Down-regulation of DPH2L gene during cellular differentiation /apoptosis: Use of mRNA differential display

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Abstract To characterize the genes associated with differentiation/apoptosis induced by all-trans retinoic acid (ATRA) in human lung cancer cells, mRNA differential display was employed. Six cDNA fragments have been isolated, and one of them corresponds to a sequence-known gene DPH2L with unknown function, which was first isolated from the critical region of deletion on chromosome 17p13.3 in human ovarian carcinoma, and regarded as a candidate tumor suppressor gene. Results show that DPH2L is a wide expressed gene, and has another major transcript besides the previously reported 2.3 kb transcript. It is proved that the DPH2L gene is down-regulated during differentiation or apoptosis in several kinds of cancer cells induced by all-trans retinoic acid and N-(4-hydroxyphenyl) retinamide (4HPR). This may suggest that DPH2L does not play a role as a tumor suppressor gene, on the contrary, its down-regulation may be functionally involved in the transition from cell growth to differentiation or apoptosis.

Keywords: mRNA differential display, retinoic acids, cell differentiation, apoptosis, tumor suppressor gene.

GENE differential expressions which occur in the processes of development, cellular growth and differentiation, and tumorigenesis, are the molecular basis for understanding variety of biological processes. Differentially expressed genes could be identified by comparing steady-state mRNA concentrations. Subtractive hybridization¹ and mRNA differential display² are two methods commonly used. Differential display is the method of choice because it needs small quantity of RNA and is able to identify both increase and decrease of mRNA levels.

Since retinoids are involved in the general maintenance or enhancement of differentiation/apoptosis and cancer is a process in which loss of differentiation/apoptosis occurs, retinoids have been considered as potent cancer chemopreventive agents³. We have previously described that all-trans retinoic acid (ATRA) induce differentiation and apoptosis in a human lung adenoma cell line GLC-82⁴. To better understand the molecular mechanisms which underlie the effect of retinoids against lung cancer by inducing cell differentiation or apoptosis, we used ATRA to treat the human lung adenoma cell line GLC82 cells and employed mRNA differential display to characterize genes which are involved in ATRA induced differentiation or apoptosis in GLC82 cells. Similar strategies such as subtractive hybridization have also been used in our laboratory to identify retinoic acid induced genes, and several novel genes have been successively cloned⁵, ⁶. This demonstrates that the combination of in vitro differentiation or apoptosis induction and techniques for gene expression pattern analysis is an effective way for elucidating the molecular mechanisms of redifferentiation and apoptosis of cancer cells.
1 Materials and methods

(i) Cell cultures and treatments. GLC82 (human lung adenoma cell line) and human lung cancer cell Calu-6 were grown in DMEM medium supplemented with 10% fetal calf serum. Human lung cancer cell A549 and Calu-1, HL-60, SK-N-BE2(c) (neuroblastoma cell line) were grown in DMEM medium supplemented with 10% fetal calf serum. The latter 5 cell lines came from ATCC. In all experiments, cells were routinely treated with 10 μmol/L of ATRA, or 10 μmol/L of 4HPR, 24 h after seeding to allow for cell adhesion. For HL-60 cells, 1 μmol/L of ATRA or 4HPR was used. Culture medium with or without the drug was replaced every 48 h.

(ii) RNA extraction and mRNA differential display. Total RNAs were isolated from cells with or without ATRA or 4HPR treatment for different time, by using TRIzol reagent (GIBCO BRL) according to the manufacturers’ instruction. First strand cDNA synthesis and mRNA differential display were conducted as described in ref. [7]. PCR products were separated on 6% non-denaturing polyacrylamide gel, and detected by staining with SYBR Green I (Molecular Probes, OR), a sensitive fluorescent dye excited by conventional ultraviolet illuminator. Excised bands with expression difference during all-trans RA treatment were reamplified and cloned into “pGEM-T vector” (Promega), and subjected to automatic sequencing or manual sequencing.

(iii) Northern blot analysis. 20 μg of total RNA of each sample was then electrophoresed in agarose/formaldehyde gel and transferred to Hybond nylon membrane (Amersham). Probe labeling, hybridization and autoradiography were conducted according to the manufacturers’ instructions. Images were recorded using Gel Doc 1000 system (BIO-RAD) and subjected to quantitative analysis, using “Molecular Analyst” software (BIO-RAD). The signal intensity of interested genes were computed and normalized against the p-actin signal obtained from the same blot. To test the expression of DPH2L gene in various human tissues, a “Human RNA Master Blot” (Clontech) was used, and hybridization was done according to the manufacturers’ instruction.

(iv) Flow cytometry. Aliquots of 5 × 10^5 cells were centrifuged at 300 × g for 10 min, the pellets were fixed with 70% ethanol overnight, then washed with PBS, resuspended in 500 μL of a solution containing 3.4 mmol/L sodium citrate, 50 μg/mL propidium iodide and 100 μg/mL RNase A, and then kept in the dark for 30 min at room temperature. Cells were then analyzed on a FACSscan flow cytometer (Becton Dickinson) according to standard protocols.

2 Results

(i) Cloning of differentiation/apoptosis-specific genes. To identify genes that are transcriptionally activated or inhibited during differentiation/apoptosis with ATRA treatment, we compared mRNA differential display patterns between untreated cells and cells treated with ATRA. We carried out amplifications by PCR with 20 different primer combinations for GLC82 untreated cells, GLC82 cells treated with ATRA for 8 h, 1 and 4 d respectively. Fig. 1 shows part of an electrophoresis of the PCR products. Six bands with expression difference have been subcloned. Sequence analysis and a search for homologous sequences in GenBank were performed. We found that a 442-bp PCR product was 99% homologous to the 3’ untranslated region of a candidate tumor suppressor gene DPH2L (data not shown).

Fig. 1. Part of mRNA expression patterns of untreated and ATRA-treated GLC82 cells by using mRNA differential display. There are 4 panels, each of them contains 4 lanes in which the same primers (indicated above the lanes) were used for PCR amplification: lane 0, untreated control cells; lane 1, cells treated with ATRA for 8 h; lane 2, cells treated with ATRA for 1 d; lane 3, cells treated with ATRA for 4 d; M, pBR322/Hae III marker. The arrow indicates the interesting band of DPH2L 3’ cDNA fragment for later cloning.