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Resveratrol represses estrogen-induced mammary carcinogenesis through NRF2-UGT1A8-estrogen metabolic axis activation

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ABSTRACT

Estrogen plays a pivotal role in the pathological development of breast cancer. Resveratrol has chemo-preventive effects against breast cancer, whereas, the mechanism of antitumor activities of resveratrol remains unanswered. In this study, we showed that estrogen homeostasis profile was disturbed in both breast cancer patients and in experimental breast cancer model rats, with carcinogenic catechol estrogens significantly accumulated in the mammary tissues. UDP-glucuronosyltransferase 1A8 (UGT1A8) is an important phase II drug-metabolizing enzymes which involved in the metabolism of catechol estrogens. Here we found that the mammary nuclear factor erythroid 2-related factor 2 (NRF2) – UGT1A8 signaling was down-regulated in breast cancer rats, whereas treatment with resveratrol could upregulate the expression of NRF2 and UGT1A8, accelerate metabolic elimination of catechol estrogens, inhibit estrogen-induced DNA damage and suppress the pathological development of breast cancer. In addition, luciferase reporter assay suggested that resveratrol activated the expression of UGT1A8 by up-regulating the transcriptional activity of NRF2. Small-interfering RNA-mediated silencing of NRF2 abolished resveratrol-mediated preventive effects indicated that the antitumor effect of resveratrol is based on NRF2-UGT1A8-estrogen metabolism axis. Taken together, we established the resveratrol regulating potential on estrogen homeostasis based on NRF2-UGT1A8 signaling pathway, and also provided a novel link between estrogen glucuronidation metabolism and breast cancer pathological development.

1. Introduction

Breast cancer is a major cause of mortality in women worldwide [1]. Accumulating evidence suggests that women with early menarche and late menopause, who have longer exposure to estrogens, may have higher risk of breast cancer [2]. Estrogens have long been recognized as the primary risk factor for the development of breast cancer, and the United States government has added steroidal estrogens to the list of known human carcinogens in 2011 [3]. Thus, the concentration of estrogens must be rigorously regulated to ensure a homeostasis status.

Estrogens are proposed to cause breast cancer by stimulating cell growth and proliferation through receptor-mediated processes and toxic metabolites [4]. Parent estrogens (estrone, E1 and estradiol, E2) are postulated to promote tumorigenesis directly through the stimulation of estrogen receptor (ER) and the downstream activation of promitogenic transcriptional programs. The endogenous conversion of estrogen to genotoxic metabolites has been reported as an alternative, potentially ER-independent mechanism for estrogen-dependent breast

Abbreviations: 2-MeOE1/E2, 4-MeOE1/E2, methoxyestrogens; 2-OHE1/E2, 4-OHE1/E2, hydroxyestrogens; 53BP1, tumor protein p53 binding protein 1; DMBA, 7,12-dimethylbenz(a)anthracene; DSBs, DNA double-strand breaks; E1, estrone; E2, estradiol; E2-3G, E2-3-glucuronide; E2-17G, E2-17-glucuronide; ER, estrogen receptor; Glu-2-OHE2, 2-hydroxyestradiol-glucuronide; Glu-4-OHE2, 4-hydroxyestradiol-glucuronide; Glu-E2, estradiol-glucuronide; H2AX, H2A histone family member X; NRF2, nuclear factor erythroid 2-related factor 2; UDPGA, UDP-glucuronic acid; UGT1A8, UDP-glucuronosyltransferase 1A8; UGTs, UDP-glucuronosyltransferases

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tumorigenesis [5]. Estrogen is hydroxylated to form the catechol estrogens 2-hydroxyestrogens (2-OHE1/E2) and 4-hydroxyestrogens (4-OHE1/E2). The catechol estrogens are either methylated by COMT to methoxyestrogens (2-MeOE1/E2, 4-MeOE1/E2) or further oxidized by CYPs to estrogen quinones, which can chemically react with the guanine and adenine of DNA, forming depurinating estrogen-DNA adducts (99%) [3]. It has been reported that urinary levels of 2-OHE2 and 4-OHE2 are elevated in patients with breast cancer compared with healthy controls [6], and 4-OHE2 concentrations have been reported to be up to three times higher in breast cancer biopsies compared with normal breast tissue [7]. Thus, it is reasonable to propose that restoration of estrogen homeostasis may represent a useful approach to the therapy of breast cancer and even the prevention of breast cancer.

Among the dietary phytoestrogens that have been reported to treat breast cancer, resveratrol stands out with a unique chemical structure that resembles mammalian E2. This structural compatibility might be responsible for its estrogen metabolic regulatory prowess which consequently explains the reasons for its usage in hormone-dependent therapy against breast cancer [8]. Previous studies have proved that the antitumor activities of resveratrol are mediated through a network of several cell signaling pathways [9]. It was also observed that resveratrol inhibits estrogen-induced breast carcinogenesis through induction of nuclear factor erythroid 2-related factor 2 (NRF2) mediated protective pathways [10]. However, the role of NRF2 in cancer therapy is controversial, with studies showing either pro-tumorigenic or anti-neoplastic effects [11]. For now, though, the mechanism(s) of resveratrol-mediated prevention of breast cancer remains largely elusive.

Resveratrol has been demonstrated to modulate phase I and phase II enzymes involved in the detoxification of endogenous hormones and carcinogens in preclinical studies [12]. UDP-glucuronosyltransferases (UGTs) is one of the phase II enzymes which catalyze the covalent addition of glucuronic acid (UDPGA), and facilitate the elimination of estrogen and its metabolisms [13,14]. In opposition to other metabolic pathways of estrogen, the UGT-mediated process leads to the formation of glucuronides that are devoid of biologic activity and are readily excreted from the tissue into the circulation [15]. It has been reported that UGTs are mainly expressed in the liver, playing a major role in hepatic glucuronidation in systematic clearance of toxic lipophilic compounds. However, UGTs were also noted in steroid target tissues, such as breast and uterine recently [16]. In addition, UGT polymorphisms with altered circulating hormones and modified risk of endometrial, breast, and ovarian cancers also indicated that glucuronidation pathway could play an important role in estrogen metabolism [17–19].

To date, it remains to be determined whether resveratrol truly has any beneficial effect on breast cancer progression and whether the promise of animal studies could be translated into the clinical, and the mechanism(s) of resveratrol-mediated prevention of breast cancer remain largely elusive [20]. Thus, the aim of the present study was to uncover the dysregulation pattern of NRF2 and UGTs in mammary gland tissues and their role in estrogen homeostasis. Furthermore, we aimed to validate whether the underlying molecular mechanisms of resveratrol in the pathological development of breast cancer have a link of NRF2-UGT1A8-estrogen metabolic axis and the regulation of estrogen homeostasis.

2. Materials and methods

2.1. Materials

7,12-dimethylbenz(a)anthracene (DMBA), resveratrol, estrone (E1), 17β-estradiol (E2), 2-hydroxyestradiol/estrone (2-OHE2/1), 4-hydroxyestradiol/estrone (4-OHE2/1), 2-methoxyestradiol/estrone (2-MeOE2/1), 4-methoxyestradiol/estrone (4-MeOE2/1), d5-E2, dansyl chloride and Vitamin C were supplied by Sigma-Aldrich (St. Louis, USA) and Steraloids (Rhode, USA). Phenol red-free DMEM/F12 medium and charcoal-dextran stripped horse serum were available from Gibco (California, USA). UGT1A8-promoter-luc plasmids, siNRF2 plasmids and NRF2 virus were obtained from Genechem (Shanghai, China). Lipofectamine 3000 transfection reagent and plasmid extraction kit were purchased from Invitrogen (Shanghai, China). Dual-Luciferase Reporter Assay Kit was procured from Promega (Madison, USA). TRIzol® and PrimeScript RT Reagent Kit were purchased from TaKaRa Bio-technology Co., Ltd. (Beijing, China). All analytical solvents were of liquid chromatography (LC) – grade and were obtained from Sigma-Aldrich (St. Louis, USA) or Merck (Darmstadt, Germany).

2.2. Working solutions and buffers

The standard stock solutions (1 mM) of E1, E2, 2-OHE2/1, 4-OHE2/1, 2-MeOE2/1, 4-MeOE2/1 and d5-E2 were respectively prepared by dissolution of methanol containing 0.1% Vitamin C and stored at −20 °C. Equal volume of above solutions were mixed evenly and diluted with methanol containing 0.1% Vitamin C to form a series of standard working solutions. The internal standard d5-E2 stock solution was diluted with methanol containing 0.1% Vitamin C to a final concentration of 50 nM.

DMBA stock solution (20 mg/ml) was prepared by dissolving the pure compound in sesame oil. Resveratrol stock solution for animal experiments was also prepared in sesame oil at a concentration of 10 mg/ml. Resveratrol stock solution (30 mM) for cell experiments was prepared in DMSO and used at a final concentration of 30 μM. Na_2CO_3/$NaHCO_3$ buffer contained 0.1 M Na_2CO_3 and 0.1 M $NaHCO_3$.

2.3. Animals and treatment

Female Sprague-Dawley rats (140–160 g) were obtained from SIPPR-BK Lab Animal Co., Ltd. (Shanghai, China). The rats were housed under controlled temperature, humidity and lighting conditions. All animal studies were approved by the Animal Ethics Committee of Xuzhou Medical University and have been carried out in accordance with the Declaration of Helsinki. After one week acclimatization, rats were randomly divided into the following four groups: Control, resveratrol (RES), DMBA and DMBA + resveratrol (RES). In brief, rats were treated with a single intragastric dose of DMBA (100 mg/kg dissolved in sesame oil). Resveratrol was given as a dose of 50 mg/kg every other day. Rats of control group were treated with sesame oil alone. At the end of the experimental time period (18 weeks), serum, urine and mammary tissues from animals were collected and frozen at −80 °C for future analyses.

2.4. Cell culture and treatment

Non-tumorigenic human breast epithelial cell line MCF-10A was purchased from American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in a humidified environment with 5% carbon dioxide at 37 °C. All cells for experiments were no longer than 15 passages. Twenty-four hours before treatment, cells were washed twice with phosphate-buffered saline (PBS) and then grown in phenol red-free DMEM/F12 (50:50) media supplemented with 5% charcoal-dextran stripped horse serum. Cells were treated with 0.1% DMSO and resveratrol (30 μM) for 48 h according to previous studies [21].

2.5. Clinical sample preparation

This study was conducted on 94 premenopausal female patients (mean age of 42.32 ± 0.91 years) who were surgically treated and pathologically diagnosed with advanced breast cancer and 54 premenopausal female healthy women (mean age of 36.85 ± 1.06 years) from June 2017 to August 2017 in the Affiliated Hospital of Xuzhou Medical University, Xuzhou, China. The patients with breast cancer were enrolled from the Department of Thyroid and Breast Surgery,

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