EFFECTS OF TCDD ON GJIC AND CELL GROWTH IN v-*ras*-TRANSFECTED RAT LIVER EPITHELIAL CELLS

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Introduction

As for a mechanism of high-responsiveness in skin tumorigenesis of Tg/AC mice to TCDD[1], we hypothesized that GJIC in the tissue expressing v-*ras* is lower than the normal tissue, as reported in case of v-*ras*-transfected cells[2,3], and TCDD might induce possible loss of GJIC[4], which may be associated with induction of *ras* gene[5], and then result in encouraging the cell growth together with activating a mitogenic signal, a ras-MAPK pathway[6]. To test this hypothesis, we examined *in vitro* the stimulatory effects of TCDD on the cell growth of the v-*ras* transfected rat liver epithelial cells (WB-RAS cells)[3] and compared with the wild-type cells (WB cells)[7], together with the analyses on the status of gap junctional intercellular communication (GJIC) and activation of the *ras*-MAPK pathway.

Materials and methods

Chemicals used in this study are shown below; TCDD (Cambridge Isotope Laboratories, Inc., Andovr, MA), D-medium (Formula No. 78-5470EF, GIBCO, Grand Island, NY), Fetal bovine serum (GIBCO, Grand Island, NY), Lucifer yellow (Molecular Probes, Inc., Eugene, ORE), Monoclonal anti-Cx43 (Chemicon International Inc., Temecula, CA), Monoclonal anti-pan-Ras (Oncogene Research Products, Cambridge, MA), Monoclonal anti-p-ERK & total-ERK (New England Biolabs. Inc., Beverly, MA)

Cells: Rat liver-derived WB cells[7], which is confirmed to express AhR by RT-PCR, and a v-ras-transfected WB clone (P9), which is morphologically transformed and show low GJIC, are used. Confluent cells were treated with TCDD (1 to 10 nM) for up to 72 h in growth media (D-medium containing 5 % FBS) at 37°C under a humidified atmosphere containing 5 %CO_2 and 95 %.

Materials and Methods

Activation of the *ras*-MAPK pathway and inhibition of GJIC and the cell proliferation by TCDD in the normal rat liver epithelial cells (WB cells) (Experiment 1) and comparison of the effect of TCDD on the cell growth of v-*ras* transfected rat liver epithelial cells (WB-RAS cells) and the wild WB cells, associated with a lower GJIC and a higher activity of MAPK (Experiment 2) were performed. Scrape-loading dye transfer (SL/DT) method was used to monitor the GJIC in culture cells. Cell growth was monitored by DNA content (OD260nm) of cell lysates in 0.1 N NaOH. Ras content was monitored by Western blotting, 1:200 dilution, 4°C O.N., ERK-activation by Western blotting; 1:1000 dilution, 4°C O.N., and modification of Cx43 Western blotting; 1:1000 dilution, 4°C O.N.

Results

Exp.1 Effects of TCDD on the GJIC and the cell growth of the WB cells; a possible involvement of Ras

1) Time-course and dose-dependent inhibition of GJIC

A transient inhibition of GJIC by 10μM TCDD was observed within 24 h, but the GJIC restored afterwards (48 h - 72 h). A dose-dependent inhibition of GJIC was observed in 24 h.

2) Cell proliferation

The DNA content was increased in the TCDD-treated cells after 48 h.

3) Induction of Ras

The increase in Ras protein level was observed after 24 h-incubation with TCDD, and the dose-dependency was evident at 72 h. The Ras-induction was not associated with the time course of the GJIC-inhibition, but paralleled with the cell growth

4) ERK-activation

A steady activation of ERK was observed from 24 h after the TCDD-treatment, and a dose-dependency was evident at 72 h, associated with the Ras-induction.

5) Cx43 expression

The up-regulation of the total Cx43 level was observed from 24 h-incubation with TCDD, associated with the increase of higher phosphorylated Cx43.

Conclusion of Exp 1.:

These findings suggest that the activation of Ras-MAPK pathway may be involved in the cell-proliferation mechanisms by TCDD, but not in the GJIC-inhibition.

Exp.2. Specific effects by TCDD on the GJIC and the cell growth of the WB-RAS (P9) cells; a role of constitutive lower GJIC and higher MAPK-activity

1) GJIC

Associated with a morphological change of WB-RAS(P9)cells to the original WB-RAS, a slight recover of GJIC, up to the level of the original WB-RAS cells, was observed by TCDD-treatment. However, the level of GJIC was steadily lower than that of the WB cells.

2) Cell proliferation

The DNA content in the WB-RAS(P9)cells was elevated much more than that in the WB cells at 72 h after the TCDD-treatment.

3) Induction of Ras

Induction rate of pan-Ras in WB-RAS (P9) cells was not different from that in WB-cells after the TCDD-treatment for 72 h.

4) ERK-activation

The constitutive activity of ERK was higher in the WB-RAS(P9) cells compared with the WB cells, and an additive activation of ERK was observed after the TCDD-treatment for 72 h.

5) Cx43 expression

A slight induction of Cx43 in the WB-RAS(P9) cells was observed, but the phosphorylated forms of Cx43 were not induced by the TCDD-treatment for 72 h, while a significant induction of Cx43 associated with an increase of the phosphorylated types was evident in the WB cells.

Conclusion of Exp. 2:
These findings suggest that TCDD may stimulate the cell growth of v-ras transfected cells specifically, in which mechanisms the constitutive lower GJIC and higher MAPK-signaling might be involved. This result also showed the different effects of MAPK on the modification of Cx43 between the normal and the v-ras-transfected cells.

Overall conclusion
Findings obtained in this study suggest that 1)*ras*-MAPK pathway may not be directly involved in GJIC-inhibition, but may contribute to the mitogenic action by TCDD, and 2) both constitutively lower GJIC and higher mitogenic signal may contribute to the growth-expansion of the cells expressing *v-ras*. This *in vitro* model may further provide useful information on the mechanism of high-responsiveness of Tg/AC mice to TCDD.

References

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