DNA microarray analysis of changes in gene expression induced by 1,25-dihydroxyvitamin D3 in human promyelocytic leukemia HL-60 cells

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ABSTRACT

Using a DNA microarray, we analyzed about 16,600 genes for changes in expression associated with the differentiation of human promyelocytic leukemia HL-60 cells induced by 1α,25-dihydroxyvitamin D3 (DVD). Many of the up-regulated genes could be correlated to differentiation-associated changes toward a monocyte/macrophage lineage, and many down-regulated genes could be correlated to repressed cell growth. The present study revealed the down-regulated gene expression of importins and exportins 1, 5, 7, and exportin-tRNA. Thus, the present results confirmed our previous findings of down-regulation of exportin 1 and exportin-tRNA by DVD. Gene expression of exportin 6 is suggested to be regulated differently from that of exportins 1, 5, 7, and exportin-tRNA. The down-regulation of nuclear transport factors may be deeply associated with the differentiation of HL-60 cells induced by DVD.

The active form of vitamin D3, 1α, 25-dihydroxyvitamin D3 (DVD), results from sequential hydroxylation of vitamin D3 in the liver and kidney and is responsible for calcium homeostasis in the body. Vitamin D3 metabolites have an important role in the proliferation of hematopoietic cells, lymphocytes, and keratinocytes, as well as other cell types (17). DVD is a potent inducer of cell differentiation in human leukemia HL-60 cells (6, 10, 18, 24). The HL-60 cell line, derived from a single individual with acute promyelocytic leukemia, provides a unique in vitro system for studying the cellular and molecular events involved in the proliferation and differentiation of normal and leukemic cells of the granulocyte/monocyte/macrophage lineage (5).

In spite of extensive studies (20, 25), it remains unclear how DVD exhibits biological activities including induction of differentiation, growth inhibition, and apoptosis. In our previous study, we examined changes in gene expression during the DVD-induced differentiation of HL-60 cells employing a DNA microarray technique and found the gene expression of the β subunit of eukaryotic translation initiation factor 2 to be suppressed, suggesting a linkage to differentiation-associated growth inhibition of these cells (7, 22). Since the microarray used covered only 425 genes, the study provided us with limited information.

In the present study, we analyzed about 16,600 genes for changes in expression associated with the DVD-induced differentiation of HL-60 cells. Reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR were used to examine the expression of some selected genes of our interest to evaluate the validity of cDNA microarray analysis.

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MATERIALS AND METHODS

Cells and cell culture. Human promyelocytic leukemia HL-60 cells were obtained from the Riken Cell Bank, Tsukuba, Ibaraki, Japan. HL-60 cells were cultured in 10% fetal bovine serum in RPMI 1640 medium containing 50 U/mL penicillin, 50 μg/mL streptomycin, and 2.5 μg/mL amphotericin B at 37°C under a 5% CO₂ atmosphere. HL-60 cells were treated with DVD at 10⁻⁷ M for up to 3 days as described previously (22).

cDNA microarray technique. Total RNA was extracted from cells using a QIAamp RNA Blood Mini Kit (QIAGEN Inc., Tokyo, Japan). Cy5- and Cy3-labeled DNA probes were prepared using mRNA prepared from DVD-treated and untreated cells, respectively, and hybridization was performed using a TaKaRa RNA Transcript SureLABEL Core Kit according to the manufacturer’s directions. The microarray used was IntelliGene HS Human Expression CHIP (TaKaRa Biomedicals, Inc., Tokyo, Japan). Scanning was performed using an Affimetrix 428 Array Scanner (TaKaRa Biomedicals) and data were analyzed using the software Bio Discovery Imagene Ver. 4.2 (TaKaRa Biomedicals).

RT-PCR. Total RNA was prepared from 1 × 10⁶ cells and mRNA was prepared using a QuickPrep™ Micro mRNA Purification Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer’s directions. RT-PCR was performed as described previously (1, 19, 22). Primers used are listed in Table 1. Amplified DNA was subjected to electrophoresis in 2% agarose, stained with SYBR Green I (Molecular Probes Ltd., Eugene, OR, USA), and imaged and calculated using a FluorImager (Molecular Dynamics Tokyo Ltd., Tokyo, Japan) as reported previously (1, 19, 22).

Real-time PCR. Quantitative real-time PCR was performed using LightCycler SYBR Green Fluorophore (Roche Diagnostics Ltd., Tokyo, Japan) as described previously (1, 19, 22). Primers used are listed in Table 1.

RESULTS

Up- and down-regulated genes
RNA was isolated from control HL-60 cells and those cultured in the presence of 10⁻⁷ M DVD, and subjected to microarray analysis. Scatter plots obtained from cells treated for 24, 48, and 72 h are presented in Fig. 1. About 16,600 genes were examined for their significant fluorescence intensities as compared with non-specific values for DNAs such as those for lambda A and Escherichia coli arabinose isomerase. The results showed that 12,725, 13,518, and 7,948 gave significant fluorescence signals after DVD-treatment for 24, 48, and 72 h, respectively, indicating significant expression of these genes in both DVD-treated and untreated cells. The numbers of genes with more than a 2-fold increase were 583, 852, and 667 in the cells treated with DVD for 24, 48, and 72 h, respectively, and those with a 2-fold decrease were 1,403, 655, and 1645, respectively.

Since significant changes of cell morphology and attachment to plastic dishes were evident by 48 h, we picked 100 up-regulated and 100 down-regulated genes in the cells treated with DVD for 48 h, and compared them with those in the cells treated for 24 and 72 h (Tables 2 and 3). Gene expression ratio in Tables represents fluorescence intensity of Cy5 for DVD-treated cells relative to that of Cy3 for control cells.

<table>
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<tr>
<th>Gene name</th>
<th>Forward primers</th>
<th>Reverse primers</th>
<th>Product size (bp)</th>
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<td>5’-AAGGTCATCCCTGAGCTGAA-3’</td>
<td>5’-CCCCTTCTTCAAGGGGTCTAC-3’</td>
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<td>Exportin 5</td>
<td>5’-TAAACCGTGCCAGAATGAGC-3’</td>
<td>5’-TGCAGGGGTTACCGAAGATT-3’</td>
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<tr>
<td>Exportin 6</td>
<td>5’-GTGTGCCCTTTCACGTGCAA-3’</td>
<td>5’-CGGAAAGAGGCTCAAACAG-3’</td>
<td>273</td>
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<tr>
<td>Exportin 7</td>
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<td>5’-ACTTCCTGAGTGGGCGGTA-3’</td>
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<tr>
<td>GAPDH</td>
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<td>5’-TGTTGGCTCATGAGTCTCTCC-3’</td>
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<td>Exportin 5</td>
<td>5’-TAAACCGTGCCAGAATGAGC-3’</td>
<td>5’-TGCAGGGGTTACCGAAGATT-3’</td>
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<td>Exportin 6</td>
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<tr>
<td>Exportin 7</td>
<td>5’-TGGCGGCTTGTAATGTGA-3’</td>
<td>5’-CACCAGATCAGTGCTCAAATA-3’</td>
<td>172</td>
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</table>
Effects of vitamin D3 on gene expression

Genes up-regulated were those for proteins related to gene expression (isoleucine-tRNA synthetase, phenylalanine-tRNA synthetase beta subunit, cytidine 5’-triphosphate synthetase, and arginine-serine-rich 7.3 kDa splicing factor), the nuclear transport protein exportin-tRNA, and the cell cycle-related protein cyclin D2. These changes may be correlated to the cell-growth inhibitory activity of DVD (6, 22).

Changes in exportin expression
Exportins are protein factors involved in the nuclear export of RNAs such as mRNA (26) and tRNA (13), and proteins such as eukaryotic translation elongation factor 1A (2), interleukin enhancer binding factor 3 (4), profilin-actin complexes (21), and GTPase-activating protein, p50RhoGAP (15).

The results of the present microarray analysis (Table 4) confirmed our previous findings that the expression of exportin-tRNA and exportin 1/CRM1 was down-regulated at both the gene and protein levels in DVD-treated HL-60 cells (23). In addition, the present study examined the gene expression of exportin 5, 6, and 7 by RT-PCR and quantitative real-time PCR to validate the results of the microarray analysis.

The microarray analysis demonstrated down-regulated gene expression of exportins 5 and 7, and no change in the expression of exportin 6 (Table 4). The results of RT-PCR and real-time PCR demonstrated the decreased mRNA levels of exportins 5 and 7 without significant change in exportin 6 gene expression (Figs. 2–4). The complete agreement of the results from three different methods indicates the high fidelity of the methods used.

Changes in importin expression
Importins are protein factors involved in the transport of macromolecules from the cytoplasm to nucleus (11). Previously, changes in importin expression have been examined at the protein level in 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated HL-60 cells (11). DVD and TPA often exhibit similar effects including inducing the differentiation of cells.
Table 2  List of up-regulated genes

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<th>Gene name</th>
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<td>CD14 antigen (CD14)</td>
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HL-60 cells toward a monocyte/macrophage lineage (16, 27).

The present microarray analysis revealed the down-regulated gene expression of most of importins (Table 5). The findings were generally consistent with those reported by Koehler et al. who demonstrated that importin proteins except for importin α5 were down-regulated in their expression in TPA-treated cells (11).
Effects of vitamin D3 on gene expression

**Changes in expression of eukaryotic translation initiation factor 2 (eIF-2)**

The results of the present microarray analysis presented in Table 6 confirmed our previous findings that DVD down-regulated the gene expression of three subunits of eIF-2 (22).

**DISCUSSION**

In our previous analysis of 425 genes using DNA microarray chips, we identified 7 up-regulated and 9 down-regulated genes in DVD-treated HL-60 cells (22). Seven of these genes were examined further by RT-PCR, and the results were compatible with those from the DNA chip analysis. The mRNA levels of interleukin-8, lysozyme, and cathepsin B were found to increase with the DVD treatment time-dependently and the findings were consistent with the monocytic differentiation of HL-60 cells (14, 18, 22). The down-regulation of cyclin D2 and proliferating cell nuclear antigen expression can be correlated to the reported cell cycle arrest of these cells treated with DVD (25).

When the results from the present and previous analyses are compared, they are mostly compatible (Table 7). It should be emphasized that the present results shown in Tables 6 and 7 confirmed our previous findings that DVD down-regulated the expression of three subunits of eIF-2 (22), suggesting a linkage to the suppressed cell growth of the DVD-treated cells (7).

Previously, we found that exportin-tRNA and exportin 1 were down-regulated at both the gene and protein expression levels (23). The present microar-

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**Table 5** Gene expression of importins in DVD-treated HL-60 cells in comparison with that in control cells

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Gene name</th>
<th>Gene expression ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>NM_002266.1</td>
<td>importin alpha 1 (karyopherin alpha 2)</td>
<td>0.43</td>
</tr>
<tr>
<td>NM_002268.3</td>
<td>importin alpha 3 (karyopherin alpha 4)</td>
<td>0.83</td>
</tr>
<tr>
<td>NM_002267.1</td>
<td>importin alpha 4 (karyopherin alpha 3)</td>
<td>0.50</td>
</tr>
<tr>
<td>NM_002264.1</td>
<td>importin alpha 5 (karyopherin alpha 1)</td>
<td>0.62</td>
</tr>
<tr>
<td>NM_002270.2</td>
<td>importin beta 2</td>
<td>0.85</td>
</tr>
<tr>
<td>NM_002271.3</td>
<td>importin beta 3</td>
<td>0.72</td>
</tr>
<tr>
<td>NM_006391.1</td>
<td>importin 7</td>
<td>0.75</td>
</tr>
<tr>
<td>NM_006390.1</td>
<td>importin 8</td>
<td>0.73</td>
</tr>
<tr>
<td>NM_018085.3</td>
<td>importin 9</td>
<td>0.57</td>
</tr>
<tr>
<td>NM_016338.2</td>
<td>importin 11</td>
<td>0.30</td>
</tr>
<tr>
<td>NM_014652.1</td>
<td>importin 13</td>
<td>0.51</td>
</tr>
<tr>
<td>NM_002265.4</td>
<td>transportin 1 (karyopherin (importin) beta 1)</td>
<td>0.52</td>
</tr>
<tr>
<td>NM_013433.2</td>
<td>transportin 2 (karyopherin beta 2b)</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Previously, we demonstrated the suppressed growth of DVD-treated HL-60 cells (22) and this can be correlated to the down-regulation of gene expression of eIF-2 (22). In addition, the down-regulation of genes of proteins related to nuclear-cytoplasmic transport such as exportins and importins can be reasonably correlated to suppressed cell growth and/or cell cycle arrest in light of reports such as those indicating that inhibitors of exportin 1 inhibit cell proliferation and cell cycle progression (12, 28).

The present microarray analysis revealed a down-regulation of the gene expression of some aminoacyl tRNA synthetases involved in the first step of protein synthesis. It would be worth studying further this down-regulation in connection with DVD-induced cellular events such as growth inhibition, differentiation, and cell cycle arrest, because there have been no reports on this issue.

Acknowledgement

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REFERENCES


