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Tissue-specific transcripts of human steroid sulfatase are under control of estrogen signaling pathways in breast carcinoma

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Abstract

Steroid sulfatase (STS) increases the pool of precursors of biologically active steroids, thereby playing an important role in breast cancer development. Mechanisms that control STS expression remain poorly understood. In present study we investigated alterations in the 5′ region of STS gene to gain insight into the mechanism(s) that regulates its expression in mammary epithelial cells. We found that at least four alternatively spliced transcripts of STS gene can be produced from at least four different leader exons. Distinct expression patterns of the STS variants were observed in human tissues. Expression profiles of estrogen receptor α (ERα)-positive and ERα-negative breast carcinomas showed that these two categories of tumors and their adjacent benign tissues display remarkably different expression of STS isoforms. Coexpression of STS isoforms with ER isotypes suggests their cell-type specific coregulation. In addition, we identified ERα as essential regulator of STS transcription and provide evidence of direct estradiol-dependent binding of ERα to multiple STS *cis*-regulatory regions *in vivo*. Our results indicate that STS isoforms are under control of estrogen signaling pathways and their differential expression may play a significant role in breast cancer biology.

Keywords: Steroid sulfatase; Steroid biosynthesis; Estrogen receptor; Breast cancer

1. Introduction

Local estrogen production is important in the development of breast malignancies [1–4]. Hydrolysis of alkyl and aryl 3β-hydroxysteroid sulfates (e.g., dehydroepiandrosterone sulfate DHEAS and estrone sulfate E1S, respectively) is catalyzed by steroid sulfatase (EC 3.1.6.2, STS, also known as arylsulfatase C) to form unconjugated DHEA and E1 which can be converted to potent estrogens [5]. Elevated DHEAS levels stimulate growth of cultured breast cancer cells [6] and high STS expression is associated with increased risk of breast cancer recurrence [7–9]. The adverse prognostic impact of STS expression is confined to estrogen receptor (ER) positive tumors and appears to affect both pre- and post-menopausal women [8,10]. Recently, a non-steroidal STS inhibitor, 667 COUMATE, has entered a Phase 1 trial in postmenopausal women with breast cancer [11,12].

Human placental STS cDNA [13] corresponds to 583 amino acids with an N-terminal signal peptide [14]. However, experimental evidence suggest the existence of tissue-specific STS isozyme(s) with different kinetic parameters for DHEAS and E1S [15–17]. Several studies demonstrate that STS enzyme activity and mRNA level are concordant [7,8,18], however the role of transcription factors, *cis*-acting elements, and the cellular context in STS regulation remain obscure. The promoter region and 5′ upstream regulatory ele-

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ments of the STS gene have been characterized in human placental cells where STS activity is high [19]. This promoter exhibits little basal activity and shows tissue-specificity, suggesting that additional regulatory elements are required to achieve high STS expression [20].

Our incomplete knowledge of STS gene regulation at the transcriptional level may be due to the fact that so far only the placental transcript has been studied. The paucity of experimental data for STS gene regulation in other tissues together with our interest in the involvement of the STS gene in breast carcinoma lead us to investigate whether there may be 5′-variability in STS transcripts. We found that the STS gene, like most of human genes [21], expresses mRNA variants as result of multiple splicing and alternative promoters. Four STS transcripts that differ at their first exons were identified in MCF-7 mammary epithelial cells. The alternative use of these leader exons is, likely, the key step in the regulation of mature STS mRNA level, which is the part of molecular mechanism conferring tissue-specific regulation of STS expression. To understand the role of STS variants in breast cancer, we explored their expression patterns in normal tissue, cancer samples and matched adjacent tissue, and have examined the associations of these with the ERα status of the primary tumor. In addition, we identified ERα as the mediator of the estradiol (E2)-dependent increase of STS transcription in MCF-7 mammary epithelial cells. Our results indicate that the changes in the balance between different STS isoforms are likely to be important to our understanding of mechanisms of breast cancer initiation and progression.

2. Materials and methods

2.1. Cell culture and reagents

MCF-7 cells obtained from American Type Culture Collection (Manassas, VA) were maintained following manufacture's instructions. Three days before cell exposure to 10^{-8} M E2 (Sigma, St. Louis, MO) or/and 10^{-6} M ICI 182,780 (Tocris Cookson, Ellisville, MI.) the medium was replaced by phenol red-free MEM containing 5% steroid-stripped serum (JR Scientific Inc., Woodland, CA). Cells were pre-treated with vehicle (DMSO) or 10^{-6} M MG132 from Sigma for 1 h followed by treatment with E2. Antibodies used in Western blot analysis performed as described previously [32], were: ERα (NeoMarkers/Lab Vision, Fremont, CA, Ab-15) and β-actin (Sigma, AC-15). Oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

2.2. Tissue specimens

Grossly dissected tissue fragments from the 21 primary breast carcinomas and paired adjacent nonneoplastic tissues were provided by Northwestern University Breast SPORE tissue bank following the Institutional Review Board approval. The age of patients ranged from 36 to 80 years, with a mean of 49.2 years. ERα measurement were made as part of the routine pathological assessment of tumors by immunohistochemistry (IHC) and scored using a cut-off value of 10% of positive tumor nuclei staining. Four specimens of normal breast tissue were obtained from cosmetic breast reduction surgery and one specimen of normal breast tissue (Ambion Inc., Austin, TX) originated from autopsy. Total RNA from breast tissues specimens was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, CA) and subjected to a single round of linear RNA amplification with a starting amount of 100 ng RNA (Full Spectrum™, Complete Transcriptome RNA Amplification Kit, System Bioscience, CA).

2.3. RNA ligase-mediated 5′ rapid amplification of cDNA ends (5′-RLM-RACE) and sequence analysis

Reverse transcription of total RNA (10 μg) and second-strand synthesis were performed using a FirstChoice RLM-RACE kit (Ambion, Austin, TX). In this system only full-length, capped mRNA is reverse transcribed. To assure this DNA-free RNA template was treated with calf intestinal phosphatase (CIP) to remove 5′-phosphates from rRNA, tRNA and fragmented mRNA. The cap structure was then removed by treatment with the tobacco acid pyrophosphatase (TAP) to ligate a synthetic RNA adapter oligonucleotide to the mRNA 5′ end with T4 RNA ligase. A random-primed reverse transcription reaction was then carried out to make a cDNA copy of the treated RNA and after RNase treatment the cDNA was size selected by column chromatography. A 0.5 ng cDNA aliquot was used as a template for PCR amplification with an antisense primer annealing to the STS exon 3 (5′-AACCGGTCGATATTGGGAGT-3′) and a primer containing the synthetic RNA adapter sequence. Then, a nested PCR was applied to the first PCR products using 5′ RACE inner primer and STS-specific primer from exon 2 (5′-AGGAGGAAAGGGATCTTCATC-3′). The final PCR products were analyzed on a 2% agarose gel and visualized by ethidium bromide staining. Gel-purified products were cloned into the pGEM-T vector (Promega, Madison, WI) for DNA sequencing. These sequences are available at GeneBank under accession numbers DQ851171–DQ851173.

2.4. Database search and secondary structure prediction

Nucleotide sequence information was subjected to the basic local alignment search tool (BLAST) homology search against the Human Genome resource database of the National Center for Biotechnology Information (NCBI) [22]. Comparing the genomic sequences to the corresponding cDNA sequences identified 5′ splice sites. The contexts of alternative ATG start codons were analyzed using software tool for prediction of translation initiation sites [23], and by comparison to the Kozak consensus sequence [24]. The secretory