PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 73 (2000) 141–145

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Elevated steroid sulfatase expression in breast cancers

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Accepted 10 March 2000

Abstract

In situ estrogen synthesis makes an important contribution to the high estrogen concentration found in breast cancer tissues. Steroid sulfatase which hydrolyzes several sulfated steroids such as estrone sulfate, dehydroepiandrosterone sulfate, and cholesterol sulfate may be involved. In the present study, we therefore, assessed steroid sulfatase mRNA levels in breast malignancies and background tissues from 38 patients by reverse transcription and polymerase chain reaction. The levels in breast cancer tissues were significantly increased at 1458.4 ± 2119.7 attomoles/mg RNA (mean $\pm$ SD) as compared with 535.6 ± 663.4 attomoles/mg RNA for non-malignant tissues ($P < 0.001$). Thus, increased steroid sulfatase expression may be partly responsible for local overproduction of estrogen and provide a growth advantage for tumor cells. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Estrone sulfate is present in blood at much higher concentrations than estrone, the unconjugated form, and is thought to play a key role in regulating several important physiological and pathological processes [1]. Estrone sulfatase may therefore make an important contribution to the supply of estrone to target tissues. A number of studies have shown that human breast cancer tissues contain this enzyme [2–5] as well as aromatase [2,5–9]. Furthermore, it has been proposed that the growth of breast cancers may be influenced by locally synthesized estrogen [8,10].

While there is still some controversy as to whether the same or distinct enzymes are responsible for hydrolysis of alkyl and aryl steroid sulfates [11–14], recent studies have indicated that steroid sulfatase is a single enzyme which hydrolyzes both types of steroid sulfate [15,16]. A potential association has been reported in terms of sulfatase activity and response to anti-estrogen [4] and recently, we found a good inverse correlation between intratumoral steroid sulfatase mRNA expression and relapse-free survival in breast cancers [17]. Currently, a number of steroid sulfatase inhibitors [18–25] are being developed as a new therapeutic strategy for breast cancers.

The present study was performed to cast light on the significance of the sulfatase pathway in breast cancer. To investigate possible differences in steroid sulfatase expression between breast cancer and background non-malignant tissue, we quantified steroid sulfatase mRNA transcripts by the reverse transcription–polymerase chain reaction (RT–PCR).

2. Materials and methods

2.1. Patients

Materials for this study were obtained from 38

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PII: S0960-0760(00)00060-1

patients with primary breast carcinomas who underwent curative operations at Fujita Health University Hospital and Marumo Hospital between 1993 and 1995. Their average age was 53.4 ± 10.2 years (mean ± SD), with a range of 37–77 years. Non-malignant breast tissues including adipocytes, fibroblasts, epithelial cells, and inflammatory cells histologically, were obtained from a distant site of the tumor-bearing breast.

These 38 tumors were classified by pathologists according to the WHO scheme for typing [26]. Histologically, there was one case each of mucinous carcinoma and invasive lobular carcinoma, and 36 of invasive ductal carcinomas. Immediately following surgical removal, the specimens were frozen in liquid nitrogen and then stored at −80°C until use.

2.2. Preparation of total RNA

Frozen tissues were homogenized in 5 M guanidine thiocyanate containing 5 mM sodium citrate and 0.5% sodium sarcosyl, and total RNA fractions were prepared from the homogenates, as described by Chirgwin et al. [27]. RNA concentrations were determined from the spectrophotometric absorption at 260 nm.

2.3. Quantitation of steroid sulfatase mRNA

Quantitative analysis of steroid sulfatase mRNA in the RNA fraction was carried out by RT–PCR using a fluorescent primer in the presence of an internal standard RNA as previously described [17]. In brief, oligonucleotides of antisense primer STS-2R (5′-AGGGTCTGGGTGTGTCTGTC-3′) for reverse transcription, and an antisense primer STS-2R (5′-AGGGTCTGGGTGTGTCTGTC-3′) and a sense primer STS-1F (5′-ACTGCAACGCCTACTTAAATG-3′) for PCR were synthesized. STS-1F was labeled with a fluorescent dye, FAM (Perkin–Elmer), after conjugation with Amino-link 2. The coding sequence between the two PCR primer sites is interrupted by a 18 kbp intron in the steroid sulfatase gene. To prepare the internal standard RNA, a modified human steroid sulfatase cDNA was constructed by inserting a 64 bp fragment of *Alu*I-digested pUC119-DNA between the two PCR primer sites. The internal standard RNA was synthesized in vitro with T7 RNA polymerase using the modified steroid sulfatase cDNA as a template, purified on an anion exchange column of QIAGEN, and then quantitated from the absorbance at 260 nm. Total RNA (1–2 μg) and an internal standard RNA (0.2 attomoles) were subjected to reverse transcription with five units of RAV-2 reverse transcriptase (Takara Shuzo, Kyoto) and the specific antisense primer STS-2R at 42°C for 40 min. The resulting cDNAs were amplified by PCR using the fluorescent dye-labeled primer STS-1F and the primer STS-2R. The PCR conditions were — denaturation at 94°C for 20 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s for 24 cycles. Fluorescent PCR products were analyzed on a 2% agarose gel with a Gene Scanner 362 Fluorescent Fragment Analyzer (Perkin–Elmer). The amounts of steroid sulfatase mRNA were calculated from the peak areas of the fluorescent products by the internal standard method.

2.4. Statistics

Mean levels of steroid sulfatase mRNA were compared using the Wilcoxon signed rank test. The cutoff for significance was taken to be $P = 0.05$.

3. Results

3.1. Steroid sulfatase mRNA in breast cancer and non-malignant tissues

Steroid sulfatase mRNA levels were determined in tissues from 38 breast cancer patients. Examples of typical RT–PCR results are shown in Fig. 1. In each case, breast cancer values were plotted against those for the respective non-malignant tissue (Fig. 2). In 33 cases, the levels in breast cancer tissues were 1.1–35.0 times higher than those in non-malignant tissues. In the other five cases, they were lower. Steroid sulfatase mRNA values obtained for all the patients are summarized in Table 1. The levels (1458.4 ± 2119.7 attomoles/mg RNA) in breast cancer tissues were significantly ($P < 0.001$) increased as compared to the non-malignant tissues (535.6 ± 663.4 attomoles/mg RNA).

4. Discussion

The importance of the estrone sulfatase pathway in human breast cancers has been suggested by several clinical or experimental studies [2,10]. The present data clearly show that steroid sulfatase mRNA levels may be significantly elevated in breast cancer as compared to non-malignant background tissues. Earlier, Vermeulen et al. [6] also demonstrated estrone sulfate concentrations to be higher in cancerous than in non-cancerous breast tissues. Furthermore, Pasqualini et al. [5] reported its concentrations in postmenopausal patients to be significantly higher in their cancerous tissue than in plasma. Thus, in situ production of estrogens may be significant in human mammary tumors.

How is this local production of estrogen in breast cancers controlled? There are several possible expla-

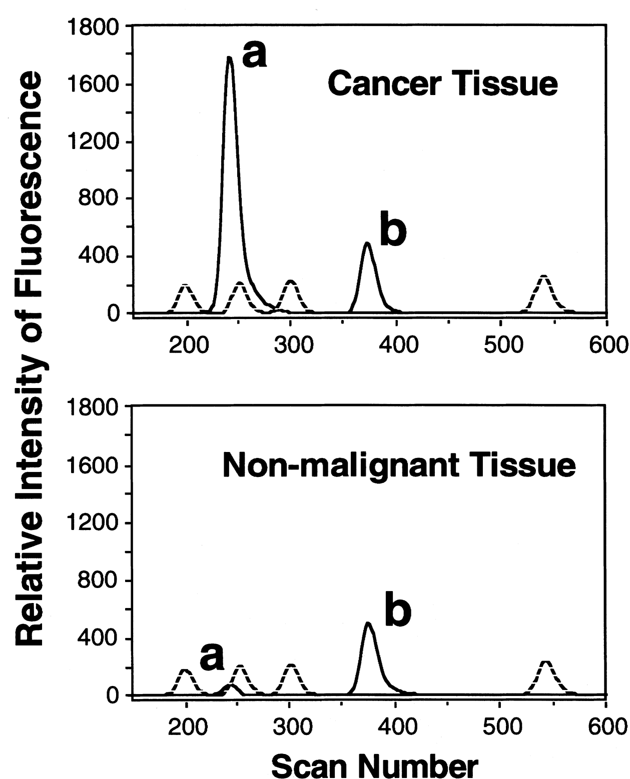

Fig. 1. Results of RT–PCR analyses of steroid sulfatase mRNAs in breast cancer and non-malignant tissues of a patient. The fluorescent PCR products (solid line) show two peaks: (a) a 290 bp product derived from steroid sulfatase mRNA; and (b) a 354 bp product derived from modified steroid sulfatase RNA as an internal standard RNA. GENESCAN-1000 ROX (Applied Biosystems) of internal size standards (broken line) shows four peaks, corresponding to 262, 293, 317 and 439 bp.

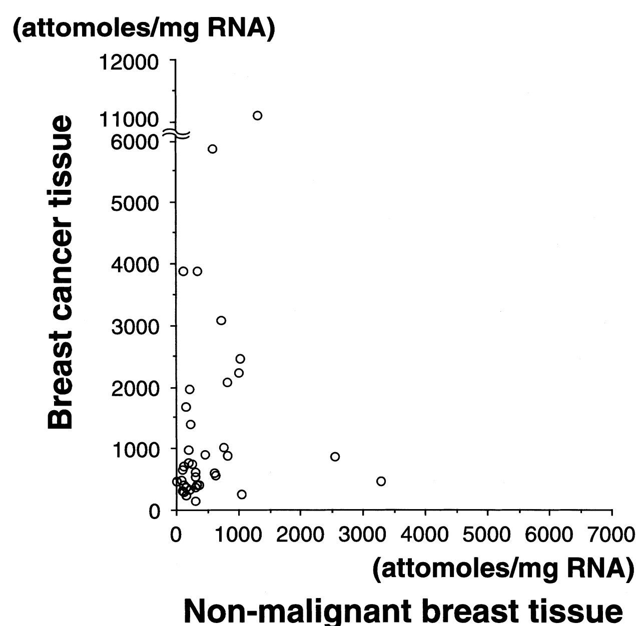

Fig. 2. Relationship between steroid sulfatase mRNA levels in breast cancer tissues and those in non-cancerous tissues obtained from 38 patients. In 33 cases, the levels in the breast cancer tissues were higher than those in the non-malignant tissues, and in only five cases they were lower.

nations to account for increased steroid sulfatase transcripts in breast tumors. Breast cancer and non-malignant breast tissues may contain very different populations of epithelial and stromal cells. Evans et al. [28] demonstrated estrone sulfatase activity to be lower in normal breast epithelial cells than in cancer cells. Regional differences in relative proportion of histological components might be a primary cause of apparent differences in steroid sulfatase expression. Several factors, such as interleukin-6 and tumor necrosis factor α have been found to stimulate estrone sulfatase activity [29–31]. Furthermore, it has been evidenced for a complex interaction between various cells within the tumors (i.e., epithelial cells, stromal cells, macrophages, and lymphocytes) and cytokines/growth factors/steroids [32,33]. They may also have mechanistic roles to play.

Table 1
Steroid sulfatase mRNA levels in breast cancer tissues and non-malignant breast tissues from 38 patients with breast cancer

Tissue	Steroid sulfatase mRNA (attomoles/mg RNA)	
	Mean ± SD	Range
Breast cancer tissue	1458.4 ± 2119.7[a]	148–11778
Non-malignant breast tissue	535.6 ± 663.4	0–3282

[a] Significantly ($P < 0.001$) higher than the non-malignant tissue value.

The present results underscore the potential importance of sulfatase inhibitors as efficacious agents for endocrine therapy of hormone-dependent breast cancer. Pasqualini et al. [34] demonstrated that progestagen Promegestone (R-5020) can inhibit enzyme activity as well as its mRNA in MCF-7 cells; it is suggested that the inhibitory effect provoked by the R-5020 in sulfatase is a complex mechanism involving not only the enzyme itself, but also transcriptional factors which express this enzyme. Selcer et al. [24] reported that estrone sulfatase inhibitors effectively depress the proliferation of estrogen-dependent MCF-7 cells in the presence of estrone sulfate as the only source of estrogen, correlating with their potential to inhibit the enzyme activity. Therefore, these agents give us the possibility of blocking estrogen delivery to tumor cells. Indirect support for importance of local sulfatase activity in carcinogenesis or tumor proliferation has also come from studies correlating tumor sulfatase activity with clinical response to anti-estrogen [4]. Recently, we found that higher steroid sulfatase mRNA levels in tumor tissues predicted shorter relapse-free survival in

breast cancers [17], which again points to a putative role of steroid sulfatase in the growth and metastasis of breast cancers.

It is concluded that steroid sulfatase expression in breast cancer tissues is generally elevated as compared to that in non-cancerous breast tissues. The physiological and clinical significance of this finding needs to be further explored.

Acknowledgements

This work was supported in part by Grants-in-Aid for Medical Research from Fujita Memorial Fund and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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