TCDD ENHANCES TGF-α-INDUCED TRANSFORMATION OF HUMAN ENDOMETRIAL CELLS IN CULTURE

Jae-Ho Yang and Sun-Young Kim

Department of Pharmacology and Toxicology, School of Medicine, Catholic University of Taegu-Hyosung, Taegu, Republic of Korea.

Introduction

Endometrial effects following exposure to environmental pollutants such as dioxins and PCBs have been a great public health concern. Incidence of endometriosis is associated with urban life in the industrialized area and the trend coincides with the increased production of PCBs and the related dioxin production since World War II[1)]. Recently, a link between TCDD exposure and severe endometriosis in rhesus monkey has been reported[2)]. While TCDD is believed to be associated with reproductive diseases such as endometriosis, there has been no data linking TCDD exposure with endometrial cancer. Since endometrial cancer can be developed from the endometriosis[3)], it is interesting to look into roles of TCDD on developing endometrial cancer. The present study has attempted to examine a possible role of TCDD in the neoplastic transformation of human endometrial cells in culture. The study showed that 4-week exposure of TCDD enhanced the TGF-α-induced transformation of the human endometrial cells.

Materials and Methods

Cell cultures; Human endometrial cells immortalized with temperature-sensitive SV40 T-antigen (a generous gift from Dr. Kaufman , Univeresity of North Carolina at Chapel Hill) were sub-cultured in our laboratory and subcultures at

passage 54 were used for the present study. Cells were maintained in 5% CO_2 incubator at 33 ^{o}C. Culture media consist of the 1:1 mixture of F-12 and I-199 with 1% FBS, glutamine, insulin and antibiotics.

Construction of retrovirus vector; The retrovirus vector was constructed to overexpress TGF-α in the human endometrial cells, as described previously[4)]. Briefly, pT7T3 vector carrying TGF-α was digested with Sma I and Sau3A to obtain TGF-a fragment. After the polyadenylation site of TGF-α cDNA fragment was removed, the fragment was inserted into Sac I and Hind III site of pGEM-11zf. pLNCX retrovirus DNA plasmid was digested with Cla I and Hind III to place TGF-a fragment under control of CMV promoter. Purified TGF-α fragment and pLNCX were ligated to make pLNCX-TGF-α.

TGF-α-induced transformation; Cells were infected with retrovirus vector carrying human TGF-α gene and then selected by G418. The status of TGF-α overexpression was identified by RT-PCR analysis. Changes of contact inhibition, anchorage-independence and cellular adhesion were evaluated to determine characteristics of neoplastic transformation. These transformation properties were measured by saturation density, soft-agar colony formation, and cell aggregation, respectively, as described previously[5,6)].

Chemical treatment; TCDD was obtained from KOR Biomedical (Cambridge, MA). TCDD was dissolved in DMSO and aliquots (100 μM) were stored at -70 ^{o}C. Both the parental and the TGF-α-transformed cells were exposed to DMSO (0.1%), 0.1 nM, 1 nM, or 10 nM TCDD for 4 weeks. Changes of transformation properties following the treatment were measured as described above.

RT-PCR analysis; Total RNAs were prepared from both the parental and the transformed cells. Overexpression of TGF-α mRNAs was evaluated by RT-PCR as described previously[7)].

Results and Discussion

TGF-α is one of dioxin-responsive genes in human keratinocytes and other cell systems[8]. It is suggested that TGF-α may play a role in the mechanism of TCDD-induced carcinogenesis[9]. When human endometrial cells were infected with retrovirus vector to overexpress TGF-α, there were significant phenotypic changes associated with neoplastic transformation. Properties of saturation density, soft-agar colony formation and cellular aggregation were significantly elevated (table 1.). The results suggest that overexpression of TGF-α may play an important role in the mechanism of endometrial carcinogenesis. RT-PCR analysis confirmed the overexpression of TGF-a mRNAs in the transformed cells (data not shown). In contrary to the human keratinocytes[10], when endometrial cells were treated with a variety of TCDD concentrations, neither the parental cells nor the transformed cells showed the induction of TGF-α mRNAs (data not shown). It is suggested that TGF-α may not be directly associated with the TCDD mechanism of action in the present cellular system. Thus, further studies under the different experimental designs may be warranted to confirm a lack of the inducibility of TGF-α mRNAs in this cell system. While TCDD did not induce the phenotypic changes of the parental cells, the TGF-α-transformed cells following the exposures to TCDD showed significant increases of neoplastic transformation properties (table 1.). Dose-dependent increases of soft-agar colony formation were observed in the transformed cells and significant increase of saturation density was shown at the highest dose. However, there was no significant changes of cell aggregation. The results suggest that, while TCDD may not be directly involved in the transformation of human endometrial cells, long-term exposures with high doses may contribute to promoting the development of endometrial cancer. Our findings seem consistent with other reports demonstrating promotional effects of TCDD on the genetically altered cells[9]. While a link between TCDD exposure and human endometrial cancer is limited at present, the present study demonstrated an evidence that TCDD may be involved in the development of

human endometrial cancer via a promotional mechanism. The study will provide valuable scientific basis to study further roles of TCDD as a possible promoter in human endometrial cancer.

Table 1. Properties of parental cells and TGF-α-transformed cells following 4-week TCDD exposures.

	Saturation density (10^5/cm^2)	Soft-agar colony formation (%)	Cell aggregation (size >0.5 mm)**
Parental cells only	1.1 ± 0.02	< 0.01	-
+ DMSO (0.1%)	1.4 ± 0.02	< 0.01	-
+ 0.1 nM TCDD	1.3 ± 0.05	< 0.01	-
+ 1.0 nM TCDD	0.9 ± 0.02	< 0.01	-
+ 10 nM TCDD	1.6 ± 0.04	< 0.01	-
Transformed cells only	3.1 ± 0.15	0.11± 0.02	+
+ DMSO (0.1%)	2.8 ± 0.12	0.15 ± 0.04	+
+ 0.1 nM TCDD	3.0 ± 0.23	0.13 ± 0.01	+
+ 1.0 nM TCDD	4.3 ± 0.18	0.23 ± 0.08*	+
+ 10 nM TCDD	5.2 ± 0.25*	0.28 ± 0.06*	+

*; $p < 0.05$, as compared to the transformed cells

**; - ; < 5 colonies, + ; ≥ 5 colonies

The data are mean ± SD with 3 different counts.

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