Characterization of Transformed Human Keratinocyte Cell line by TCDD

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TCDD, one of the most toxic man-made compounds, bioaccumulates in animals and humans and is widely dispersed in the environment. Although TCDD is a powerful carcinogen in several species, limited model systems exist to study carcinigenicity of this compound at the cellular level. TCDD acts as a promoter on mouse embryonic fibroblasts C3H10T1/2 and rat tracheal epithelial cells initiated with MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). So far, there has been no report of its carcinogenic potentials in human cells in culture. The present study attempted to study the carcinogenic potential of TCDD in human cells and the mechanisms of carcinogenesis.

We used a nontumorigenic, immortalized human epithelial keratinocyte cell line (RHEK-1). The RHEK-1 cell line was established from primary foreskin epidermal keratinocytes after infection with the Ad12-SV 40 hydrid virus[1]. Since TCDD effects are target organ and species- specific and one of the sensitive target organ in humans is skin, use of this particular cell system offers an advantage in evaluating human toxicity to TCDD.

Growth and maintenance medium for RHEK-1 cells consisted of DMEM with 10% fetal bovine serum, hydrocortison (5ug/ml), penicillin G (50u/ml) and streptomycin (50ug/ml). Primary epithelial keratinocytes were grown in keratinocyte growth medium (Clonetics, San Diego, CA). TCDD was obtained from KOR biochemical (Cambridge, MA). TCDD was dissolved in DMSO and aliquots (100uM) were stored at -70^{0}C. Various concentrations of TCDD were prepared by direct dilution of aliquot into appropriate media. All media including control contained a final concentration of less than 0.1% DMSO.

One day after primary human keratinocytes or RHEK-1 line were plated at 10^6 cells per T-75 Falcon plastic flask, the medium was removed and replaced with medium containing the various ranges of TCDD in DMSO. After cells were exposed for 2 weeks, the cultures were washed, fed again with TCDD-free medium and passaged by trypsin treatment every 10 to 14 days. Cultures were observed biweekly for changes in morphology and growth patterns.

After RHEK-1 cells were treated with TCDD for 4 days or 2 weeks, ethoxyresorufin - O-deethylase (EROD) were measured as described by Hebert et al[2].

When primary human epithelial cells were exposed to TCDD, no evidence of transformation phenotypes was observed in treated and non-treated cells. The cells progressively deteriorated and died after 2 or 3 subcultures (data not shown). In contrast, RHEK-1 cells exposed to 0.1 nM or above for 2 weeks showed apparent morphological changes of cells and an abnormal pattern of growth after the 5th to 7th subcultures, 70 to 90 days after treatment. While the morphology of control cells remained unchanged, the transformed cells began to pile up in focal areas, form small projection and release round cells from the foci. These transformed

cells were characterized by the quantitative difference in growth properties, such as saturation density and soft agar colony-forming efficiency associated with the neoplastic phenotypes. The saturation density of transformed cells was 1.5 to 2 times higher than untransformed cells. In addition, these transformants grew in soft agar with colony-forming efficiencies of 0.04 to 0.2 percent, whereas the untransfomed cells did not grow in soft agar (Table 1).

When athymic nude mice were subcutaneously inoculated with 10^7 TCDD-transformed cells, the animals developed tumors within 8 weeks. These tumors were diagnosed as squamous cell carcinoma and confirmed as human origin by karyological analysis. In contrast, subcutaneous injection of 10^7 untransformed cells into nude mice produced regressing cystic nodules containing epidermal cells[3].

Induction of cytochrome p-450 has been suggested as a surrogate to measure sensitive responses to dioxins in all apecies. In particular, induction of EROD activity has been used as a marker for TCDD responsiveness and CYP 1A1 induction. In the present study, concentration-dependent induction of EROD activity by TCDD was observed in this particular cell line (Table 2). Data indicate that RHEK-1 cell line is responding to TCDD in a similar way to other responsive cell lines such as SCC lines. There was no significant difference in induction level of EROD activity between 4 day and 2 week exposure (Table 2). This indicates that induction of EROD activity in this particular cell line is not a sensitive marker in assessing effects by the long-term exposure.

The present study suggests that at least two and possibly more alterations in cell growth properties is required for the malignant transformation. A measurable event was the acquisition of unlimited growth potential as a result of Ad12-SV40 infection. Treatment of immortalized cells with TCDD resulted in further changes in their properties. Morphological alterations as well as the ability to grow in soft agar and to form squamous carcinoma in athymic nude mice appeared to be concomitantly acquired properties of the TCDD-transformed cells.

It is reported that TCDD induces transforming growth factor-alpha in human keratinocytes and its overproduction may play a crucial role in tumor-promoting action of TCDD[4]. Thus, the present study attempted to compare the secreted amount of TGF-a in the culture medium and its m-RNA levels in the parental cells and tumor cells established from the nude mice. There was no significant increase of the secreted TGF-a in the culture medium of tumor cells and its m-RNA levels in the tumor cells, as compared to the parental cells (data not shown). Elisa assay for mutant p53 (Oncogene Science, NY) showed no significant differences between the parental RHEK-1 cells and TCDD-induced tumor cells, indicating that p53 mutation may not be a major event in this transformation. Further studies on other oncogenes and growth factors are in progress.

The induction of transformation and EROD activity in higher dose groups strongly suggests that this cell line has the Ah receptor and that the transformational events may be a receptor-mediated process.

Considering that interspecies differences in response to TCDD are an especially important factor in evaluating human risks from experimental animals, the use of human epithelial cells, from which most of cancer origins are derived, may add further significance of the present findings to risk assessment. Since this cell line is responding to TCDD and, in particular, can be neoplastically transformed by this copound, this in vitro system provides a valuable tool to study mechanisms of TCDD-induced carcinogenesis in human cells.

Table 1. Biological properties of the RHEK-1 human epidermal line transformed by TCDD.

TCDD(nM)	Saturation density[a] (x105/cm2)	Soft agar colony[b] formation	Animals with tumors/[c] Animals inoculated
control	2.3	0.01	0/4
0.03	2.1	0.01	0/4
0.1	3.8	0.04	4/4
0.3	4.9	0.19	4/4[d]
1.0	4.9	0.21	4/4
3.0	4.5	0.16	N.D.

[a]Saturation density was measured as the maximum number of cells obtained after initial plating with $5x10^3$ cells /cm^2 followed by incubation at 37^0C with growth medum changed every 3 days.

[b]Cell suspension ($1x10^4$cells/ml) in 5ml of 0.35% noble agar was overlaid in a 60-mm dish containing a 0.6% agar base. Viable colonies were scored at 21 days.

[c]Nude mice were inoculated with 10^7 cells

[d]Tumor were re-established in tissue culture and confirmed as human. Their resemblance to the cell of origin was determined by karyological analysis.
N.D. = not done

Table 2. Induction of EROD activity in RHEK-1 cell line treated with TCDD.

TCDD (nM)	Exposure for 4 days (pmol/min/mg)	Exposure for 2 weeks (pmol/min/mg)
control	18 ± 2.34	16 ± 1.85
0.03	5 ± 1.40	9 ± 2.51
0.3	42 ± 4.54	38 ± 3.22
3	96 ± 7.50	94 ± 6.97
30	216 ± 17.28	203 ± 18.40
300	120 ± 11.78	170 ± 12.52

References

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