Analysis of gene expression in lung and thymus of TCDD treated C57BL/6 mice using differential display RT-PCR

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1. Introduction

Many effects associated with exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) are believed to occur through Ah-receptor mediated changes of gene expression in responsive cells. Most of the genes yet known to be affected by TCDD are upregulated and can be subdivided by function in two major classes: 1. drug metabolizing enzymes of phase I and II and 2. growth factors and cytokines[1,2]. Altered expression of these genes was mainly studied in in vitro systems. Less is known about cell specific factors mediating tissue specific responses following receptor binding in vivo.

We used the PCR based differential display technique[3] to isolate new candidate genes which are involved in mediating adverse effects of TCDD on lung and thymus of female C57BL/6 mice. This method, unlike substractive hybridization simultaneously identifies up- and downregulated genes in the same experiment.

2. Methods

Animals and treatment: Female C57BL/6 mice at 6-8 weeks of age were i.p. injected with 10μg TCDD/kg body weight, control animals received corn oil. Three days after treatment mice were sacrificed.

DDRT-PCR: Analysis were performed as described by Liang et al.[4]. In short, total RNA from control and TCDD treated animals was reverse transcribed with $T_{12}XV$ primers (X= degenerate mixture of G, A, C; V= either G, A, T or C), amplified by PCR with additional 10-mer random primers[5] and seperated on a standard sequencing gel. Differentially expressed PCR products were cut out from the gel, reamplified and subsequently used as probes in RNA slot blot analysis to confirm differential expression.

Slot blot hybridization: Equal amounts of total RNA (20μg) of control and TCDD treated animals were applied to nylon membranes using a slot blot manifold. Prehybridization and hybridization were carried out at 42°C in DIG Easy Hyb solution (Boehringer). The cDNA fragments were purified on a 1.5% low-melting agarose gel and labeled with [α-^{32}P]dCTP using a random-primer DNA labeling kit (Pharmacia).

Cloning and sequencing: The reamplified cDNA bands were cloned using the pCR-Script SK(+) Cloning kit (Stratagene). Both strands of the cDNA fragments were sequenced with the ^{T7}Sequencing kit from Pharmacia.

3. Results

DDRT-PCR was carried out to compare differences in expression of mRNAs in lung and thymus between TCDD treated and control C57BL/6 mice. Sixty different combinations of primer sets made of 4 anchored oligo-dT primers and 15 short random primers were used to obtain differential displays. Each differential display lane yielded about 100 bands allowing evaluation of 6,000 mRNA species representing over 45% of the estimated 10,000-20,000 cellular mRNAs[6].

In 35 cases obvious differences between control and TCDD treatment were reproducible upon two seperate DDRT-PCRs. For further analysis the bands were recovered from the dried denaturating polyacrylamid gel and reamplified using the corresponding primer sets. These cDNAs were used as probes in RNA slot blot hybridization to confirm differential expression.

Altered expression was noted in 6 clones, 3 of which show up- and 3 downregulation. The other differential display signals were either not confirmed by slot blot hybridization due to undetectable signals or lack of regulation, and some of them need to be further characterized. Subsequently positive cDNA fragments were subcloned into Bluescript SK(+) vector and sequenced. The results revealed that the isolated bands contained more than one species and it is under current investigation which species within the fragment is differentially expressed.
Up to now, searches to DNA data bases revealed no significant sequence similarities of the isolated clones with known genes. For further characterization the differentially expressed clones have to be elongated by RACE or by screening of a cDNA library.

4. Conclusions

Compared with standard methods such as substractive hybridization this method requires only small amounts of total RNA, is less time consuming and up- and downregulation can be observed in the same experiment. One important problem is the high incidence of false positives. Furthermore, since this method provides clones of only 100-400 bp in length from the extreme 3´-end of mRNAs, it is difficult to find identities to known genes because non-coding 3´-regions of mRNAs show great species to species variation.
In summary this method offers a means to screen for mRNAs which are transcriptionally regulated following TCDD treatment[7] in vivo. Further characterization of the isolated clones may provide further clues to the mechanism of TCDD action on lung and thymus.

5. References

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