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Differentiation-associated alteration in gene expression of importins and exportins in human leukemia HL-60 cells

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ABSTRACT

Employing the DNA microarray technique, we previously reported the alteration in gene expression of nucleocytoplasmic transport factors, importins and exportins, induced by 1,25-dihydroxyvitamin D3 (DVD) in human leukemia HL-60 cells. Here, we used the quantitative reverse transcription-polymerase chain reaction method to confirm such previous findings, and compared them with those from the cells treated with all-*trans*-retinoic acid (ATRA). The results indicated that the gene expression of the transport factors examined was mostly down-regulated following differentiation induced by DVD and ATRA, but importin α5 gene expression was up-regulated in either case. The differences were found in the gene expression of importin α3 and exportin 6 between the cells after treatments with DVD and ATRA. These variations may be related to the difference between HL-60 cell lineages differentiating into monocytes/macrophages and granulocytes. The present findings provide further evidence to support the important roles of importins and exportins in cell differentiation.

In eukaryotic cells, the nucleus is separated from the cytoplasm by the nuclear envelope. Macromolecules such as RNA transcripts generated in the nucleus are exported from the nucleus to the ribosomes in the cytoplasm, and proteins synthesized in the cytoplasm, such as histones, polymerases, and transcription factors, are imported into the nucleus. The importin/exportin transport system is involved in the nucleocytoplasmic transport of such cargo molecules (1–4, 6–8). In this system, proteins to be imported or exported have generally a nuclear localization signal (NLS) sequence or a nuclear export signal (NES) sequence. Importin α binds to NLS within protein cargoes and links them to importin β to enter into the nucleus. In some cases, the cargo molecule with NLS directly binds to β and is transported into the nucleus.

In an analogous fashion, exportin recognizes NES in the cargo protein and the complex is exported from the nucleus by binding to the GTP-bound form of guanine nucleotide binding protein Ran. There are at least 18 importin and 6 exportin genes in humans and 15 importin and 6 exportin genes in mice. Alteration of the expression level of importins and exportins would affect the transport efficiency, and play a crucial role in development, differentiation, and transformation.

It is well-known that human promyelocytic leukemia HL-60 cells can be induced to differentiate into monocytes/macrophages and granulocytes by 1,25-dihydroxyvitamin D3 (DVD) and all-*trans*-retinoic acid (ATRA), respectively (5). Employing the DNA microarray technique, we previously reported the alteration in gene expression of importins and exportins induced by DVD in HL-60 cells (11). In the present study, we used the quantitative real-time polymerase chain reaction (Q-PCR) method to con-

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firm these previous findings, and compared them with those from cells treated with ATRA.

MATERIALS AND METHODS

Chemicals. DVD was kindly provided by Dr. Ryushi Nozawa of University of Shizuoka, and ATRA was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Cells. HL-60 cells were obtained from the Riken Cell Bank (Tsukuba, Ibaraki, Japan) and 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% CO_2 atmosphere. These cells were treated with 100 nM DVD for 72 h, as described previously (11) or with 1 μM ATRA for 24, 48, and 72 h. The concentration of ATRA used was reported to be sufficient to induce maximal granulocyte differentiation (5).

Q-PCR. Total RNA was extracted from cells cultured under various conditions, and mRNA was prepared using a QIAamp RNA Blood Mini Kit (Qiagen Ltd., Tokyo, Japan). To prevent possible contamination, samples were treated with deoxyribonuclease (RT-grade; Wako Pure Chemical Industries Ltd.), as recommended by the manufacturer (11). Q-PCR was performed using the Thermal Cycler Dice (TaKaRa Bio., Tokyo, Japan) according to the manufacturer's directions. Primers were purchased from TaKaRa Bio., and are listed in Table 1.

RESULTS

The results of Q-PCR showed the alteration in the expression of importins in HL-60 cells before and after DVD treatment for 72 h (Fig. 1). Gene expression of importins α1, α3, β1, and 3 was down-regulated in association with differentiation, while that of importin α5 was up-regulated.

When cells were treated with ATRA, the expression of importins α1, β1, and 3 was down-regulated time-dependently, although the temporal up-regulation was noted for importins α1 (Fig. 2). The gene expression of importin α3 was down-regulated at 48 h, but restored to the control level after 72 h. The gene expression of importin α5 was up-regulated in association with ATRA-induced differentiation.

Gene expressions of all exportins examined were down-regulated by ATRA (Fig. 3). For comparison, data on the cells treated with DVD for 72 h (9, 11) are included in Fig. 1. The data show that DVD also down-regulated the expression of most exportins, but that the expression level of exportin 6 is maintained in contrast to the case of ATRA.

DISCUSSION

Our previous cDNA microarray analysis (11) revealed the down-regulated expression of most importins and exportins in DVD-treated HL-60 cells (Table 2). The changes in the expression of exportins have been confirmed by Q-PCR (9, 11).

The present study using Q-PCR for importins generated results in accordance with the previous findings shown in Table 2, with one exception. Although microarray analysis failed to show an alteration in the gene expression of importin α5 (Table 2), the present study revealed that it was up-regulated (Fig. 1). The changes in expression of nucleocytoplasmic transport factors may be correlated with the differentiation of HL-60 cells toward a monocyte/

Table 1 *Primers used for Q-PCR*

Gene name	Forward primer	Reverse primer
Importin α1	5'-AACAAGCTGTCTGGGCTCTAGGAA-3'	5'-CAGTGGGTCAACTGCACCGTA-3'
Importin α3	5'-GCTATGAAGCAGTGTGTGAATGGA-3'	5'-ACAACCACGCTCTCGTACACAGTC-3'
Importin α5	5'-AAGAGCCCTGCAAATTCTGTGTC-3'	5'-GGTGCTGATGGTAAGCAGGAGTC-3'
Importin β1	5'-CATTGCTGGAGATGAGGATCACA-3'	5'-TCATGGATCATTGGCCTAGCTTC-3'
Importin 3	5'-CCAGCTTTGTTGCAGCCTCA-3'	5'-GCCACTGGAGCACACAGGAA-3'
Exportin 1	5'-TCTTCAGGAATATGTGGCTAATCTC-3'	5'-TCAGAAGTGTCTTCACCTGCAA-3'
Exportin 5	5'-CACAGGCAGGGTCAGATTGAGA-3'	5'-GATCGCCTTCACGATCCACA-3'
Exportin 6	5'-TCCTGACCAGCTGTGATGGTG-3'	5'-GTCTGTAGTAGCGCAGGTCGTTGA-3'
Exportin 7	5'-GCAGCTGTCACGTAGCACCAA-3'	5'-GATGATGTTCAGCACCGTGGA-3'
Exportin t	5'-TGGGATGAGCGAAGTTATAGCAA-3'	5'-CAAATCCCACTGGTCCATCTTTAC-3'
GAPDH*	5'-TGTGGTCATGAGTCCTTCCA-3'	5'-CATGGGTGTGAACCATGAGA-3'

*Glyceraldehyde-3-phosphate dehydrogenase

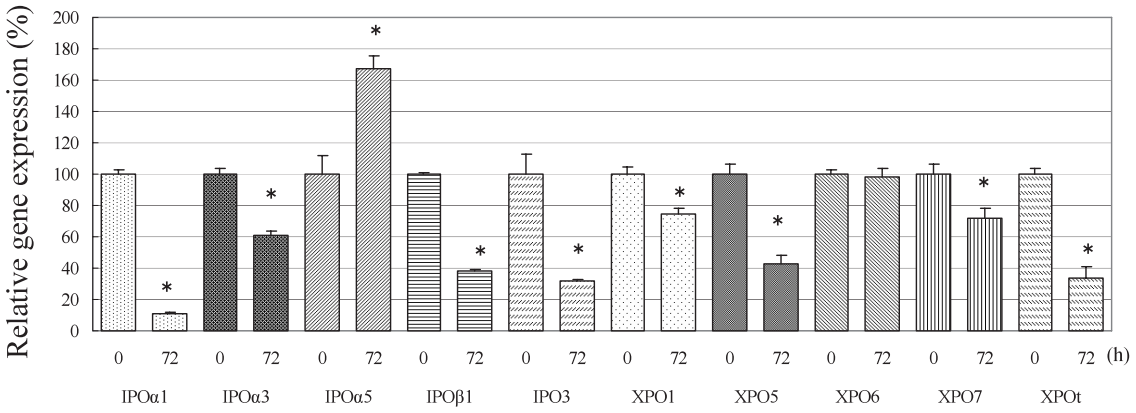

Fig. 1 Effects of DVD on expression of importins and exportins in HL-60 cells, as determined by Q-PCR. RNA was extracted from HL-60 cells untreated or treated with DVD at 100 nM for 72 h and examined by Q-PCR for the expression of importins and exportins. Gene expression is normalized using glyceraldehyde-3-phosphate dehydrogenase, and the results (72 h) are expressed as a percentage relative to that for the untreated control (0 h) from three determinations (mean ± S.E). Data for exportins are from the previous studies (9, 11). IPO, importin; XPO, exportin. *Significantly different from control (0 h) at $p < 0.05$ (ANOVA).

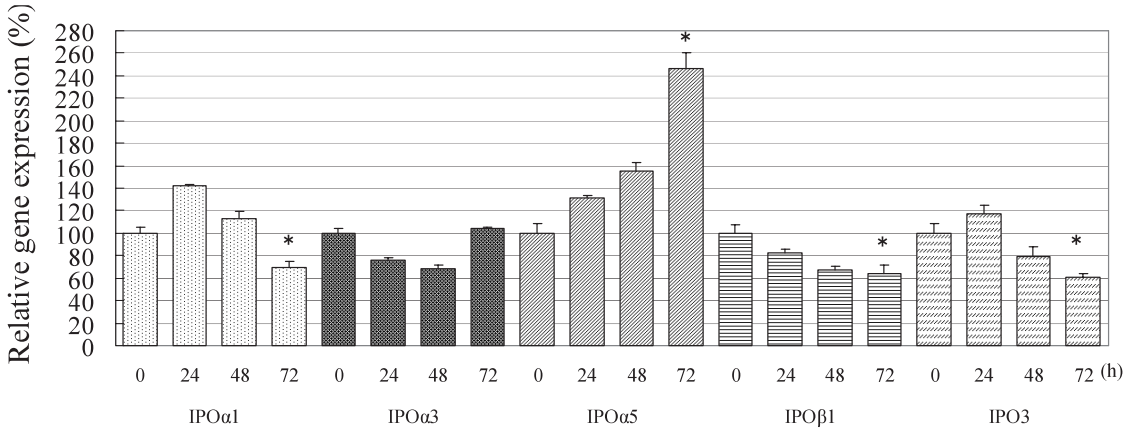

Fig. 2 Effects of ATRA on importin expression, as determined by Q-PCR. RNA was extracted from HL-60 cells treated with 1 μM ATRA for 0, 24, 48, and 72 h and examined by Q-PCR for the expression of importins, as indicated. The results are expressed as a percentage relative to that for the control (0 h) from three experiments (mean ± S.E). IPO, importin. *Significantly different from control (0 h) at $p < 0.05$ (ANOVA).

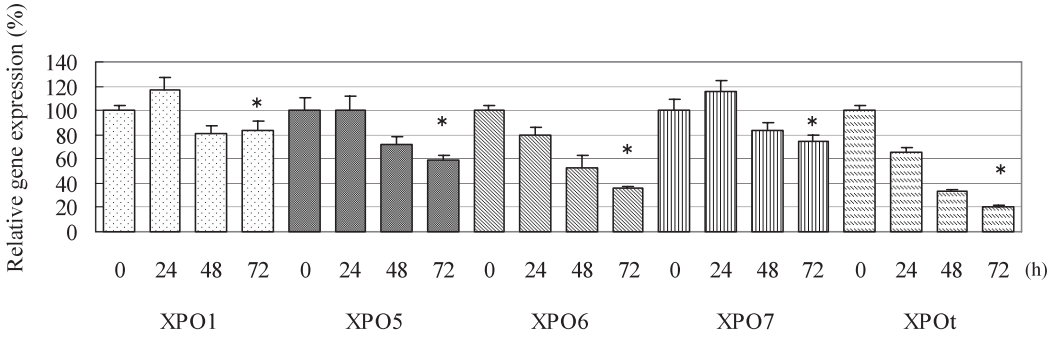

Fig. 3 Effects of ATRA on exportin expression, as determined by Q-PCR. RNA was extracted from HL-60 cells treated with 1 μM ATRA for 0, 24, 48, and 72 h and examined by Q-PCR for the expression of exportins, as indicated. The results are expressed as a percentage relative to that for the control (0 h) from three experiments (mean ± S.E). XPO, exportin. *Significantly different from control (0 h) at $p < 0.05$ (ANOVA).

Table 2 *DVD-induced changes in gene expression of importins and exportins in HL-60 cells, as examined by cDNA microarray**

Gene name (Duration of DVD treatment)	Ratio of gene expression in DVD-treated to that in control cells (24 h)	(48 h)	(72 h)
Importin α1 (karyopherin α2)	0.43	0.86	0.62
Importin α3 (karyopherin α4)	0.83	0.91	0.78
Importin α5 (karyopherin α1)	0.62	0.74	0.95
Importin β1	0.52	1.27	0.45
Importin 3 (karyopherin beta 2b)	0.31	0.44	0.54
Exportin 1 (CRM1)	0.73	0.64	0.38
Exportin 5	0.26	0.42	0.34
Exportin 6	0.88	1.17	0.99
Exportin 7	0.93	0.34	0.84
Exportin t	0.31	0.19	0.34

*Data cited from Reference (11).

macrophage lineage.

When HL-60 cells were incubated with DVD, their cell growth was inhibited (5, 10). Therefore, it is reasonable to speculate that the down-regulated gene expression of proteins related to nuclear cytoplasmic transport is correlated with suppressed cell growth. It has been shown that leptomycin B, an inhibitor of exportin 1, inhibits cell growth (13). RNA interference technology to specifically down-regulate the expression of ubiquitously expressed human α importins in HeLa cells has revealed that the down-regulation of importins α3, α5, α7, and β strongly inhibited HeLa cell proliferation (8).

It is well-known that ATRA induces the differentiation of HL-60 cells toward a granulocyte lineage (5). The expression levels of most of the nucleocytoplasmic transport factors examined were decreased in association with ATRA-induced differentiation. The down-regulated expression of exportins examined in ATRA-treated cells (Fig. 2) was similar to that in DVD-treated cells (Fig. 1).

However, in contrast to DVD, ATRA did not lead to down-regulated importin α3 gene expression after 72-h treatment, although it caused a transient down-regulation. This difference may be related to the difference between HL-60 cell lineages to monocytes/macrophages and to granulocytes, as has been proposed by Köhler *et al.* (2). These authors demonstrated by the immunoblotting method that 12-*O*-tetradecanoylphorbol-13-acetate caused the down-regulation of importin α3 protein expression in HL-60 cells, while dimethylsulfoxide did not promote such a change. Differences were also found in the exportin 6 expression in addition to the difference in importin α3, suggesting that this variation may also contribute to the differentiation into the different cell lineage of HL-60 cells.

The down-regulated expression of importin α1 accompanied by the up-regulation of importin α5 was observed in the case of differentiation induced by both DVD and ATRA. The findings are in line with a recent report that this switching triggers the neural differentiation of embryonic stem cells (12), suggesting the possibility that this switching is a hallmark of cell differentiation. Thus, the present findings provide further evidence to indicate the important roles of nucleocytoplasmic transport proteins in cell differentiation.

REFERENCES

1. Cook A, Bono F, Jinek M and Conti E (2007) Structural biology of nucleocytoplasmic transport. *Annu Rev Biochem* **76**, 647–671.
2. Köhler M, Fiebeler A, Hartwig M, Thiel S, Prehn S, Kettritz R, Luft FC and Hartmann E (2002) Differential expression of classical nuclear transport factors during cellular proliferation and differentiation. *Cell Physiol Biochem* **12**, 335–344.
3. Köhler M, Haller H and Hartmann E (1999) Nuclear protein transport pathways. *Exp Nephrol* **7**, 290–294.
4. Kutay U and Güttinger S (2005) Leucine-rich nuclear-export signals: born to be weak. *Trends Cell Biol* **15**, 121–124.
5. Miyaura C, Abe E, Suda T and Kuroki T (1985) Alternative differentiation of human promyelocytic leukemia cells (HL-60) induced selectively by retinoic acid and 1 alpha,25-dihydroxyvitamin D3. *Cancer Res* **45**, 4244–4248.
6. Poon IK and Jans DA (2005) Regulation of nuclear transport: central role in development and transformation? *Traffic* **6**, 173–186.
7. Pouton CW, Wagstaff KM, Roth DM, Moseley GW and Jans DA (2007) Targeted delivery to the nucleus. *Adv Drug Deliv Rev* **59**, 698–717.
8. Quensel C, Friedrich B, Sommer T, Hartmann E and Kohler M (2004) In vivo analysis of importin alpha proteins reveals

cellular proliferation inhibition and substrate specificity. *Mol Cell Biol* **24**, 10246–10255.
9. Suzuki T, Koyama Y, Hayakawa S, Munakata H and Isemura M (2006) 1,25-Dihydroxyvitamin D3 suppresses exportin expression in human promyelocytic leukemia HL-60 cells. *Biomed Res* **27**, 89–92.
10. Suzuki T, Koyama Y, Ichikawa H, Tsushima K, Abe K, Hayakawa S, Kuruto-Niwa R, Nozawa R and Isemura M (2005) 1,25-Dihydroxyvitamin D3 suppresses gene expression of eukaryotic translation initiation factor 2 in human promyelocytic leukemia HL-60 cells. *Cell Struct Funct* **30**, 1–6.
11. Suzuki T, Tazoe H, Taguchi K, Koyama Y, Ichikawa H, Hayakawa S, Munakata H and Isemura M (2006) DNA microarray analysis of changes in gene expression induced by 1,25-dihydroxyvitamin D3 in human promyelocytic leukemia HL-60 cells. *Biomed Res* **27**, 99–109.
12. Yasuhara N, Shibazaki N, Tanaka S, Nagai M, Kamikawa Y, Oe S, Asally M, Kamachi Y, Kondoh H and Yoneda Y (2007) Triggering neural differentiation of ES cells by subtype switching of importin-alpha. *Nat Cell Biol* **9**, 72–79.
13. Yoshida M, Nishikawa M, Nishi K, Abe K, Horinouchi S and Beppu T (1990) Effects of leptomycin B on the cell cycle of fibroblasts and fission yeast cells. *Exp Cell Res* **187**, 150–156.