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Differential Display Analysis of Gene Expression Induced under DCA Treatment in Rat Liver

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Abstract: The expression of genes induced by Dichloroacetate (DCA) treatment was analyzed by mRNA differential display. Purified total RNAs from rat liver treated with saline or DCA (100 mg/100 g b.w.) were reverse transcribed by using a set of oligonucleotide primers. The PCR products were resolved on a denaturing sequencing gel. PCR band representing mRNA expressed specifically in DCA-treated liver was excised and reamplified by PCR. A 120-bp c-DNA clone named IC1 was isolated and the DNA sequence of IC1 was analyzed. IC1 revealed 50% homology with 3' end of a mouse fibroblast growth factor mRNA. This result indicates that DCA induces the expression of a gene which has a 50% homology with a mouse fibroblast growth factor, and expression of this gene might be involved in non genotoxic process caused by DCA.

Key words: dichloroacetate (DCA) treatment, differential display analysis, gene expression.

DCA is regarded as one of several potential candidates for oral antidiabetic agents which can reduce blood glucose and lipids without stimulating insulin secretion (Stacpoole and Greene, 1992). Additionally DCA is known to be an effective lactate-lowering compound in conditions of marked metabolic acidosis such as sepsis, severe trauma and liver failure (Record *et al.*, 1972; Kreisberg, 1980; Wolfe *et al.*, 1991; Shangraw and Winter, 1992). Congenital lactic acidosis in infants and children has been clinically treated with DCA to improve morbidity (Stacpoole, 1989). Despite extensive physiological and pharmacological studies on intermediary metabolic effect of DCA (Nicholl *et al.*, 1991), the mechanism of the adverse effects of DCA at the molecular level has not been elucidated. Most *in vitro* and *in vivo* studies have shown that DCA has little or no genotoxic activity by itself (Herbert *et al.*, 1980). However, DCA was shown to be a peroxisome proliferator (Elliot and Elcombe, 1987; DeAngelo *et al.*, 1989) and reported to have properties of hepatocellular proliferation (Bull *et al.*, 1990), and many of peroxisome proliferating agents possess mitogenic and hepatotrophic properties (Grasso and Sharrat, 1991). Therefore, there exist possibilities of cancer promotion through the stimulation of growth of the initiated tumor cells. In order to understand the molecular mechanisms involved in cancer promotion, expression of genes that are induced during the DCA treatment should be analyzed.

mRNA differential display is a recently developed method, reported first by Liang and Pardee (1992) to select for novel genes expressed in mammalian tumor cells but not in normal cells (Liang *et al.*, 1993). The method is based upon comparisons of most mRNAs expressed in two or more cell populations, by running their reverse transcribed PCR products on sequencing gels in adjacent lanes. The differential expression can be readily detected in X-ray film, the bands cut out of the gels, eluted, and after PCR reamplification used directly as probes in Northern blot analysis for verification, and to recover the full length clones from cDNA libraries. Recently it has been used to identify glucose regulated genes (Nishio *et al.*, 1994) and an oncogene (Shen *et al.*, 1995) in animal cells, and sucrose regulated genes (Tseng *et al.*, 1995) in plant cells.

Compared with the subtractive hybridization (Owens *et al.*, 1991), a conventional differential screening method, mRNA differential display is simple, quick and has higher sensitivity. So, we adopted this method to screen and clone differentially expressed genes under DCA treatment. From this we can obtain more information about which genes are induced by DCA, leading to the elucidation of DCA effect in nongenotoxic cancer development.

Materials and Methods

Animals and treatments

Male Sprague-Dawley rats, 3 weeks old, were obtain-

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ed from Korea Research Institute of Chemical Technology. The animals were housed individually in stainless-steel wire bottom cages, and received semipurified diet and distilled water *ad libitum* for 5 weeks. DCA (Sigma, St. Louis, USA) was dissolved in deionized water and neutralized with 0.1 N NaOH. Control rats received 0.9% NaCl solution and experimental rats DCA solution (100 mg/100 g b.w.) intraperitoneally. One hour after receiving DCA, rats were anesthesized under ether and liver samples were removed promptly and frozen with liquid N_2.

Total RNA isolation

Total RNAs were purified from saline or DCA-treated rat liver tissue using the Ultraspec RNA isolation kit (BIOTECX, USA). DNA was removed from total RNA using RNase-free DNase (Ambion, USA). The integrity of total RNA prepared was examined by formaldehyde agarose gel electrophoresis.

Differential display of total RNA

Purified total RNAs (0.2 μg) from rat liver treated with saline or DCA were reverse transcribed using 2 μM of 5′-AAGCTTTTTTTTTTTC-3′ as primer (reverse transcriptase and primers from GenHunter, USA). cDNAs were amplified and labeled by including one-tenth volume of the reverse transcription reaction with the 2 μM of 5′-AAGCTTTTTTTTTTTC-3′ as 3′ primer, 2 μM of either 5′-AAGCTTGATTGCC-3′, 5′-AAGCTTCGACTGT-3′, 5′-AAGCTTTGGTCAG-3′, 5′-AAGCTTGTTGGTA-3′, 5′-AAGCTTAACTGAG-3′, 5′-AAGCTTCTAGCAT-3′, 5′-AAGCTTTCGAATC-3′, or 5′-AAGCTTAGTTGCT-3′ as 5′ primers, 25 μM dNTPs, 5 μCi α-^{35}S-dATP (1200 Ci/mmol), 2 unit Taq DNA polymerase (Perkin Elmer, USA). Forty PCR cycles were run with the following parameters: 94℃ for 30 seconds; 40℃ for 120 seconds; 72℃ for 45 seconds, followed by 5 min elongation at 72℃. Aliquots were heated for 2 min at 80℃ with loading dye and separated by electrophoresis on a 6% denaturing polyacrylamide gel. Gel was vacuum-dried at 80℃ and exposed to X-ray film for 72 h.

Recovery, reamplification, and cloning of cDNA

Selected cDNA was excised from the dried sequencing gel, eluted in 100 μl of distilled water, reamplified using the same primers according to GenHunter's manual. The reamplified PCR product was visualized on a 1.5% agarose gel, stained with ethidium bromide, and recovered by glass milk extraction (Bio-101, USA). Reamplified PCR product was cloned into the *Xcm*I-digested pDK101 vector which has a single T base tail at both ends (Kovalic *et al.*, 1991), and DNA was sequenced by dideoxy chain termination reaction with Top DNA sequencing system (Korea BioTech Inc., Korea).

Northern hybridization

Total RNA was fractionated by electrophoresis through a 1% formaldehyde agarose and transferred to nitrocellulose membrane (S & S, Germany). As a probe, a 120-bp fragment cloned into pDK101 vector was *in vitro* transcribed with MAXIscript*in vitro* transcription kit (Ambion, USA). The membrane was prehybridized for 30 min and hybridized for 1 h in ExpressHyb hybridization solution (CLONTECH, USA) at 68℃ and washed in 0.1X SSC, 0.1% SDS at 58℃. The membrane was exposed to X-ray film (Fuji, Japan) with intensifying screen at −70℃.

Results and Discussion

Identification of DCA-induced mRNA

To investigate the nature of DCA hepatoxicity at the molecular level, we isolated total RNA from rat liver tissue after DCA treatment or saline injection as a control, and utilized an mRNA differential display technique on these two different RNA samples. This technique has the advantage that it requires relatively little starting RNA materials for analysis and is not limited by redundancy of highly expressed mRNAs or underrepresentation of rare mRNAs in a cDNA library. First strand cDNAs were synthesized from saline or DCA-treated rat liver RNAs using 5′-AAGCTTTTTTTTTTTC-3′ as the priming oligonucleotide. Theoretically, this primer should produce cDNAs from about one-third of all the mRNAs present in a given sample (Liang *et al.*, 1994). A limited set of arbitrary 5′-primers (8 primers) was then used to amplify the first strand cDNAs. The PCR products, labeled with α-^{35}S-dATP, were resolved on a denaturing sequencing gel and the pattern of bands for the two samples compared for each arbitrary primer combinations. PCR band representing mRNA expressed specifically or at higher levels in DCA-treated versus control rat liver was identified using a small number of primer combinations. Fig. 1 shows a polyacrylamide gel pattern where a DCA-treated rat liver specific band was detected. The arrow points to a band where there was distinctly specific PCR amplification of RNA isolated from DCA-treated rat liver, whereas surrounding bands were roughly equivalent for the two RNA samples.

The band indicated by an arrow was excised from the dried gel, eluted in distilled water, and reamplified by PCR. A 120-bp cDNA clone corresponding to the selected band, named IC1, was isolated and used as a probe for Northern blot analysis and sequenced to

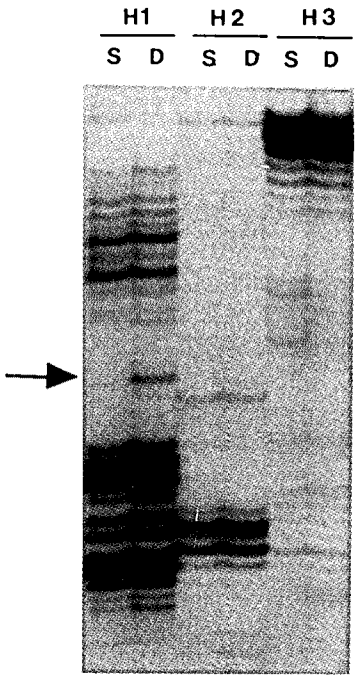

Fig. 1. A denaturing polyacrylamide gel showing differential display of mRNAs from rat liver at 1 h after saline (S) and DCA (D) treatment. Anchor primer HT11C and arbitrary primers H1, H2, H3 (sequences described in Materials and Methods) were used. The arrow indicates the IC1 band appeared only in mRNA from DCA-treated rat liver. The length of this IC1 DNA fragment is 120-bp.

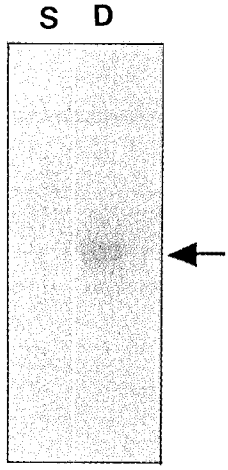

Fig. 2. Expression of IC1 clone. Total RNAs isolated from rat liver treated with saline (S) or DCA (D) were subjected to RNA blot hybridization analysis using the IC1 cDNA as a probe.

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  1 GAT TGC CCA CAC ACC CTC TCT CAG CTG GAG AGC TTA
 37 TTG GAC TGA AGG CCT GGC TCT CCC TTT CAA TAT ACT
 73 GCT TGC TTG CTG TCT CCA ATA AAG TTT TAC CTT GAA
109 AAA AAA AAA
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Fig. 3. Nucleotide sequence of IC1 clone. Underlined in the sequence are the primers that were used to identify and amplify the clone. The double-underlined sequence represents a likely polyadenylation signal of the transcript.

provide information for homology search of the DNA databases.

RNA gel blot analysis

To verify the authenticity of the differentially displayed band, IC1 cDNA clone corresponding to the selected band was used to probe a membrane blot containing total RNA from normal and DCA-treated rat liver. The result in Fig. 2 indicates that IC1 clone hybridizes specifically to 1.5-kb RNA isolated from DCA-treated tissue, confirming that the expression of IC1 gene is induced by DCA.

DNA sequence analysis

IC1 clone described above was subjected to nucleotide sequence analysis. The nucleotide sequence, shown in Fig. 3, has flanking primer sequences identical to those used in the differential display and a putative polyadenylation signal, AATAAA. Searching the GenBank and EMBL DNA database revealed that IC1 has about 50% nucleotide identity with 3′ end of a mouse fibroblast growth factor mRNA (GenBank accession No. M92416). No other significant similarities were found in these databases. Since nucleotide sequence for 120-bp cDNA clone is restricted to 3′-end, and possibly due to incomplete sequence information for this clone, no definite homologies were identified at the amino acid or nucleotide level. The induction of IC1 by DCA might be involved in the nongenotoxic process through increasing hepatocellular proliferation. Fibroblast growth factor is known to stimulate DNA synthesis in cultured rat hepatocytes (Hayashi *et al.*, 1992). Further research is required to clarify that IC1 product is directly involved in hepatocellular proliferation and the mechanism by which hepatocellular proliferation leads to the nongenotoxic process caused by DCA treatment.

We have found that mRNA differential display can be a useful supplement to or replacement for the more routinely used subtractive hybridization and differential plaque hybridization techniques (St. John and Davis, 1979). However, there are still problems to be solved. For example, the quality of RNA used can significantly influence the results that are obtained. Identifying a large number of differentially expressed mRNAs may require more primer sets and chemicals, which increase experiment cost. Also, we have found that some of the probes made from eluted, reamplified bands fail to detect corresponding mRNAs on Northern blots (data not shown). These bands may represent mRNAs of very low abundance or 'false positives' of the procedure (Liang *et al.*, 1993). However, despite such problems, the mRNA differential display technique holds

promise for the study of differential gene expression in eukaryotes.

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References

Bull, R. J., Sanchez, I. M., Nelson, M. A., Larson, J. L. and Lansing, A. (1990) *Toxicology* **63**, 341.

DeAngelo, A. B., Daniel, F. B., McMillan, L., Wemsing, P., Savage, Jr., R. E. (1989) *Toxicol. Appl. Pharmacol.* **101**, 285.

Elliot, B. M. and Elcombe, C. R. (1987) *Carcinogenesis* **8**, 1213.

Grasso, P. and Sharrat, M. (1991) *Ann. Rev. Pharmacol. Toxicol.* **31**, 253.

Hayashi, Y., Yamamoto, I. and Gohda, E. (1992) *Chem. Pharm. Bull.* **40**, 452.

Herbert, V., Gardner, A. and Coleman, N. (1980) *Am. J. Clin. Nutr.* **33**, 179.

Kovalic, J. H., Kwak J. H. and Weisblum, B. (1991) *Nucl. Acids Res.* **19**, 4560.

Kreisberg, R. A. (1980) *Ann. Inter. Med.* **92**, 227.

Liang, P. and Pardee, A. B. (1992) *Science* **257**, 6189.

Liang, P., Averboukh, L. and Pardee, A. B. (1993) *Nucl. Acids. Res.* **21**, 3269.

Liang, P., Zhu, W., Zhang, X., Guo, Z., O'Connel, R. P., Averboukh, L., Wang, F. and Pardee, A. B. (1994) *Nucl. Acids Res.* **22**, 5763.

Nicholl, T. A., Lopaschuk, G. D. and McNeill, J. H. (1991) *Am. J. Physiol.* **261**, H1053.

Nishio, Y., Aiello, L. P. and King, G. L. (1994) *FASEB J.* **8**, 103.

Owens, G. P., Hahn, W. E. and Cohen, J. J. (1991) *Mol. Cell. Biol.* **11**, 4177.

Record, C. O., Iles R. A., Cohen, R. D. and Williams, R. (1972) *Gut* **16**, 144.

Shangraw, R. E. and Winter, R. (1992) *Anesthesiol.* **77**, A235.

Shen, R., Su, Z., Olsson, C. A. and Fisher, P. B. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 6778.

Stacpoole, P. W. and Greene, Y. J. (1992) *Diabetic Care* **15**, 785.

Stacpoole, P. W. (1989) *Metabolism* **38**, 1124.

StJohn, T. P. and Davis, R. W. (1979) *Cell* **16**, 443.

Tseng, T. C., Tsai, T. H., Lue, M. Y. and Lee, H. (1995) *Gene* **161**, 179.

Wolfe, R. R., Jahoor, F., Herndon, P. N. and Miyoshi, H. (1991) *Surgery* **110**, 54.