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Involvement of peroxiredoxin IV in the 16α-hydroxyestrone-induced proliferation of human MCF-7 breast cancer cells

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Abstract

A variety of investigations on peroxiredoxins (Prxs) in different types of cancer have been carried out, but the estrogen-related function of Prxs in breast cancer has not yet been studied. In order to study the involvement of Prxs in the growth of breast cancer cells by estrogen, we evaluated the effect of mitogenic estrogen metabolites on the expression of Prx isoforms (I to VI) in MCF-7 cells and found that the transcript/protein expression of Prx IV was significantly induced by 16α-hydroxyestrone (OHE1) under both serum-free and serum conditions. In addition, treatment with Prx IV-specific siRNA significantly inhibited the 16α-OHE1-induced proliferation of MCF-7 cells. These results suggested that Prx IV involved in the 16α-OHE1-induced proliferation of MCF-7 cells has a proliferative effect and may be related to cancer development or progression.

Keywords: Breast cancer; 16α-Hydroxyestrone; Peroxiredoxin; Cell proliferation

1. Introduction

Estrogens are known to play an important role in the development and progression of breast cancer (McGuire et al., 1976; Clemons and Goss, 2001). 17β-Estradiol (E2) is the most biologically active estrogen in breast tissue and its administration in rodent models has shown to be carcinogenic. Hydroxylated metabolites of E2 such as 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2), and 2-methoxyestradiol (2-ME) also have estrogenic activities and are carcinogenic (Schneider et al., 1982; Lippert et al., 2000; Liu and Zhu, 2004), but 16α-hydroxyestrone (16α-OHE1) has more potent estrogenic activity than E2 (Fishman and Martucci, 1980; Kim et al., 2005). Abnormal 16α-hydroxylation of E2 and increased activity of 16α-hydroxylase, the enzyme involved in the formation of 16α-OHE1, have been observed in a population of women with breast cancer (Schneider et al., 1982; Fishman et al., 1984).

Oxidative stress is important not only for normal cell physiology but also for many pathological processes, such as cancer (Ambrosone, 2000). To reduce oxidative stress, cells have developed different kinds of reparative or defensive systems including antioxidant enzymes such as superoxide dismutases, catalase, glutathione peroxidases, and a quite recently found, rapidly growing family of peroxiredoxins (Prxs). Interestingly, there are several reports indicating that a carcinogenic estrogen shows strong oxidant potential, whereas a weakly-carcinogenic estrogen shows poor oxidant potential (Patel and Bhat, 2004). In addition, the treatment of estrogen receptor (ER)-positive human breast MCF-7 cells with E2 resulted in a marked decrease in their ability to metabolize peroxide, which paralleled an alteration in activity of antioxidant enzymes and level of total glutathione (Mobley and Brueggemeier, 2002). These alterations were not observed in ER-negative human MDA-MB-231 breast cancer cells, suggesting that E2 might be

Abbreviations: DC, dextran–charcoal; E2, 17β-estradiol; ER, estrogen receptor; FBS, fetal bovine serum; 2-ME, 2-methoxyestradiol; 16α-OHE1, 16α-hydroxyestrone; 2-OHE2, 2-hydroxyestradiol; 4-OHE2, 4-hydroxyestradiol; PR, progesterone receptor.

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capable of inducing an increase in sensitivity to oxidative stress with the alteration in the levels of antioxidants and antioxidant enzymes through an ER-mediated mechanism.

Prxs are a ubiquitous family of peroxidases containing high antioxidant efficiency. The mammalian Prx family has six distinct members located in various subcellular locations (Wood et al., 2003). Recently, the increased expression of Prxs, especially III, IV and V, has been reported in breast malignancy, suggesting that high Prx levels in cancer may be because of proliferative and antiapoptotic functions that may participate in tumorigenesis (Karihtala et al., 2003). Therefore, we have evaluated the effect of mitogenic estrogen metabolites on the expression Prx isoforms in MCF-7 cells in order to evaluate the involvement of Prx family in the growth of breast cancer cells by E2 metabolites.

2. Materials and methods

2.1. Cell culture and E2 metabolites

Human MCF-7 breast cancer cells (American Type Culture Collection number: HTB-22) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, MD, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, UT, USA), 100 U/ml of penicillin, and 100 mg/ml streptomycin with a change of medium every 3 days in humidified atmosphere of 5% CO_2 in air at 37 °C. Cells were plated in DMEM with 10% FBS and incubated for 2 days. The medium was replaced with phenol red-free DMEM with 5% dextran/charcoal (DC)–FBS to remove endogenous steroids from serum and exclude the weak estrogen-agonist activity of phenol red (Darbre et al., 1983; Ernst et al., 1989). After 1 day, cells were incubated with E2 metabolites under either serum (5%) and serum-free (0.1%) conditions. E2 metabolites (Steraloids Inc., Newport, RI, USA) were initially dissolved in dimethyl sulfoxide (DMSO) and 10 mM stock solutions were further diluted with serum-free and phenol red-free medium. DMSO (below 0.001%) was used as control in all experiments.

2.2. Real-time quantitative PCR (QPCR)

All experiments were performed according to the manufacturer's protocol. Total RNA was isolated with TRIzol reagent (Life Technologies) and the concentrations of total RNA were calculated from the absorbance at 260 and 280 nm with BioPhotometer (Eppendorf AG, Germany). First-strand cDNA was synthesized with 2 μg of total RNA, 1 μM of oligo-dT_{18} primer and 10 U RNase inhibitor RNasin (Promega, Madison, WI, USA) using an Omniscript RT kit (Qiagen, Valencia, CA, USA). The SYBR green-based QPCR was performed using Stratagene Mx3000P Real-Time PCR system and Brilliant SYBR Green Master Mix (Stratagene, La Jolla, CA, USA) with first-strand cDNA diluted 1:50 and 20 pmol of primers. Primers were determined by an on-line primer design program to set the most suitable PCR amplification conditions (Rozen and Skaletsky, 2000) and the primer sets used in this study are shown in Table 1. The PCR reaction consisted of three segments. The first segment at 95 °C for 10 min was for the activation of the polymerase and the second one corresponded to 3-step cycling (40 cycles) at 94 °C for 40 s (denaturation), 60 °C for 40 s (annealing), and 72 °C for 1 min (extension). The third segment was for the generation of PCR product temperature dissociation curves (also called 'melting curves') at 95 °C for 1 min, 55 °C for 30 s, 95 °C for 30 s. All reactions were run in triplicate, and data were analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). To assess differential transcript expression between groups, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control gene. Significance was determined by Student's *t*-test using the GAPDH-normalized $2^{-\Delta\Delta CT}$ value, and expression differences were considered significant at $P < 0.05$.

2.3. Western blot analysis

Cells were homogenized in buffer consisting of 10 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.05% (v/v) Tween 20, 1 mM PMSF, and one protease inhibitor cocktail tablet (Roche, Germany) at 4 °C and centrifuged at 10,000 × *g* for 15 min. The BCA protein assay kit (Pierce, Rockford, IL, USA) was used to determine the concentration of protein in the supernatant. Protein samples (20 μg) were mixed with sample buffer (100 mM Tris–HCl, 2% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 2% glycerol, 0.01% bromophenol blue, pH 7.6), incubated at 95 °C for 15 min, and loaded onto 10% polyacrylamide gels. Electrophoresis was performed using the Mini Protean 3 Cell (Bio-Rad, CA, USA). The membrane was incubated in blocking buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20, 3% non-fat dry milk) and

Table 1
Primer sequences used in this study

Target gene	Forward (5′–3′)	Reverse (5′–3′)
Prx I	CAACTGCCAAGTGATTGGTG	TGATCTGCCGAAGAATACCC
Prx II	GCGTGCTGAAAACAGATGAG	ACGTTGGGCTTAATCGTGTC
Prx III	GTTGTCGCAGTCTCAGTGGA	TAGGAGAATCCGGTGTCCAG
Prx IV	CTCCCTGCACCTAAGCAAAG	ATCCTTATTGGCCCAAGTCC
Prx V	TGTGCTGTTTGGAGTTCCTG	ATGCCATCCTGTACCACCAT
Prx VI	CAGTGTGCACCACAGAGCTT	TGCTGTCAGCTGGAGAGAGA
GAPDH	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA