# Malignant TCDD-transformed Human Cells Exhibit Altered Expressions of Growth Regulatory Factors

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## Abstract

Neoplastic transformation of human cells in culture with exposure to TCDD has recently been reported. In the present study, effects of growth factors and cytokines in the TCDD-transformed cells were analyzed to understand mechanisms of TCDD-induced carcinogenesis of human cells. Transcriptional levels of growth factors and dioxin-responsive genes were compared between parental cells and the transformed cells, using a sensitive RT-PCR technique and western blot analysis. While levels of CYP1A1 mRNA, or Ah receptor protein showed no significant difference between parental and TCDD-transformed cells, altered expression of growth regulartory factors were observed in the transformed cells. Transcriptional levels of TGF-$\beta_1$ or PAI-2 in the TCDD-transformed cells were 4 times or 20 times lower than that of the parental cells, respectively. mRNA level of TNF-$\alpha$ was 3 times higher in the transformed cells than in the parental cells. Taken together, the present study represents a first evidence that altered regulations of growth factors and cytokine are associated with a mechanism of TCDD-induced carcinogenesis of human cells.

## Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of the most potent carcinogens ever tested in animal assays and bioaccumulates in animals and humans[1)]. Despite an increasing evidence on human cancers, mechanisms of TCDD-induced carcinogenesis in humans remains unclear. TCDD forms few DNA adducts and is negative in mutagenicity assays. In addition, TCDD is reported to induce tumor promotion in a fashion similar to TPA, which acts by alteration of growth regulatory genes[2)]. Because these growth regulatory factors are involved in cell proliferation and differentiation, disruption of these pathways by TCDD are suggested to be a plausible mechanism of carcinogenic action[3)]. However, most of these alterations observed shortly after TCDD exposure tend to return to normal when the chemical exposure is ceased. Thus, it has been difficult to know whether the alterations of growth regulartory factors observed right after the exposure play a role in developing the

malignancy, which requires fixation of altered gene expression through clonal expansion of the damaged cell.
The present study used the TCDD-transformed cells as a cellular model to study roles of growth regulartory factors in TCDD-induced carcinogenesis.

## Experimental Methods

Cell cultures and treatment: The parental cell line was established from human foreskin epidermal keratinocyte with infection of Ad12-SV40 hybrid virus[4]. The TCDD-transformed cell line was obtained from the parental cell line after 2 week exposure of TCDD and 6 subsequent subcultures[5]. Maintenance media consist of DMEM with 10 % FBS, hydrocortisone( 5μg/ml), penicillin G (50u/ml) and streptomycin (50μg/ml). Both cell lines were treated with 0.1, 1, 10, 100 nM or 0.1% DMSO for 24hrs, respectively.
RT-PCR analysis: Total RNAs were prepared with a RNA isolation kit. cDNA was synthesized with reverse transcriptase and an aliquot of the synthesized cDNA was used as a template for PCR as described previously[6]. Analysis of radiolabeled PCR products were performed by the image analyzer (Bio Rad, U.S.A.). The densitometric values of mRNA were normalized to GAPDH.
Western blot: Proteins (25 μg) of the lysates from the parental cells or the transformed cells were prepared on 8 % SDS-PAGE. The nitrocellulose sheet was blocked with 3 % non-fat dry milk in Tris-buffered saline. Affinity purified polyclonal antibody to Human AHR ( a generous gift from Dr. Hankinson, U.S.A.) was used as a primary antibody. The AHR antibody bound to the protein on the nitrocellulose sheet were detected with goat anti-rabbit IgG conjugated with peroxide, using the ECL system of Amersham Life Science.
Statistical analysis: Student *t*-test was used to compare individual means. The level of significance was $p < 0.05$.

## Results and Discussion

The present study has attempted to determine if alteration of growth factors and cytokines are associated with TCDD-mediated carcinogenesis by analyzing human epithelial cells malignantly transformed by TCDD. RT-PCR analysis of the transformed cells showed a 20 fold decrease of PAI-2 mRNA level, as compared to the parental cells. When the transformed cells were treated with various concentration of TCDD for 24 hrs, there was no significant increase of PAI-2 mRNA level over the doses. However, treatment of the parental cells with TCDD revealed dose-dependent increase of PAI-2 mRNA(Fig. 1.). Thus, it seems that loss of PAI-2 inducibility through clonal expansion rather than immediate response after the exposure is one of genetic events responsible for the transformation. Regulation of PAI-2 expression is closely implicated with tissue degradation and cancer progression[7]. However, link between carcinogenesis and TCDD-mediated regulation of PAI-2 expression remains unclear. A 20 fold reduction of PAI-2 expression in the TCDD-transformed cells suggests that downregulation of PAI-2 expression may be involved in the transformation process. It is plausible that imbalanced control of u-PA / PAI-2 system resulted from downregulation of PAI-2, which leads to alteration of PA/plasmin system, may contribute to phenotypic changes of the parental

cells. TCDD is known to increase TNF-α production and its toxicity is modulated by TNF-α in TCDD-treated mice[8]. TNF-α stimulates the growth of normal cells as well as certain cancer cells in culture. Its expression could contribute tumor progression and spread[9]. However, a role of TNF-α in TCDD-induced malignant transformation of human cells remains unknown. RT-PCR analysis showed that mRNA level of TNF-α in the transformed cells was 3 times higher than that of the parental cells. However, neither the parental cells nor the transformed cells treated with TCDD for 24 hrs showed any change of TNF-α expression (data not shown). This finding suggests that TNF-α is not a TCDD-responsive gene in the current cellular system, but its modulation as a result of clonal expansion of the damaged cells may be responsible for the transformation.

Members of TGF-β family are potent and reversible inhibitors of epithelial proliferation, including keratinocytes. Modulation of TGF-β expression affects homeostasis of epithelia and is also associated with tumorigenesis[10]. While transcriptional level of TGF-$\beta_1$ was 4 times lower in the transformed cells than in the parental cells (Table 1.), the treatment of these cells with TCDD for 24 hrs did not affect mRNA level of TGF-$\beta_1$ (data not shown). The result suggests that while responses after short-term exposure may not be directly associated with cell transformation, substantial decrease of inhibitory action of TGF-$\beta_1$ following successive cell division may cause uncontrolled proliferation of epithelial cells leading to tumorigenesis. Constitutive mRNA levels of CYP1A1 gene were similar between the parental cells and the transformed cells (data not shown). When both cell lines were analyzed for CYP1A1 induction after 24 hr exposure of TCDD, inducibility of the gene was greatly dampened in the transformed cells (Fig. 2.). In contrast to a reduced inducibility of the gene in the transformed cells, western blot analysis showed that levels of Ah receptor are similar between these two cell lines ( Table 1.). This finding suggests that functional changes of Ah receptor following clonal expansion may play a role in the altered expression of growth regulartory factors observed in the present study.

The present study indicates that, since changes of cytokines and growth factors observed right after short-term exposure are not fixed in the genom following successive cell division, their changes may help mediate activation of another set of genes responsible for the transformation via yet unknown mechanism. Our results revealed that alteration of cytokines and growth factors such as PAI-2, TNF-α and TGF-β are associated with TCDD-induced carcinogenesis of human cells and provides additional evidence that dysregulation of growth regulartory factors could be a plausible mechanism of TCDD-induced carcinogenesis.. Multiple cell division is an essential component of chemical carcinogenesis. Without replication, DNA damage indirect or direct would not be fixed in the genome and the clonal expansion of gentically altered cells would not occur. Thus, altered expression of growth regulartory factors following the clonal expansion of the TCDD-damaged human cells will help improve our understanding on the mechanism of TCDD-induced carcinogenesis in human cells..

## Acknowledgments

This work was supported by grants from the Korean Science and Engineering Foundation # 951-0709-096 and the Ministry of Environment in Korea.

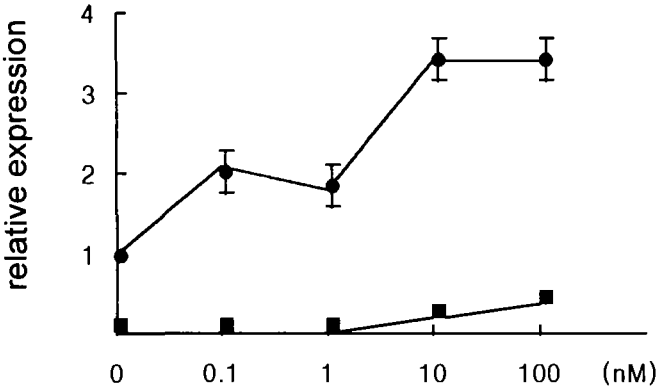


Fig 1. Alterations of PAI-2 expression in the parental cells ( ● ) and TCDD-transformed cells( ■ ) treated with various concentration of TCDD for 24hr. The data are means + SD from three different experiments

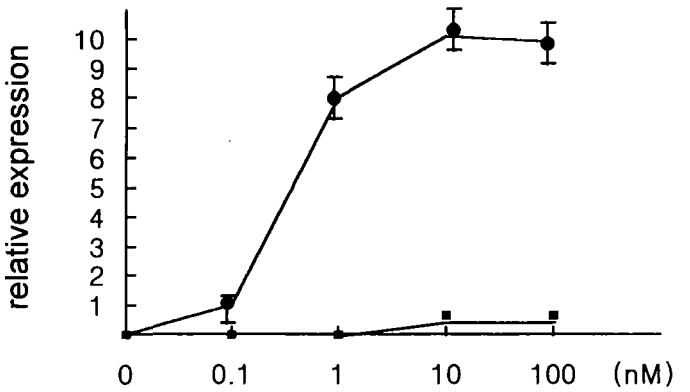


Fig 2. Relative expression of CYP1A1 in the parental cells( ● ) and TCDD-transformed cells( ■ ) treated with various concentration of TCDD for 24hr. The data are means + SD from three different experiments

Table 1. Alterations of growth factors and AHR in the parental cells and the TCDD-transformed cells. Densitrometric tracings were normalized to GAPDH. Values are means ± SD from three separate experiments.

| Cell line | TGF-$\beta_1$ | TNF-$\alpha$ | AHR |
|---|---|---|---|
| parental | 8524 ± 180 | 622 ± 257 | 6383 ± 54 |
| Transformed | 2105 ± 3.5* | 1754 ± 120* | 6411 ± 38 |

* $p < 0.05$

## References


(1) Yang, J.H.; Rhim, J.S. *Crit. Rev. Oncol. Hemato*l. 1995, **18**, 111-127.
(2) Poland, A.; Glover, E. *Cancer Res.* 1976, **39**, 3341-3344.
(3) Huff, J.; Lucier, G.; Tritscher, A. *Annu. Rev. Pharmacol. Toxicol.* 1994, **34**, 343-372.
(4) Rhim, J.S.; Arnstein, P.; Price, F.M.; Sanford, K.K.; Aaronson, S.A. *Science*, 1985, **227**, 1250-1252.
(5) Yang, J.H.; Thraves, P.T.; Dritshilo, A.; Rhim, J.S. *Cancer Res.* 1992, **52**, 3478-3482.
(6) Doehr, O.; Vogel, C.; Abel, J. *Arch. Biochem. Biophys.* 1995, **321**, 405-412.
(7) Ishikawa, N.; Endo, Y.; Sasaki, T. *Jpn. J. Cancer Res.* 1996, **87**, 480-487.
(8) Clark, G.C.; Taylor, M.J.; Tritscher, A.M.; Lucier, G.W. *Toxicol. Appl. Pharmacol.* 1991, 422-431.
(9) Malik, S.K.; Griffin, D.B.; Fiers, W.; Balkwill, F.R. *Int. J. Cancer*, 1989, **44**, 918-925.
(10) Massague, J. *Annu. Rev. Cell. Biol.* 1990, 597-641.