Pesticide Biochemistry and Physiology 120 (2015) 5–13

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

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

The aryl hydrocarbon receptor (AhR) mediates resistance to apoptosis induced in breast cancer cells

Kanae Bekki [a], Helena Vogel [a], Wen Li [a], Tomohiro Ito [a], Colleen Sweeney [b], Thomas Haarmann-Stemmann [c], Fumio Matsumura [a,d], Christoph F.A. Vogel [a,d,*]

[a] Center for Health and the Environment, University of California Davis, One Shields Avenue, Davis, CA 95616, USA
[b] Cancer Research Center, Basic Science Research, Medical School, University of California, Davis, CA 95817, USA
[c] Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany
[d] Department of Environmental Toxicology, University of California Davis, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 19 September 2014
Accepted 17 December 2014
Available online 23 December 2014

Keywords:
Breast cancer
AhR
Apoptosis
IDO
NF-κB
TCDD

ABSTRACT

The aryl hydrocarbon receptor (AhR) is well known as a ligand binding transcription factor regulating various biological effects. Previously we have shown that long-term exposure to estrogen in breast cancer cells caused not only down regulation of estrogen receptor (ER) but also overexpression of AhR. The AhR interacts with several cell signaling pathways associated with induction of tyrosine kinases, cytokines and growth factors which may support the survival roles of AhR escaping from apoptosis elicited by a variety of apoptosis inducing agents in breast cancer. In this study, we studied the anti-apoptotic role of AhR in different breast cancer cells when apoptosis was induced by exposure to UV light and chemotherapeutic agents. Activation of AhR by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in AhR overexpressing breast cancer cells effectively suppressed the apoptotic response induced by UV-irradiation, doxorubicin, lapatinib and paclitaxel. The anti-apoptotic response of TCDD was uniformly antagonized by the treatment with 3′methoxy-4′nitroflavone (MNF), a specific antagonist of AhR. TCDD's survival action of apoptosis was accompanied with the induction of well-known inflammatory genes, such as cyclooxygenase-2 (COX-2) and NF-κB subunit RelB. Moreover, TCDD increased the activity of the immunosuppressive enzyme indoleamine 2, 3-dioxygenase (IDO), which metabolizes tryptophan to kynurenine (Kyn) and mediates tumor immunity. Kyn also acts as an AhR ligand like TCDD, and kyn induced an anti-apoptotic response in breast cancer cells. Accordingly, our present study suggests that AhR plays a pivotal role in the development of breast cancer via the suppression of apoptosis, and provides an idea that the use of AhR antagonists with chemotherapeutic agents may effectively synergize the elimination of breast cancer cells.
© 2014 Elsevier Inc. All rights reserved.

1. Introduction

The recently found phenomenon of aryl hydrocarbon receptor (AhR) overexpression in breast cancer [1] has raised several important questions. The immediate question being raised by us is "does the AhR contribute to cancer progression?" There is a plethora of knowledge regarding this question. Brooks and Eltom [2] addressed this question by artificially overexpressing AhR in a human mammary epithelial cell, and thereby showed that they indeed convert this type of cells into that exhibiting the phenotypic characteristics of highly transformed breast cancer cells such as increased proliferation, matrigel invasion, epithelial to mesenchymal transition and apoptosis resistance. Among them, several mechanisms of anti-apoptotic action of AhR have already been reported [3]. In some mammary epithelial cells, for instance, AhR activation promotes cells to produce transforming growth factor α (TGFα) and/or activate epidermal growth factor receptor (EGFR) signaling, which helps cells to survive under apoptotic conditions [4]. In addition, modulation of epithelial and endothelial tyrosine kinases (ETK) [5], p53 [6], NF-κB, phosphatidyl-inositol-3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK), TGFβ [7] and E2F [8] have been reported as mechanisms underlying AhR action for anti-apoptosis. Interestingly, inhibition of AhR in keratinocytes has been found to result in down-regulation of E2F1 and checkpoint kinase-1 (CHK1) protein expression, which was accompanied by an increase in UV-induced apoptosis [9]. These data provide the information for

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; MNF, 3′methyl-4′nitroflavone; COX-2, cyclooxygenase-2; IDO, indoleamine 2, 3-dioxygenase; Kyn, kynurenine; MNF, 3′methoxy-4′nitroflavone; TGFα, transforming growth factor α; EGFR, epidermal growth factor receptor; ETK, endothelial tyrosine kinase; PI3K, phosphatidyl-inositol-3-kinase; ERK, extracellular signal-regulated kinase; IL-8, interleukin 8; CEBPβ, CCAAT/enhancer-binding protein β.

* Corresponding author. Center for Health and the Environment, University of California Davis, One Shields Avenue, Davis, CA 95616, USA. Fax: +1 530 752 5300.
E-mail address: cfvogel@ucdavis.edu (C.F.A. Vogel).

http://dx.doi.org/10.1016/j.pestbp.2014.12.021
0048-3575/© 2014 Elsevier Inc. All rights reserved.

the possibility of AhR's function as a broad-spectrum pro-survival factor for cancer cells to enhance survival via multiple means.

As for the probable cause for such an effect of AhR, while the simplest assumption may be that AhR is making cancer cells more susceptible to dioxin-like environmental pollutants, there is now an increasing body of evidence indicating that the phenomenon of losing ER (estrogen receptor) α is intimately associated with overexpression of AhR. During the course of our previous studies, we found that the increase of AhR expression is closely linked to continuous exposure of breast cancer cells to 1 nM estradiol (E_2) for a number of passages as shown in both MCF-7 [10] and MCF10AT1 [11] cells. Contrary to AhR expression, this long-term exposure to E_2 resulted in the reduction of ERα. Although it has not been fully investigated, it may be based on the fundamental negative relationship between the functions and expression of these two receptors [12].

The current recommendation for first line chemotherapy includes an anthracycline-based (including doxorubicin) and/or taxane-based (including paclitaxel) regimen. Of these two therapies, the anthracycline-based approach appears to be more effective against ErbB2-overexpressing subtypes of breast cancer than the taxane-based therapy, particularly in older patients [13]. Unfortunately, the effective duration typically lasts less than 1 year for over 90% of patients. For those patients with ErbB2 positive breast cancer, trastuzumab (=Herceptin) is considered as one of the most effective treatments. However, this effect is usually not long-lasting because resistance typically develops within 1 year. In addition, it has been found that ErbB2 protein level is frequently elevated in ER-negative breast tumors associated with increased levels of AhR, RelB and interleukin 8 (IL-8) [14]. These results also indicate that the AhR-overexpressing tumors are found more frequently than previously thought.

The primary objective of the present study is the investigation about the role of AhR in the development of breast cancer and the possibility that AhR becomes a therapeutic target of breast cancer treatment. In assessing the influence of AhR we chose 6 different cultured breast cancer cell lines. P35E and P20E were generated by exposure to E_2 from MCF-7 and MCF10AT1 cells, respectively, and express high levels of AhR while P20C and P35C were mock selected control cells [10,11]. MDA-MB-231 and SKBR3 cells also have relatively high level of AhR, and especially SKBR3 cells are known as ErbB2 over-expressing type of breast cancer cells [15]. We also used the AhR antagonist MNF (3′methoxy-4′nitroflavone) in order to elucidate AhR-dependent function of anti-apoptosis in breast cancer cells.

2. Material and methods

2.1. Materials

Doxorubicin, dimethyl sulfoxide (DMSO), E_2, L-kynurenine (kyn) and paclitaxel were purchased from Sigma-Aldrich (St. Louis, MO). 3′-Methoxy-4′nitroflavone (MNF) was kindly provided by Dr. Gabriele Vielhaber (Symrise GmbH & Co.KG, Holzminden, Germany). TCDD (>99.99% purity) was obtained from Dow Chemicals Co. (Midland, MI). Polyclonal antibody against actin of goat origin and polyclonal antibodies against human IDO1 and IDO2 of rabbit origin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Lapatinib was purchased from LC laboratories (Woburn, MA).

2.2. Breast cancer cell lines and culture conditions

The details of E_2 selection process for MCF10AT1 cells (generation of P20E and the mock selected control; P20C) and for MCF-7 cells (generation of P35E and the mock selected control; P35C) have been described previously [10,11]. Briefly, MCF10AT1 and MCF-7 cells were cultured with 1 nM E_2 for 20 and 35 passages in order to establish AhR overexpressing breast cancer cells P20E and P35E, respectively. P20C and P35C cells were generated as the corresponding control cells by culturing each cell line for the same number of passages with the vehicle only. MDA-MB-231 cells were obtained from Dr. Josef Abel. SKBR3 (overexpressing ErbB2, derived from MCF-7 cells) was obtained from American Type Culture Collection (ATCC) (Manassas, VA). Both P20C and P20E cell lines were cultured in phenol red free DMEM/F12 medium (Invitrogen Co., Carlsbad, CA) containing 2.5% fetal bovine serum (FBS) (Invitrogen) treated with charcoal dextran (Gemini Bio-Products, West Sacramento, CA), 20 ng/ml human recombinant epidermal growth factor (EGF) (EMD Chemicals, Gibbstown, NJ), 100 μg/ml penicillin and 100 μg/ml streptomycin (Invitrogen). Serum-free medium for P20C and P20E were DMEM/F12 contains no serum but still included 20 ng/ml of EGF, 100 μg/ml penicillin and 100 μg/ml streptomycin. Both P35C and P35E cell lines were cultured in phenol red free DMEM medium (Mediatech, Inc., Manassas, VA) containing 5% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin. MDA-MB-231 and SKBR3 cell lines were cultured in DMEM medium supplemented with 10% FBS, 100 μg/ml penicillin and 100 ug/ml streptomycin. All cell lines were maintained at 37 °C with 5% CO_2 and medium was changed every 3 days.

2.3. Apoptosis assay on UV-irradiated or apoptosis-inducing agent-treated cells

For UV-irradiation study, cells (5×10^5 cells) were seeded in a 6 cm dish. After 24 h, cells were exposed to TCDD 1 h prior to UV-irradiation with changing the medium. UV was irradiated for 3 min at 3 mJ/cm^2 by using UV Crosslinker (FB-UVXL-1000, Fisher Scientific; emission peak at 254 nm), followed by being incubated for additional 4 h, and the number of cells showing apoptosis was counted. For apoptosis studies each chemical (doxorubicin, lapatinib or paclitaxel) was added to the cell culture at approximately 70% confluence after receiving the last medium change and incubated for additional 24 h. Apoptosis was detected by annexin V staining as described previously [10].

2.4. Knockdown of AhR by siRNA transfection

Knockdown of AhR by siRNA was performed as described previously with slight modifications [16]. Briefly, siRNA specific for human AhR was transfected into P20E and P20C cells in 6 cm dishes by using jetPEI (PolyTransfection; Qbiogene, Irvine, CA). After 24 h, medium was changed, and cells were further incubated for 24 h with fresh medium. The effect of AhR knockdown on apoptosis induction was estimated by annexin V staining as described [10].

2.5. Quantitative RT-PCR (qPCR)

Total RNA was extracted from cells using Quick RNA MiniPrep Kit (Zymo Research Co., Orange, CA). Reverse transcription was conducted with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc., Foster City, CA) according to manufacturer's protocol. Two microliters of each cDNA was mixed with Fast SYBR Green Master Mix (Applied Biosystems) and 10 pmol of each primer pair in a 20 μl total reaction volume, and then real-time PCR was performed using LC480 (Roche, Indianapolis, IN). The primer pairs for each gene were the following: GAPDH FP: 5′-GAGTC AACGGATTTGGTCGT-3′, GAPDH RP: 5′-TTGATTTTGGAGGGATCTCG-3′, COX-2 FP: 5′-TTTGTTGAGTCATTCACCAGACAGAT-3′, COX-2 RP: 5′-CAGTATTGAGGAGAACAGATGGGATT-3′, RelB FP: 5′-TGA TCCACATGGAATCGAGA-3′, RelB RP: 5′-CAGGAAGGGATATGGAAGCA-3′ IDO1 FP: 5′-CAGGCAGATGTTTAGCAATGA-3′, IDO1 RP:

Download English Version:

https://daneshyari.com/en/article/2009089

Download Persian Version:

https://daneshyari.com/article/2009089

Daneshyari.com