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Human glutathione S-transferase P1-1 functions as an estrogen receptor α signaling modulator

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ABSTRACT

Estrogen receptor α (ERα) plays a crucial role in estrogen-mediated signaling pathways and exerts its action as a nuclear transcription factor. Binding of the ligand-activated ERα to the estrogen response element (ERE) is a central part of ERα-associated signal transduction pathways and its aberrant modulation is associated with many disease conditions. Human glutathione S-transferase P1-1 (GSTP) functions as an enzyme in conjugation reactions in drug metabolism and as a regulator of kinase signaling pathways. It is overexpressed in tumors following chemotherapy and has been associated with a poor prognosis in breast cancer. In this study, a novel regulatory function of GSTP has been proposed in which GSTP modulates ERE-mediated ERα signaling events. Ectopic expression of GSTP was able to induce the ERα and ERE-mediated transcriptional activities in ERα-positive but GSTP-negative MCF7 human breast cancer cells. This inductive effect of GSTP on the ERE-transcription activity was diminished when the cells express a mutated form of the enzyme or are treated with a GSTP-specific chemical inhibitor. It was found that GSTP inhibited the expression of the receptor interacting protein 140 (RIP140), a negative regulator of ERα transcription, at both mRNA and protein levels. Our study suggests a novel non-enzymatic role of GSTP which plays a significant role in regulating the classical ERα signaling pathways via modification of transcription cofactors such as RIP140.

1. Introduction

Estrogen receptor α (ERα) is a transcription factor crucial for estrogen-responsive gene transcription. The dimers of ligand-bound ERα interact with the specific DNA sequences such as the estrogen response element (ERE; GGTCAcagTGACC), activating protein (AP)-1 binding sequences (ATGAGTCAT), or GC rich specificity protein (Sp) binding sequences ((G/T)GGGCGG(G/A)(G/A)(C/T))-located in the regulatory region of target gene promoters [1]. Interaction of ERα with the specific responsive elements leads to the induction of target gene transcription depending on the cell type and promoter context by recruiting a variety of coregulatory proteins that stabilize the transcription initiation complex or alter chromatin structure.

ERα target genes including cyclin D1, progesterone receptor, and trefoil factor 2 are tightly regulated since these genes are closely implicated in cell proliferation, survival and/or death, thus playing vital roles in development, growth, and tumorigenesis [2]. The classical mechanism of ER action involves binding of ERα to the ERE and is attributed to approximately two thirds of estrogen activated ERα action [3]. Thus, regulation of the classical ERα action is a critical part of in the management of receptor-mediated disease conditions.

Human glutathione S-transferase P1-1 (GSTP; EC 2.5.1.18) catalyzes thioether conjugation of glutathione (GSH) with potentially toxic electrophile reactive intermediates, acting as the detoxifying enzyme [4]. In addition, GSTP has a diversity of regulatory functions such as JNK signaling pathways and Cdk5 kinase activity, and it is therefore involved in cell death and/or survival mechanisms [5,6]. Elevated GSTP expression of has been demonstrated in tumors and believed to play a role in drug resistance. For

Abbreviations: AF, activating function; AP, activating protein; ANOVA, analysis of variance; DMSO, dimethylsulfoxide; ERα, estrogen receptor α; ERE, estrogen response element; GSTP, human glutathione S-transferase P1-1; 4-OHT, 4-hydroxytamoxifen; IgG, immunoglobulin G; PBS, phosphate buffered saline; PVDF, poly vinylidene fluoride; RIP, regulatory interacting protein; SERM, selective estrogen receptor modulators; Sp, specificity protein; TBST, tris-buffered saline with 0.5% Tween-20.

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example, a study showed that GSTP expression indicates chemotherapy resistance and has been associated with a poor prognosis in breast cancers [7].

The recent studies show that the presence of GSTP predicts poor pathological complete response in ERα-negative breast cancer during neoadjuvant chemotherapy [8]. Various studies also indicate that GSTP expression accompanying loss of ERα at the either mRNA or protein level may contribute to poor prognosis in breast cancer and the mechanisms of drug resistance [9,10]. Although the regulation of GSTP at either the gene or protein level via ERα and its role in the development of endocrine therapy resistance remain unclear, GSTP expression is increased with decreased expression of ERα and has been associated with an altered response of selective estrogen receptor modulators (SERM) to ERα [11,12]. It was shown that GSTP protein expression occurs in the presence of ERα in drug-resistant cancer cell lines, implying that GSTP may play a role in the activation of ERα signaling leading to an abnormal response to chemotherapeutic agent such as tamoxifen.

In the present study, we demonstrate that the ectopic expression of functionally complete GSTP enhances the classical ERα signaling activities in ERα-positive but GSTP protein-negative human mammary epithelial cancer MCF7 cells. ERE-mediated ERα transcriptional activities were increased in the presence of GSTP and GSTP enzyme inhibitor decreased the ERE-mediated ERα transcriptional activities. One of molecules involved in GSTP-induced modulation of ERα signaling has been identified as receptor interacting protein 140 (RIP140). Our study proposes a novel non-enzymatic role of GSTP in regulating ERα classical signaling events, which supports the idea that GSTP has additional nonenzymatic functions other than regulation of kinase signaling pathways.

2. Materials and methods

2.1. Chemical and reagents

All chemicals and reagents were purchased from Sigma (St. Louis, MO) unless stated otherwise. Ezatiostat hydrochloride was purchased from APExBio (Boston, MA). The compounds were dissolved in DMSO and stored at −20 °C.

2.2. Cell culture

All cell culture reagents were purchased from Invitrogen (Grand Island, NY) unless stated otherwise. The MCF7 human breast cancer cell line (ATCC® HTB-22™) and HeLa human cervical cancer cell line (ATCC® CCL-2™) were obtained from American Type Culture Collection (Manassas, VA). Cells were routinely maintained in DMEM (HyClone, GE Healthcare Life Sciences, Logan, UT) containing 10% fetal bovine serum (FBS), 1% nonessential amino acids at 37 °C in 5% CO_2. Estrogen-free media were prepared by supplementing 3 × dextran-coated charcoal-treated FBS to phenol-red free DMEM (HyClone) while other components remained the same.

2.3. Plasmids and transfection

The sequences for the primers and siRNA oligonucleotides are listed in Table S1. The expression vector coding for GSTP (GenBank Accession No. NM_000852.3) was generated by inserting the 0.6 kbp long full-length GSTP into the EcoRI/BamHI sites of pIRES-neo vector (Clontech, Mountain View, CA) to produce the pIRES-neo/GSTP plasmid. The ERE-luciferase plasmid contains three copies of the *Xenopus laevis vitellogenin* A2 ERE upstream of the fire fly luciferase [13] and is a gift from Dr. V.C. Jordan (Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC). The pSG5 plasmid containing the wild-type human ERα was originally engineered by Professor P. Chambon (Institut National de la Sante et de la Recherche Medicale, Strasbourg, France). Knockdown of the target gene was performed through the transfection of siRNA duplexes to cells. siRNA oligonucleotides with TT overhang to the end of 3′ position were purchased from Bioneer (Daejeon, South Korea). Scrambled inhibitory RNA (siControl) was derived from a message transcribed from the chloroplast genome of *Euglena gracilis* (GenBank Accession No. X70810; position 24750–24768). HeLa cells were incubated in estrogen-free media for 24 h before seeding in the 24-well plates. siRNA duplexes (30 nM) were transfected using Lipofectamine RNAiMAX reagent (Invitrogen) according to manufacturer's instruction. Six hours after transfection, the cells were put into fresh estrogen-free media and incubated for another 18 hr. ERE-luciferase plasmids (0.25 μg/well) were transfected using Lipofectamine 2000. MCF-7 or HeLa cells were cultured in estrogen-free DMEM media for 4 days before transfection and plated (1.5×10^5 cells/well) in triplicate in a 24-well plate. Cells were transiently transfected with pIRESneo/GSTP (0.5 μg/well) and ERE-luciferase plasmid (0.25 μg/well) and with an internal control plasmid pRL-Tk (0.1 μg/well) using Lipofectamine 2000 Reagent (Invitrogen).

2.4. Luciferase reporter assay

The cells were harvested with Passive Lysis buffer (Promega, Madison, WI). Luciferase activity present in the cell lysates was measured using the Dual Luciferase Assay kit (Promega) with a VICTOR3™ (PerkinElmer, Waltham, MA) in a 96 well plate format. Data are reported as relative luciferase activity (firefly luciferase reading divided by the Renilla luciferase reading).

2.5. Total RNA isolation and PCR analysis

The MCF7 cells grown in estrogen-free media were treated with appropriate test compounds or vehicle control. Total RNA was extracted using RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA (5 μg) was reverse transcribed with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Real-time PCR was performed at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 30–35 cycles (Palm-Cycler™, Corbett Life Science, Sydney, Australia) in a reaction mixture with a total volume of 10 μl using the real-time PCR SYBR green kit (QIAGEN). Rotor-Gene 6.1 software was used for the estimation of the CT parameter. The $2^{-\Delta\Delta}$CT method was applied for the quantification of the gene of interest. Data normalization was performed by dividing the expression level of a *RIP140* gene by that of *18S rRNA*. Finally, results were expressed as fold-induction where the gene expression level in vehicle control-treated cells was set as 1.

2.6. Western blot

Cells were trypsinized, pelleted, washed in PBS, resuspended in lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 10 mM β-glycerophosphate, 10% glycerol, and 0.5% NP-40, pH 8.0) containing protease inhibitors (Complete protease inhibitor cocktail tablets, Roche, Indianapolis, IN), mixed, and centrifuged at 12,000×*g* for 10 min. Protein concentrations were measured by Bradford assay (Bio-Rad). Equal amounts of protein were electrophoresed on a 10% polyacrylamide gel and transferred to a PVDF membrane using Trans-Blot Turbo transfer system (Bio-Rad). The membrane was blocked in TBST containing 10% nonfat dry milk at 4 °C overnight and then incubated for 2 h with anti-human GSTP (3F2) mouse antibody (dilution 1:1000, Cell Signaling Technology, Danvers, MA), rabbit anti-RIP140 antibody (dilution 1:200, Santa Cruz Biotechnology, Dallas, TX) or rabbit anti-ERα