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Molecular mechanisms of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced inverted U-shaped dose responsiveness in anchorage independent growth and cell proliferation of human breast epithelial cells with stem cell characteristics

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Abstract

Although 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has a variety of carcinogenic and noncarcinogenic effects in experimental animals, its role in human carcinogenicity remain controversial. A simian virus 40-immortalized cell line from normal human breast epithelial cells with stem cells and luminal characteristics (M13SV1) was used to study whether TCDD can induce AIG positive colony formation and cause increased cell numbers in a inverted U-shaped dose–response manner. TCDD activated Akt, ERK2, and increased the expression of CYP1A1, PAI-2, IL-lb mRNA, and ERK2 protein levels. TCDD was able to increased phosphorylation and expression of ERK2 in same dose–response manner as AIG positive colony formation. Thus, TCDD induced tumorigenicity in M13SV1, possibly through the phosphorylation of ERK2 and/or Akt. Further, cDNA microarray with 7448 sequence-verified clones was used to profile various gene expression patterns after treatment of TCDD. Three clear patterns could be delineated: genes that were dose-dependently up-regulated, genes expressed in either U-shape

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and/or inverted U-shape. The fact that these genes are intrinsically related to breast epithelial cell proliferation and survival clearly suggests that they may be involved in the TCDD-induced breast tumorigenesis.

Keywords: Dioxin; TCDD; Human breast cancer; Anchorage independent growth; Breast tumorigenesis; Inverted U-shaped responsiveness; MAP kinase; Cell signaling

1. Introduction

The International Agency for Cancer Researh on Cancer (IARC) have classified 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a human carcinogen. TCDD is a prototype and the most potent chemical of the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (dioxins). A animal exposure to TCDD can result in various adverse effects which includes carcinogenesis, endometriosis, and immunotoxicicty [1–3]. Developmental neurobehavioral (cognitive) dysfunctions and developmental reproductive (reduction of sperm number, female urogenital malformations) abnormalities are widely reported [4–6]. However, there is an ongoing debate as to the human carcinogenic potentials of the exposure to dioxin [7,8]. Thus, it is important to assess the molecular mechanisms that could mitigate the carcinogenic potentials of TCDD in humans making use of the immortalized human breast epithelial cell line (MCF-10A), human keratinocyte cell line (HaCaT) and endometrial cell line. Immortalized human cell systems have both infinite life span and normal human cell characteristics. Immortalized human cell systems continue to be suggested for use in the screening human carcinogens as well as in the study of their molecular mechanisms [9–12]. As such, these groups of cells may not only facilitate detection of carcinogenicity but provide valuable insights into the carcinogenic process in humans. Our laboratory have established normal human breast stem cells from reduction mammoplasty and human breast immortalized cell line, namely M13SV1, simian virus 40-immortalized cell line from normal human breast epithelial cells with stem cells and luminal characteristics [13,14]. These cells were used to examine MHC expression in a human adult stem cell line and its down-regulation by hCMV US gene transfection [15]. The ability of TCDD to induce tumorigenicity in these non-tumorigenic immortalized human breast epithelial cells was assessed. Indeed TCDD was able to induce anchorage-independent growth (AIG) positive colony formation in M13SV1 cells in an inverted U-shaped dose–response manner, and which correlates with its ability to increase cell number. Further, TCDD was found to increase the expression of CYP1A1, PAI-2, IL-1 mRNA, and ERK2 protein as well as activating Akt and ERK2. More importantly, TCDD increased the phosphorylation and expression of ERK2 in same dose–response manner as AIG positive colony formation.

2. Materials and methods

2.1. Chemicals and reagents

TCDD was purchased from GL Sciences, Inc. (Tokyo, Japan). Rabbit polyclonal Akt and phospho-Akt antibodies were purchased from Cell Signaling Technology. Rabbit polyclonal MAP kinase antibody was purchased from Zymed Laboratories, Inc. (South San Francisco, CA, USA). Rabbit polyclonal phospho-MAP kinase and phospho-p38 kinase antibodies were purchased from Promega Corporation (Medison, WI, USA). Mouse monoclonal p38 kinase, rabbit polyclonal JNK1 and mouse monoclonal phospho-JNKl antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). FBS was from Gibco Laboratory (Carlsbad, CA, USA).

2.2. Cell culture

The immortalized human breast luminal epithelial cell line (M13SV1) generated by transfection of Type I normal human breast epithelial cells (HBEC) from women undergoing reduction mammoplasty with SV40 DNA in a previous study [13] was used. M13SV1 cells were cultured as previously described [14]. Early passage cells were thawed from liquid nitrogen storage and were cultured in MSU-1 medium containing 10% fetal bovine serum (FBS). Cultures were maintained in a 5% CO^2/95% humidified air at 37 °C.