Chemico-Biological Interactions 191 (2011) 206–216

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# Expression of estrogen and progesterone receptors and estrogen metabolizing enzymes in different breast cancer cell lines

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ARTICLE INFO

*Article history:*
Available online 21 December 2010

*Key words:*
17β-Hydroxysteroid dehydrogenases (17β-HSDs)
Aldo-keto reductases (AKRs)
Aromatase
Sulfatase
Sulfotransferases
Catechol-O-methyl transferase (COMT)
UDP Glucuronosyl transferase (UGT)

ABSTRACT

Prolonged exposure to estrogens is a significant risk factor for the development of breast cancer. Estrogens exert carcinogenic effects by stimulating cell proliferation or through oxidative metabolism that forms DNA-damaging species. In the present study, we aimed to provide a better understanding of estrogen metabolism and actions in breast cancer, and to characterize model breast cancer cell lines. We determined the expression profiles of the genes for the estrogen and progesterone receptors, and for 18 estrogen-metabolizing enzymes in eight cell lines: MCF-7, MCF-10A, T47D, SKBR3, MDA-MB-231, MDA-MB-361, Hs-578T and Hs-578Bst cells. Similar gene expression profiles of these receptors and enzymes for the formation of estradiol via the aromatase and sulfatase pathways were observed in the MCF-7 and T47D metastatic cell lines. The MDA-MB-361 cells expressed *ESR1*, *ESR2* and *PGR* as well, but differed in expression of the estrogen-metabolizing enzymes. In the MDA-MB-231 and SKBR3 cells, all of these estrogen-forming enzymes were expressed, although the lack of *ESR1* and the low levels of *ESR2* expression suggested that the estrogens can only act via non-ER mediated pathways. In the non-tumorigenic MCF-10A cell line, the key enzymes of the aromatase pathway were not expressed, and the sulfatase pathway also had a marginal role. The comparison between gene expression profiles of the non-tumorigenic Hs-578Bst cells and the cancerous Hs-578T cells revealed that they can both form estrogens via the sulfatase pathway, while the aromatase pathway is less important in the Hs-578Bst cells. The Hs-578T cells showed low levels of *ESR1*, *ESR2* and *PGR* expression, while only *ESR1* and *ESR2* expression was detected in the Hs-578Bst cells. Our data show that the cell lines examined provide the full range of model systems and should further be compared with the expression profiles of breast cancer specimens.


## 1. Introduction

Breast cancer represents 23% of all female cancers, and it is by far the most frequent cancer among women in the developed and developing world [1]. Although mortality rates remain much less than incidence rates, breast cancer is still the most frequent cancer-related death in women [1]. Sex steroid hormones have a major role in the growth and development of the mammary gland, and a clear correlation between pathogenesis of breast cancer and cumulative exposure to estrogens has been demonstrated [2–4]. Approximately 60% of premenopausal and 75% of post-menopausal patients with breast cancer have estrogen-dependent disease [5]. In their dual roles of ligands and substrates, the estrogens can simultaneously increase the number of DNA replication errors, by stimulating cell proliferation and gene expression, and cause DNA damage, via their oxidation products, the catechol estrogens [6–9].

In premenopausal women, the synthesis of the estrogens estradiol (E2) and estrone (E1) occurs in the ovaries and peripheral tissues, such as adipose tissue, bone and skin, among others [2,5,10]. Estrogens in target tissues can thus originate from ovarian secretion via an endocrine mechanism, from peripheral formation through circulating E1-sulfate, and from local formation in the target tissue itself [5,9]. In postmenopausal women, estrogens can only be produced in peripheral sites. Estrogens in breast tissue can be formed via the so-called aromatase pathway, or from circulating E1-sulfate via the sulfatase pathway [9,11,12]. Progesterone acts via the progesterone receptors (PRA, PRB), and it has a dual role in breast tissue: it can either stimulate cell proliferation or have differentiating effects [13,14].

The estrogens can be formed from the adrenal or ovarian androgens dehydroepiandrosterone (DHEA) and DHEA-sulfate, or androstenedione and testosterone. This can occur through the actions of steroid sulfatase (STS), 3β-hydroxysteroid dehydrogenases (3β-HSDs) types 1 and 2, aromatase (CYP19A1) and the

*Abbreviations:* E1, estrone; E2, estradiol; ER, estrogen receptor; PR, progesterone receptor; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; E1-S, estrone-sulfate; E2-S, estradiol-sulfate; COMT, catechol-O-methyltransferase; SULT, sulfotransferase; UGT, UDP glucuronosyl transferase; STS, steroid sulfatase; GST, glutathione S-transferase.

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doi:10.1016/j.cbi.2010.12.013

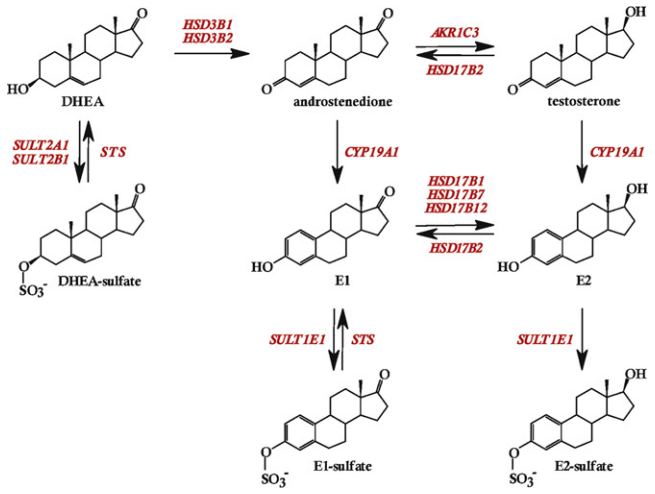


**Fig. 1.** Estrogen biosynthesis. Estrogens formation from dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S), and androstenedione and testosterone, via the actions of steroid sulfatase (STS), 3β-hydroxysteroid dehydrogenases (3β-HSD) types 1 and 2, aromatase (CYP19A1) and the reductive 17β-hydroxysteroid dehydrogenases (AKR1C3 and 17β-HSDs types 1, 7 and 12). The oxidative 17β-HSD type 2 catalyzes inactivation of E2, while SULT1E1, SULT2A1 and SULT2B1 catalyze conjugation of E1 and DHEA, respectively.

reductive 17β-HSD types 1, 7 and 12. Estrogens can also be formed from E1-sulfate by the actions of STS and the reductive 17β-HSDs [5,9,15,16]. Oxidative 17β-HSD type 2 and SULT1E1 catalyze inactivation of E2 and conjugation of E1 and E2, respectively, while SULT2A1 and SULT2B1 convert DHEA to DHEA-sulfate [9,17,18]. E2 that is formed locally can exert proliferative effects via the estrogen receptors (ERα and ERβ) [2]. All of the enzymes necessary for the local production of estrogens are expressed in human breast tissue, but the balance between estrogen-forming and inactivating enzymes determines levels of potent E2 (Fig. 1) [9,11,12].

Endogenous estrogens can also undergo extensive oxidative metabolism at different positions, catalyzed by various cytochrome P450 (CYP) isoforms, known as phase I metabolizing enzymes. These metabolic pathways form mainly A-ring catechol estrogens by 2- and 4-hydroxylation and, to a lesser extent, D-ring hydroxyl estrogens by 16α-hydroxylation (Fig. 2) [8,19]. Catechol estrogens, and particularly the 4-hydroxyestrogens, can form reactive semiquinones and 3,4-quinones that react with DNA, forming mainly unstable N3-adenine and N7-guanine DNA adducts. Reduction of estrogen quinones back to semiquinones provides an opportunity for redox cycling, which forms the reactive oxygen species (ROS), superoxide anion radical ($O_2^{-\bullet}$) and then $H_2O_2$. Hydroxyl radicals that are formed from $O_2^{-\bullet}$ and $H_2O_2$ in the presence of $Fe^{2+}$ are responsible for protein and DNA damage [7,8,19–21]. These genotoxic modifications, along with the oxidative DNA damage, can lead to mutations, and thence tumor initiation. The 2-hydroxy estrogens can also form o-quinones; however, the 2,3-quinones have shorter half lives and are apparently less carcinogenic [19,20].

Detoxification of catechol estrogens and quinones is catalyzed by what are known as the phase II metabolizing enzymes: catechol-O-methyltransferase (COMT) [21], sulfotransferases (SULTs) [17,20,22], UDP glucuronosyl transferases (UGTs), and glutathione S-transferases (GSTs) [20]. COMT, which is found in soluble and membrane-bound forms, inactivates catechol estrogens by conjugation to the non-carcinogenic methoxyestrogens (MeO-estrogens), where 2-MeO-estrogens even act as tumor-suppressors [23,24]. Among the SULTs, SULT1E1, and to a lesser extent SULT2B1, are the enzymes that inactivate estrogens and catechol estrogens [22]. UGTs can form different inactive estrogens glucuronides, while UGT2B7 mainly catalyzes glucuronidation of 4-OH-estrogens [8]. GSTs, and especially the pi-class enzyme GSTP1, conjugate both, catechol estrogens and estrogen quinones [25]. Conjugated estrogens are less active, more polar and more water-soluble, and they can therefore be more easily excreted in the bile and urine [26]. Clearly, the balance between the phase I and phase II metabolic pathways determines the levels of estrogen quinone and ROS formation [27].

Estrogen carcinogenesis is believed to be a step-wise process that consists of initiation and promotion (cell proliferation), with three mechanisms of estrogen carcinogenesis under consideration at present. A generally accepted mechanism is ER-mediated cell proliferation that increases the risk of genomic mutation during DNA replication [27]. The second mechanism involves membrane-associated ERs that appear to regulate extranuclear estrogen signaling pathways [7]. The third mechanism is tumor initiation through oxidative metabolism of estrogens to the electrophilic/redox-active estrogen quinones, with concurrent formation of ROS [7,28]. With their mitogenic and mutagenic effects with regard to breast cancer, the carcinogenicity of estrogens and catechol estrogens has been widely reported [3,21,29–31].

In the present study, we have studied the expression levels of ER and PR and for 18 estrogen-metabolizing enzymes across eight cell lines, including non-tumorigenic cell lines (MCF-10A, HS-578Bst cells), a primary cancer cell line (Hs-578T cells), and metastatic cancer cell lines (T47D, MCF-7, SKBR3, MDA-MB-231, MDA-MB-361 cells) (Table 1). Our real-time PCR analysis included genes encoding ERα and ERβ (*ESR1*, *ESR2*) and PRA and PRB (*PGR*), and those encoding 12 estrogen biosynthetic enzymes (3β-HSD types 1 and 2, 17β-HSD types 1, 2, 7, 12, AKR1C3, CYP19A1, STS, SULT2A1, SULT2B1, SULT1E1) and six phase I and phase II metabolic enzymes (phase I, CYP1A1, CYP1A2, CYP1B1; phase II, COMT, UGT2B7, GSTP1). The aim of our study was to provide a better understanding of estrogen metabolism and action in hormone-dependent and independent breast cancer. Additionally, we aimed to complement the characterization of these cell lines that are often used as models for the study of breast cancer.

## 2. Materials and methods

### 2.1. Cell culture

The T47D hormone-sensitive breast cancer cell line was originally purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). All of the other cells were originally obtained from the American Type Culture Collection. The cell lines were maintained at 37 °C and 5% $CO_2$ and passaged at 1:3 dilutions, except for the Hs-578Bst cells that were passaged at 1:2. For each cell line, the culture conditions, receptor status, patient age and source, and tumor type are shown in Table 1. The T47D cells were provided by Dr. Petra Kocbek, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia, and the other breast cancer cell lines were provided by Dr. Toni Petan and Prof. Dr. Igor Križaj, Institute Jozef Stefan, Ljubljana, Slovenia.

### 2.2. RNA isolation

Total RNA was isolated from these breast cancer cell lines using Tri Reagent (Sigma), according to the manufacturer's instructions. The samples were treated with the DNase I (Sigma), as stated in the instructions. The quality of the RNA samples was determined using a Nanodrop ND-1000 spectrophotometer (Agilent), with 260:280 and 260:230 ratios of approximately 2.0. Total RNA was reverse transcribed using SuperScript® III reverse transcriptase (Invitrogen). One μg of total RNA was converted into cDNA