis.

In summary, we report that the expression of 11β-HSD type 1, the enzyme which converts cortisone to cortisol, as evaluated by immunocytochemistry, is decreased in breast cancer cells as compared to the expression in adjacent normal epithelial cells. The lower expression of the enzyme involved in the local production of the potent glucocorticoid cortisol might favor the development and/or progression of cancer by decreasing the intratumoral concentrations of cortisol known to exert an inhibitory influence on breast cancer.

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