2,3,7,8-TETRACHRODIBENZO-p-DIOXIN-INDUCED AIG POSITIVE COLONY FORMATION IN A BELL-SHAPED DOSE RESPONSE MANNER IS INVOLVED IN MAPK ACTIVATION ON HUMAN BREAST EPITHELIAL CELLS

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototype and the most potent chemical of the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (dioxins). A variety of studies on the toxic effects of dioxin and related compounds have been conducted internationally since 1990. Various adverse effects such as endometriosis, developmental neurobehavioral (cognitive) effects, developmental reproductive (reduction of sperm number, female urogenital malformations) effects and immunotoxic effects, have been reported in animal studies following exposure to TCDD

We have successfully isolated normal human breast stem cells from reduction mammoplasty[1,2] and established human breast immortalized cell lines, namely M13SV1[3]. Even if 2,3,7,8-tetrachlorodibenzo-ρ -dioxin (TCDD) was known as a potent tumor promoter in several experimental animal species[4], the tumorigenic effect and mechanism in human breast has not been clearly understood. Using M13SV1, Simian virus 40-immortalized cells line from normal human breast epithelial cells with stem cells and luminal characteristics, The present study showed that TCDD was capable of inducing tumorigenicity in these non-tumorigenic immortalized cells.

Materials and Methods

Cells were treated with 0.1% DMSO or 0.01, 0.1, 1, 10, or 100 nM TCDD for two weeks and then grown in soft agar for 28 days. Protein was prepare for analyze the expressionspf ERK1/ERK2 and p53 by western blot after treatment with TCDD. mRNA was extracted for examining the expression of TCDD- dependent genes such as cytochrome P450 1A1, plasminogen actiator inhibitor-2, interleukin-1β after treatment with TCDD by RT-PCR.

Results and Discussion

Anchorage independent growth (AIG) of cells was determined by colony formation in soft agar. On 28 day after growing cells in soft agar, TCDD induced the increase of large colonies in dose-dependent manner. One nanomolar TCDD was most effective. However, the AIG+ large colonies diminished in higher concentrations of TCDD (100nM). To identify the responsive gene of TCDD, we performed the reverse transcription and polymerase chain reaction. Cytochrome P450 1A1 (CYP1A1) mRNA was increased in dose-dependent manner and maximum increase of CYP1A1 mRNA was observed at 100nM TCDD. Inductions of plasminogen actiator inhibitor-2 (PAI-2) and interleukin-1β (IL-1β) mRNA were also increased in response to TCDD-treatment. In Western

blot, extracellular signal-regulated kinase 2(Erk2) was increased in the same manner of the result of AIG assay, while the expression of Erk1 and p53 remained in steady level. In this study, we showed TCDD promoted the proliferation and anchorage-independency of the immortalized human breast epithelial cell. These results suggested that TCDD alone might promote neoplastic transformation involving the elevation of Erk2 expression in human breast. It is concluded that TCDD may be a tumor-promoter during human breast carcinogenesis.

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