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Sulfotransferase 2B1b in human breast: Differences in subcellular localization in African American and Caucasian women

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ABSTRACT

Breast cancer (BC) is the most commonly diagnosed cancer among American women; however, the development of post-menopausal BC is significantly lower in African Americans as compared to Caucasians. Hormonal stimulation is important in BC development and differences in the conversion of dehydroepiandrosterone (DHEA) into estrogens may be involved in the lower incidence of post-menopausal BC in African American women. DHEA sulfation by sulfotransferase 2B1b (SULT2B1b) is important in regulating the conversion of DHEA into estrogens in tissues. SULT2B1b is localized in both cytosol and nuclei of some tissues including cancerous and associated-normal breast tissue. Immunohistochemical staining was used to evaluate the total expression and subcellular localization of SULT2B1b in African American and Caucasian breast tissues. Cell fractionation, immunoblot analysis and sulfation assays were used to characterize the subcellular expression and activity of SULT2B1b in BC tissues and T-47D breast adenocarcinoma cells. Immunohistochemical analysis of SULT2B1b showed that African Americans had a significantly greater amount of SULT2B1b in epithelial cells of associated-normal breast tissue as compared to Caucasians. Also, more SULT2B1b in African American associated-normal breast epithelial cells was localized in the nuclei than in Caucasians. Equivalent levels of SULT2B1b were detected in breast adenocarcinoma tissues from both African American and Caucasian women. Nuclei isolation and immunoblot analysis of both BC tissue and human T-47D breast adenocarcinoma cells demonstrated that SULT2B1b is present in nuclei and cytoplasm.

1. Introduction

Breast cancer is the most commonly diagnosed cancer among American women with approximately 200,000 cases diagnosed in 2005. Breast cancer is the second leading cause of cancer death in women with approximately 40,000 deaths in 2005 [1]. There is a slightly higher incidence of breast cancer in pre-menopausal African American women as compared to Caucasians. In contrast, the incidence of breast cancer in post-menopausal African American women is significantly lower than that of Caucasian women [1]. The reasons for the lower incidence in the post-menopausal African American women are not well understood.

Estrogenic stimulation is recognized as an important factor in the development of breast cancer [2]. Prior to menopause the majority of estrogens are synthesized and secreted from the ovaries whereas in post-menopausal women almost all estrogens are derived from the adrenal androgen dehydroepiandrosterone (DHEA) [3]. The enzymes necessary for the conversion of DHEA to β-estradiol (E2) are present in breast tissues [3]. Additionally, DHEA has been reported as being capable of binding and activating estrogen and androgen receptors directly [4,5]. Therefore, mechanisms that inhibit the metabolism of DHEA may be important in regulating its estrogenic activity in post-menopausal breast tissues. One of the important mechanisms regulating DHEA activity and metabolism and therefore its hormonal activity is sulfation [6].

Sulfation involves the transfer of the sulfonate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an acceptor compound [7]. The primary sites of sulfation are hydroxyl groups resulting in the formation of sulfate esters. The enzyme family responsible for the sulfation of drugs, xenobiotics and many small endobiotics is the cytosolic sulfotransferase (SULT) family. The SULTs are Phase II or conjugation enzymes involved in drug and xenobiotic metabolism. The conjugation of most small compounds with a sulfonate group inhibits their biological activity and increases their hydrophilicity and excretion [7].

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The major SULT isoform in human breast responsible for DHEA sulfation is SULT2B1b [8]. The SULT2B1 gene encodes two different isoforms resulting from the use of different start sites of transcription resulting in the inclusion of different first exons [9]. SULT2B1b is slightly longer than SULT2B1a allowing for their resolution and detection by immunoblot analysis. SULT2B1b protein has been detected by immunoblot analysis in several human tissues including placenta, breast, prostate, lung and skin [8,10–12]. In contrast, although message for SULT2B1a has been found in human tissues [9,13], immunoreactive protein has not yet been detected in a human tissue [8].

SULT2B1b is selective for the conjugation of 3β-hydroxysteroids and does not sulfate most 3α-hydroxysteroids, estrogens or the D-ring hydroxyl groups of steroids [14]. SULT2B1b is the only SULT isoform that is localized in the nuclei of some human tissues. SULT2B1b has been localized to the nuclei of placental and breast cells but not lung or prostate cells [8,10,11]. In human BeWo choriocarcinoma cells, nuclear localization of SULT2B1b is associated with serine phosphorylation of a unique carboxyl-terminal proline and serine-rich sequence [15]. The functional role for nuclear localization of SULT2B1b has not been determined although its expression in estrogen responsive tissues would be expected to limit the conversion of DHEA to active estrogens.

In this study, the biochemical characterization of SULT2B1b in both T-47D breast cancer cells and human breast tissue was analyzed. Subcellular localization was evaluated by immunoblot and histochemical analyses, and SULT2B1b enzymatic activity was monitored with DHEA as substrate. Additionally, associated-normal and cancerous breast tissues from African American and Caucasian women were evaluated by histochemical staining to determine SULT2B1b levels as well as to investigate its subcellular localization.

2. Materials and methods

2.1. Cell culture

T-47D breast cancer cells were obtained from the ATCC and were maintained in RPMI 1640 supplemented with 10% FBS (Atlanta Biologicals) and 4 mg/ml insulin (Sigma). Cells were grown at 37 °C in a humidified atmosphere with 5% CO_2. Medium was replaced every 3 days and cells were passed upon reaching 80% confluency.

2.2. Biochemical characterization of SULT2B1b in T-47D cells and breast cancer tissue

For biochemical characterization of SULT2B1b, samples were prepared from T-47D cells and fresh breast cancer tissues from patients as follows. T-47D cells were plated in 100 mm plates and upon confluency, the cells were washed with PBS and scraped from the plates. Cytosolic and nuclear fractions were then prepared from the cells using the Nuclei Isolation Kit (Sigma). For the analysis of the biochemical localization of SULT2B1b in breast tissue, cytosol and purified nuclear fractions were prepared from fresh human breast cancer tissue using Optiprep gradient density centrifugation (Sigma) as per the manufacturer's instruction. Optiprep density medium is a 60% iodixanol solution used to create a self-forming iodixanol gradient during centrifugation to isolate nuclei by isopycnic banding [16,17].

For immunoblot analysis, nuclear and nuclei-free cytosolic fractions were prepared as described above. Protein concentrations were determined using the Bradford protein assay with γ-globulin as the protein standard. Nuclear and cytosolic fractions (100 μg) of the T-47D cells and breast cancer tissue were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. The immunoblot protocol involved using 5% non-fat milk for blocking, primary antibody at a 1:1000 dilution and the appropriate secondary antibody, either goat anti-rabbit or goat anti-mouse IgG HRP conjugate (1:60,000). The following primary antibodies were used: polyclonal rabbit anti-SULT2B1b antiserum generated in our laboratory [15], mouse monoclonal anti-β-tubulin IgG (Sigma), and mouse monoclonal anti-histone IgG (Chemicon International). Bound antibodies were visualized using the SuperSignal West Pico chemiluminescent substrate system (Pierce).

To determine the enzymatic activity in intact T-47D cells, cells were plated in six-well plates. When cells reached 80% confluence, 3 μM [^{3}H]-DHEA in 1% stripped FBS phenol red-free RPMI 1640 media (approximately 500,000 cpm/well) was added to the cells. Reactions were incubated for various times after which aliquots of the medium were removed and [^{3}H]-DHEA-sulfate quantified by scintillation counting of an aliquot of the aqueous phase after the removal of unsulfated [^{3}H]-DHEA by chloroform extraction. SULT2B1b activity was expressed as pmol/mg protein.

Sulfation activity was evaluated using the same samples described above. T-47D and breast cancer nuclear and cytoplasmic fractions (100 μg) were incubated with 3 μM [^{3}H]-DHEA (approximately 30,000 cpm/reaction), 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, and 25 μM PAPS at 37 °C for 20 min to detect SULT2B1b activity. DHEA-sulfate formation was quantified by scintillation counting of an aliquot of the aqueous phase after removal of unsulfated steroids by chloroform extraction [18]. SULT2B1b activity was expressed as pmol/min/mg protein.

2.3. Immunohistochemical staining of SULT2B1b in breast tissue and T-47D cells

Paraffin embedded normal and cancerous breast tissues were obtained from the Archives of the Department of Pathology at the University of Alabama at Birmingham after pathological diagnosis in accordance with Institutional Review Board approval. The breast tissues were from individuals that were self-identified as being African American or Caucasian. Following oven-drying, 5 μm breast tissue sections mounted on glass slides were deparaffinized and rehydrated by a series of xylene and graded ethanol washes. Low temperature antigen retrieval was used to optimize staining conditions [19]. Following endogenous peroxidase quenching with 3% H_2O_2 and non-specific biotin/avidin blocking with 1% goat serum (Sigma) for 1 h, sections were incubated with polyclonal rabbit anti-SULT2B1b primary antibodies (1:100) for 1 h [14]. Bound antibodies were then visualized by treating samples with biotin-SP-conjugated AffiniPure goat anti-rabbit IgG (H + L) followed by peroxidase-conjugated streptavidin (Jackson ImmunoResearch). The resulting antigen–antibody complex was then visualized using the diaminobenzidine tetrachloride Super Sensitive Substrate Kit (BioGenex). Sections were then counterstained with hematoxylin, rinsed with tap water, dehydrated with graded ethanol and washed in xylene followed by addition of a cover slip. Negative controls were processed along with stained sections except for the addition of primary antibodies. Images were acquired on an Axioskop 2 plus microscope equipped with an AxioCam digital camera and AxioVision Software (Zeiss, version 4.4). Sections were evaluated by two individual judges, a board certified histopathologist and experienced research technician. The sections were assigned a score of 0–4 based on intensity of staining in the nuclei and cytoplasm as described by Talley [20].

For immunohistochemical evaluation of T-47D cells, cells were plated on coverslips in a six-well plate and allowed to grow to confluency. The cells attached to the coverslips were fixed in 70% ethanol for 2 h, permeabilized with acetone and quenched with