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Progesterone regulates catechol-*O*-methyl transferase gene expression in breast cancer cells: Distinct effect of progesterone receptor isoforms

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Abstract

There is strong evidence that catechol-*O*-methyl transferase (COMT) protects breast cells against estrogen-induced cancer by detoxifying catecholestrogens, the carcinogenic estrogen metabolites. COMT gene expression is controlled by two promoters – a proximal promoter (COMTP1) and a distal promoter (COMTP2) – that regulate the expression of soluble (S-COMT) and membrane-bound (MB-COMT) isoforms, respectively. We investigated the transcriptional regulation of the COMT gene by progesterone/progesterone receptors in breast cancer cells. Our results indicated that progesterone (P4) downregulates COMT gene expression in breast cancer cell lines. In addition, the COMTP1 and COMTP2 harbor several progesterone response elements (PREs). Electrophoretic mobility shift assay (EMSA) indicated that nuclear extracts of T47D cells bind to the identified PREs in COMTP1. Site-directed mutagenesis of PREs in COMTP1 not only reversed the P4-induced inhibition of COMTP1, but also increased its basal activity. The two progesterone receptor isoforms, PR-A and PR-B, were found to have opposite effects on the regulation of P4 in COMT expression; PR-A is associated with P4-induced upregulation of COMT, while PR-B is associated with P4-induced downregulation of COMT. In summary, our data demonstrated that P4 downregulates the COMT gene expression through multiple PREs in the COMT promoters and that different progesterone receptor isoforms have distinctive effects on COMT gene expression. Published by Elsevier Ltd.

Keywords: COMT; Breast cancer; Progesterone; Progesterone receptor isoforms; Estrogen metabolism

1. Introduction

Breast cancer is the most common malignancy and the second most frequent cause of cancer death among women in the United States [1]. It is well-established that prolonged exposure to estrogen is an important risk factor for genesis and promotion of breast cancer [2,3]. Epidemiological studies suggest that the addition of progestins in hormone replacement therapy increases the risk of breast cancer relative to estrogen use alone [4,5]. The World Health Organization International Agency for Research and Cancer has classified combination (estrogen and progesterone) hormone contraception and menopausal therapy as carcinogenic in humans [6]. A potential mechanism of estrogen carcinogenicity is based on its mitogenic effect and stimulation of cell division, thus increasing random errors during DNA replication [7]. Additionally, oxidative metabolism of estrogen plays a detrimental role in the initiation and/or promotion of estrogen-related cancer [8,9]. The initial step in estrogen metabolism is oxidation by cytochrome P450 (CYP450) 1A1 and 1B1 to produce 2-hydroxyestradiol (2-OHE_2) and 4-hydroxyestradiol (4-OHE_2), respectively [10–12]. 4-OHE_2 has a potent estrogenic effect and was found to be carcinogenic in animal models [13–15]. The carcinogenicity of 4-OHE_2 resides in its vulnerability for auto-oxidation to the corresponding orthoquinone derivative (3,4-OHE2-*o*-quinone) [16]. These catecholestrogens-derived orthoquinones are electrophilic compounds capable of reacting with DNA to form mutagenic and carcinogenic covalent adducts [9,17]. In extrahepatic tissues, the principal pathway for detoxification of catecholestrogens is *O*-methylation by catechol-*O*-methyl transferase

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(COMT) [18]. COMT-mediated conjugation reactions render catecholestrogens more water soluble and easily excreted, thus impeding further oxidation into the corresponding quinones [18,19]. Consistent with the pivotal role of COMT in the development of breast cancer, genetic epidemiological studies suggest that the COMTLL genotype, which is associated with low COMT enzymatic activity, confers a significantly increased risk for breast cancer [20–22].

COMT is expressed in different mammalian tissues [23–26] where it exerts various biological functions. There are two isoforms of COMT with similar functions—soluble (S-COMT) and membrane-bound (MB-COMT). Both are encoded by a single gene located in chromosome 22q11.2 [23,25]. The expression of MB-COMT and S-COMT is regulated by two separate promoters: the proximal promoter (COMTP1), which regulates S-COMT, and the distal promoter (COMTP2), which regulates MB-COMT [26–29]. Since COMT plays a critical role in the detoxification of carcinogenic estrogen metabolites, studying the transcription regulation of COMT expression is of particular interest in the understanding of the pathobiology of estrogen-related cancer and in designing chemopreventive strategies. Previous work from our laboratory and from other groups indicates that the expression of COMT is regulated by sex steroid hormones [29–31]. The current study was undertaken to systematically investigate the potential role of progesterone/progesterone receptors in the regulation of COMT gene expression in breast cancer cell lines.

2. Materials and methods

2.1. DNA vectors, chemicals, and reagents

The PGL3 luciferase (Luc) vector, pSV-β-galactosidase control vector, *pfu* DNA polymerase, luciferase enzyme assay system, and β-galactosidase enzyme assay system were purchased from Promega (Madison, WI). Restriction enzymes, bovine serum albumin (BSA), and *Escherichia coli* DH5α competent cells were obtained from Life Technologies, Inc. (Gaithersburg, MD). [γ-^{32}P]ATP was purchased from Amersham (Buckinghamshire, United Kingdom). All of the chemicals and reagents were of the highest commercially available grade.

2.2. Cell culture

Human breast cancer cell lines MCF-7 and T47D (positive for progesterone receptors), and MDA-MB-231 (negative for progesterone receptors) were obtained from American Type Culture Collection (ATCC, Manassas, VA). MCF-7 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS). T47D cells were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic Acid (HEPES), 1.0 mM sodium pyruvate, 0.2 U/ml bovine insulin, 1% penicillin/streptomycin, and 10% FBS. MDA-MB-231 cells were grown in Leibovitz's L-15 medium. For treatment with progesterone or RU486, cells were maintained in the indicated growth medium without phenol red and supplemented with 10% dextran–charcoal-treated FBS.

2.3. Construction of chimeric COMT promoters-luciferase reporters

Chimeric COMTP1 and COMTP2 reporters were constructed according to the method described by Xie et al. [29] with some modification. The forward primers contained the Mlu1 recognition site at the 5′ end, and the reverse primers contained the recognition site for BglII restriction enzyme at the 5′ end. The amplified COMTP1 and COMTP2 were digested with Mlu1 and BglII enzymes and cloning into Mlu1 and BglII linearized PGL3-luciferase reporter vector. The identity of the cloned inserts was confirmed by sequencing and comparison with published sequences.

2.4. Site-directed mutagenesis of PREs in COMTP1

To further examine the functionality of the various PREs identified in the COMTP1 (which controls the S-COMT expression), the following three putative PRE sites – PRE I (5′-TGTGCC-3′, located from −48 to −53); PRE II (5′-TGTGCC-3′, located from −705 to −711); PRE III (5′-TGTTCT-3′, located from −1201 to −1206) – were mutated individually or in combination using a PCR-based strategy. PRE sites were changed either from 5′-TGACCT-3′ (sites I and II) to 5′-CGGCCG-3′ or from 5′-TGTTCT-3′ (site III) to 5′-CCGATA-3′. Mutation of the PREs, individually or in combination, resulted in a total of seven constructs harboring site-directed mutations: mutant I (mutated at PRE I), mutant II (mutated at PRE II), mutant III (mutated at PRE III), mutant IV (mutated at PRE I and PRE II), mutant V (mutated at PRE I and PRE III), mutant VI (mutated at PRE II and PRE III), and mutant VII (mutated at PRE I, PRE II, and PRE III).

2.5. Mammalian cell transfection with luciferase and β-galactosidase plasmids

The effect of progesterone or RU486 on activities of COMTP1-luciferase (COMTP1-Luc), COMTP2-Luc, or the mutated COMTP1-Luc constructs was assessed in transiently transfected MCF-7 and T47D breast cancer cell lines. In each transfection experiment, the cells (60–70% confluent) were cotransfected with luciferase reporter constructs (10 μg), and Promega pSV-β-galactosidase control vector (1 μg) using FuGENE 6 Transfection Reagent (Roche Applied Science, Indianapolis, IN), according to the manufacturer's protocol. The transfected cells were treated with different concentrations of progesterone (10^{-6}, 10^{-7}, or 10^{-8} M) or the antiprogestin RU486 (10^{-6} M) in 0.01%