# CARCINOGENICITY OF DIOXIN IN HUMAN *IN VIRTO* SYSTEMS; AN OVERVIEW

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## Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin(TCDD) has been known to be an extraordinarily potent tumor promoter and a complete carcinogen in rodent bioassay [1,2]. Recently, IARC classified this compound as a human carcinogen. However, human carcinogenicity from dioxin exposure still remains controversial and challenged. Since the nature of human subject studies is pretty complicated and epidemiological studies often have uncontrollable compounding variables, the classification of this compound as a human carcinogen may be in dispute, at least, for the time being.

In an effort to assess the carcinogenic potential of TCDD in humans and understand its mechanism, immortalized human cell systems have recently been used. Since Immortalized cell systems give the cells an infinite life span, which facilitates a detection of carcinogenicity, these cellular systems have been used for examining carcinogenic potentials.

## History

More than 40 years ago, Shein and Koprowski demonstrated for the first time that SV40, a DNA tumor virus of polyomavirus family, could morphologically transform human fetal and adult skin firboblasts, respectively [3,4]. Subsequently, Girardi was successful in obtaining the progenitors of immortal cell line from the SV40-transformed human fibroblasts[5]. Initially, the transformation of mammalian cells by SV40 was thought to require expression of the early region of the viral genome, which encodes two proteins, large T-antigen (94kd) and small t-antigen (17kd)[6]. It is now known that the large T-antigen is responsible for the immortalization of rodent and human cells. Human cells infected with SV40 express Large T antigen, develop an altered morphology and a reduced serum requirement for growth and acquire a extended lifespan following the senescence. Other DNA tumor viruses such as HPV16, 18 and adenovirus are also used for immortalization steps[7]. Some chemical carcinogens or x-ray after a lengthy exposure only are reportedly responsible for the immortalization[8], but are usually unsuccessful.

The immortalized human cell lines demonstrated their usefulness as a tool to dissect biological events leading to cancer, following the exposures of biological or chemical carcinogens. The initial finding on chemical carcinogen-induced neoplastic transformation of human cells was reported from the non-tumorigenic human osteosarcoma (HOS) cell line[9]. The MNNG or 3-MC-transformed human cells showed *ras*-activation on NIH3T3 assay, providing an initial evidence that a carcinogenicity of chemical carcinogens can be reproduced in a human *in vitro* system[10, 11]. Efforts to investigate neoplastic conversion of normal human cells by carcinogenic agents have been aided by the development of various tissue culture systems. Such efforts have recently been expedited with an advent of retroviral vector system, which provides a high efficiency of gene transfer. Several in vitro multistep models derived from the various human tissues, with various

immortalizing agents, are now available for the study of human cell carcinogenesis[12]. The recent development of immortalized human cell lines from different tissues may provide a basis for understanding tissue specificity of TCDD in humans at the molecular level and aid in explaining the molecular mechanisms involved in carcinogenesis.

Since suitable models for examining the carcinogenic potentials of TCDD in humans are very limited, recent efforts to demonstrate a carcinogenic potential in human cell systems are introduced in this report. In particular, an initial finding on neoplastic transformation of human cells with exposure to TCDD and possible mechanisms involved are highlighted.

**Neoplastic transformation of human cells**

Yang et al. reported neoplastic transformation of human epidermal cells in culture with exposure to TCDD[13]. This was a first finding on the neoplastic conversion of immortalized human cells with exposure to TCDD. In an attempt to obtain the established cell line, primary epidermal keratinocytes derived from human foreskin were infected with Ad12-SV40 hybrid virus. Subsequently, those primary cells acquired infinite life span in culture but did not undergo malignant transformation and became an established cell line. This cell line had a number of epithelial cell markers and contained the SV40 T antigen. These cells were non-producer of virus, had 'flat' epithelial morphology and were non-tumorigenic in nude mice tumorigenicity assay.

When primary epidermal keratinocytes were treated with TCDD, no evidence of transformation phenotypes such as foci formation was observed. These primary cells could not grow beyond two or three subcultures and showed a marked increase of differentiation. However, when immortalized cells were treated with the same concentrations of TCDD, morphological transformation and growth alterations were observed in a dose-dependent manner. Control cells and cells treated with 0.03nM of TCDD did not show any changes of morphology. In contrast, cells treated equal to or above 0.3nM began to form small projections, pile up in focal area and release round cells from the foci. In nude mice tumorigenicity assay, animals inoculated with these transformed cells developed a squamous cell carcinoma. Karyotype analysis of cultures established from this tumor confirmed that tumor was derived from TCDD-transformed human cells.

**Possible Mechanisms**

As compared to the control cells, stimulation of second messengers such as intracellular free calcium and $IP_3$ in response to histamine or ATP was significantly lower in the transformed cells. The transformed cells did not show as much accumulation of cAMP as the control cells with exposure to $PGE_2$. It is suggested that down-regulation of PLC-coupled signaling pathway or desensitization of cAMP-mediated responses to extracellular signals may be involved in the transformation process. In addition, a variety of PKC isoforms were elevated in the transformed cells[14].

mRNA levels of growth regulatory factors such as PAI-2, TGF-$\beta_1$, and TNF-$\alpha$ were significantly altered in the transformed cells. In particular, constitutive levels of PAI-2 mRNA was 25 times lower in the transformed cells. Altered expression of theses growth regulatory factors may be some of the genetic events fixed in the genome following the successive cell divisions of TCDD-damaged cells[15].

Microarray analysis revealed the altered patterns of gene expressions associated with signal transduction, cytoskeletons, or growth regulatory factors, suggesting the further studies on these genes. Altered expression of oncogenes was detected but was not as much as expected. Ras, p21, was significantly lowered in the transformed cells. It is interesting to note that cleft palate-

asscociated transmembrane protein 1 was elevated more than 100 times in the transformed cells. Further studies on this area are required in the future.

A significance of this study is to provide a unique opportunity to observe a role of TCDD in human cell carcinogenesis. Combined action of Ad12-SV40 hybrid virus and TCDD in neoplastic transformation indicates that TCDD acts as a tumor promoter in this human cell system. Delayed appearance of growth alterations in TCDD-treated cells suggests that multiple cell divisions are required for fixation and expression of the transformed phenotypes in response to TCDD. It is possible that more than one genetic lesion may be required as well. Cooperating cellular or viral oncogenes have been known to induce malignant transformation of embryonic rodent fibroblasts[16,17]. In addition, the combined action of tumor virus and chemical carcinogens has been demonstrated to produce neoplastic transformation[18]. Therefore, malignant transformation of Ad12-SV40 altered human epithelial cells by TCDD provides an additional support to the role of viral-chemical interactions for the neoplastic transformation

**Other immortalized human cell systems**

Tumorigenic potentials of TCDD have been also observed in other immortalized human cell lines. A human keratinocyte cell line spontaneously immortalized from the skin, HaCaT, with 2 week exposure of TCDD showed a significantly increased level of micronucleus formation. In addition, Chang's liver cell immortalized with SV40 also demonstrated an increase of micronucleus formation as well as foci formation, following 2-week exposure of TCDD. These results indicate a potential of TCDD to induce numerical chromosomal abnormality in human cells in culture.

SV40-immortalized human breast epithelial cell line, M13SV1, was also susceptible to an increase of the anchorage-independent growth, following TCDD exposure[19].

**Discussion**

While widely accepted in animals, carcinogenicity of TCDD remains disputable in humans, due to the lack of suitable models to compare the interspecies differences. The transformation studies of human cell models may provide a clue that humans are not different from animals, at least, at the cellular levels in terms of carcinogenic potentials of TCDD. Considering that a clonal expansion following a single cell genetic legion is one of possible mechanisms of human carcinogenesis, demonstration of TCDD carcinogenicity at the human cellular levels strongly suggests that this compound may have a carcinogenic potential in humans. In addition, these studies may add a biological significance to the epidemiological findings that have demonstrated carcinogenicity of TCDD in humans. While the results of epidemiological studies often remained equivocal, these human cell studies clearly demonstrated the carcinogenicity of this controversial compound. Thus, immortalized human cell systems may be a useful tool for screening human carcinogens as well as studying their mechanisms.

**References**


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