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Identification of genes differentially expressed in monocyte-derived dendritic cells with 1α,25-dihydroxyvitamin D_3 using cDNA arrays

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Abstract: In order to study the molecular mechanism of the inhibitory effect of 1,25-dihydroxyvitamin D_3 on dendritic cells, experiments were performed using Atlas cDNA expression arrays from Clonetech to identify the differentially expressed genes of dendritic cells by 1,25-dihydroxyvitamin D_3. Analysis of cDNA arrays revealed changes in the expression of 9 genes, including those involved in DNA binding and transcription, extracellular cell signaling and communication, intracellular transducers, as well as cell adhesions. The results indicated that a multiple molecular network is involved in the inhibitory role of 1,25-dihydroxyvitamin D_3 on dendritic cells. The Atlas Array technology may facilitate the elucidation of complex pharmacological process of 1,25-dihydroxyvitamin D_3 on dendritic cells.

Key words: 1,25-dihydroxyvitamin D_3, Dendritic cells, Gene expression, cDNA array
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INTRODUCTION

1α,25-dihydroxyvitamin D_3[1,25$(OH)_2D_3$], the biologically active metabolite of vitamin D_3, is a secosteroid hormone that not only regulates bone and calcium/phosphate metabolism but also regulates a number of other biological activities, including modulation of the immune response via specific receptors expressed in antigen presenting cells (APC) and activated T cells. Recently, increasing evidence showed that the modulatory role of 1,25$(OH)_2D_3$ on T cell phenotype and function is mediated by inhibiting differentiation and maturation of dendritic cells (DC), which are the most potent APC playing a key role in the initiation of immune response (Penna and Adorini, 2000; Piemonti *et al.*, 2000; Berer *et al.*, 2000). But the mechanism of the inhibitory effect of 1,25$(OH)_2D_3$ on DC is not yet identified.

The aim of our work was to study patterns of gene expression in DC influenced by 1,25$(OH)_2D_3$. This can be accomplished using RT-PCR or Northern blot analysis. But these methods focus on only a few genes at a time. Here we employed a recently developed method, human cDNA array, adopted for high-throughput analysis of gene expression.

MATERIALS AND METHODS

Cells

Human dendritic cells were generated from peripheral blood monocytes according to the cell culture method described by us (Zhu *et al.*, 2001). The cells obtained were so-called monocyte derived dendritic cells (MoDC).

Preservative-free 1,25$(OH)_2D_3$ (Calbiochem-Novabiochem, Marburg, Germany) was disolved in 96% analytical grade ethanol to a concentration of 10^{-3} mol/L and further diluted in culture medium. It

was added to the cell culture from the beginning with a concentration of 10^{-8} mol/L. An ethanol solvent control (0.01% v/v) was included at the same time.

RNA extraction, cDNA probe and array hybridization

Total RNA was extracted from cells using an Atlas™ Pure Total RNA Isolation Kit (Clonetech, USA). Cell lysates were processed according to the instruction manual by performing phenol/chloroform extractions, precipitating the purified total RNA and treatment with Rnase free Dnase I. Total RNA yield was evaluated by UV absorbance at 260 nm.

Ten micrograms of each total RNA was converted to ^{32}P-labeled DNA as specified in the Atlas™ Expression Array User Manual (Clontech, USA). Each probe was purified using a gravity column provided with the Atlas™ Expression Array kit.

Atlas™ Human Cancer cDNA Expression Array membranes (Clonetech, USA) containing 588 cancer-related cDNAs were treated as specified by the manufacturer by performing a 2-min wash with dH_2O. Prehybridization using 5 ml of prewarmed ExpressHyb (Clonetech, USA) containing 0.5 mg heat-denatured sheared salmon DNA was performed at 68 °C for 30 min with agitation. 1×10^6 cpm of each cDNA probe was prepared and hybridized with the array membrane at 68 °C overnight with continuous agitation. After hybridization, membranes were washed 4 times with 200 ml of 2×SSC (1×SSC: 0.15 mol/L NaCl and 0.015 mol/L sodium citrate), 1% SDS at 68 °C for 30 min, and once with 200 ml of 2×SSC at room temperature as specified in the in struction manual. The membranes were then exposed to *X*-ray film for 80 h.

Array analysis

Autoradiogrphs were digitized with a scanner and analyzed using AtlasImage™ 1.01 software (Clonetech, USA). The ratio of signal intensity between the test and control membranes outside 0.8−1.2 was considered to represent a significant difference in gene expression following $1,25(OH)_2D_3$ treatment.

RESULTS

Viability of MoDC treated with $1,25(OH)_2D_3$

Cell viability was evaluated by trypan blue exclusion test. Viability of MoDC treated with 10^{-8} mol/L $1,25(OH)_2D_3$ harvested after 5 d culture was >90%, not differing from those with ethanol or medium.

cDNA microarray analysis in $1,25(OH)_2D_3$ treated MoDC

cDNA microarray analysis was used to determine the differential gene expression pattern in MoDC treated with 10^{-8} mol/L $1,25(OH)_2D_3$. The ^{32}P-labelled cDNA probes were synthesized with reverse transcriptase as described in Materials and Methods, and hybridized to filters containing 588 genes including 57 oncogenes and tumor suppressors, 44 cell cycle proteins, 88 modulators/effectors /intracellular transducers, 7 stress response proteins, 64 apoptosis-related proteins, 34 DNA synthesis, repair and recombination proteins, 98 DNA binding/ transcription/transcription factors, 58 cell receptors, 40 cell surface antigens and adhesion molecules, and 98 extracellular cell signaling and communication proteins. Hybridization signals were detected and evaluated using a phosphorimager. The mean values of two independent assays listed in Table 1 shows that 9 genes were differentially expressed, of which 4 were up-regulated and 5 were down-regulated by $1,25(OH)_2D_3$. The differentially expressed genes identified in this assay have been reported to function in a variety of cellular processes including extracellular cell signaling, DNA binding and transcription, cell adhesion, intracellular trans duction, and other cellular functions.

DISCUSSION

It is well known that $1,25(OH)_2D_3$ exerts its immunomodulatory properties via intracellular vitamin D receptors present in monocytes/macrophages, T cells, B cells and NK cells (Kreutz *et al.*, 1993; Zerwekh *et al.*, 1993). In addition to these immune cells, DC is considered to be relatively more important in immune system due to its unique property

Table 1 List of differentially expressed genes with treatment of $1,25(OH)_2D_3$

Gene code	Genbank	Gene name	Ratio
B4i	U03688	Dioxin-inducible cytochrome p450 (CYR1B1)	0.59
D4e	M62810	Transcription factor 1 mitochondrial	1.28
D4f	M62829	Transcription factor ETR103 (early growth response protein)	1.28
D4g	M62831	Transcription factor ETR101(T-lymphocyte activated protein)	0.70
D6f	U09579	Cyclin-dependent kinase inhibitor 1A($p21^{WAF1}$)	0.49
E5h	J03132	Intercellular adhesion molecule-1	0.71
F4d	M92381	Thmosin beta-10	0.51
F5m	K02770	Monocyte interleukin 1β	1.47
F6b	X04602	Interleukin-6 (B cell differentiation factor)	1.54

to activate T cells and being required for the induction of a primary response (Banchereau and Steinman, 1998); for which reasons the effect of immunosuppressive $1,25(OH)_2D_3$ on DC has attracted interest in the recent years. Several studies established that DCs are a primary target of the activity of $1,25(OH)_2D_3$, which affects all major stages of the DC life cycle: differentiation, maturation, activation, function and survival (Penna and Adorini, 2000; Piemonti *et al.*, 2000; Berer *et al.*, 2000). To provide mechanistic information on the molecular basis for $1,25(OH)_2D_3$ on DC, it is essential to identify genes with differential expression in DC and $1,25(OH)_2D_3$ treated DC.

Since a direct relationship between the presence of DC within various cancers and their prognosis has been found in the past few years (Lotze and Jaffe, 1999) and $1,25(OH)_2D_3$ has been demonstrated to inhibit cancer cell growth, angiogenesis and metastasis (van Leeuwen and Polos, 1999), we in this study evaluated the potential of cDNA arrays and focused on the expression of 588 known genes from 11 different classes involving those related to cell proliferation, apoptosis, differentiation, growth control, signal transduction, and so on. We found that overall, 9 genes were differentially expressed between MoDC and 10^{-8} mol/L $1,25(OH)_2D_3$ treated MoDC, of which 4 genes were overexpressed and 5 genes were underexpressed by $1,25(OH)_2D_3$. Genes regulated by $1,25(OH)_2D_3$ were those encoding proteins of DNA binding and transcription, extracellular cell signaling and communication, intracellular transducers, as well as cell adhesions. ICAM is one of the cell adhesion molecules playing a major part in leukocyte homing to sites of chronic inflammation. $1,25(OH)_2D_3$ demonstrated multiple effects on cell proliferation/differentiation by expressing MHC and ICAM-1 on monocytes, lymphocytes and epithelial cells (Kreft *et al.*, 1996). In this study, we found that $1,25(OH)_2D_3$ inhibited ICAM-1 gene expression by MoDC. The beta-thymosins comprise a family of structurally related, highly conserved acidic polypeptides, originally isolated from calf thymus. Thymosin beta-10 is one of the extracellular cell signaling and communication proteins. Its overexpression has been detected in a large variety of human neoplastic tissues and cell lines (Santelli *et al.*, 1999). Cytochrome P450 1B1 (CYP1B1), one of the intracellular transducers, is a dioxin-inducible gene expressed in a wide range of cancers and can activate numerous chemically diverse carcinogens (Murray *et al.*, 1997). So far, no data on $1,25(OH)_2D_3$ activity on thymosin beta-10 and cytochrome P450 have been documented. They were down-regulated by $1,25(OH)_2D_3$ in MoDC in the present study. IL-1β and IL-6 are cytokines involved in extracellular cell signaling. $1,25(OH)_2D_3$ was reported to inhibit IL-1β and IL-6 production by human monocytes at the post-transcription level and this was considered to be important in $1,25(OH)_2D_3$-mediated inhibition of lymphocyte functions in vitro (Muller *et al.*, 1992). On the contrary, to the effect of $1,25(OH)_2D_3$ on monoytes, IL-1β and IL-6 was found by us to be up-regulated in MoDC. A wide range of transcription factors were investigated to elucidate the modulatory role of $1,25(OH)_2D_3$ on

different cell types such as monocytes (Wang and Studzinski, 2001), colon carcinoma cells (Palmer *et al.*, 2001), keratinocytes (Johansen *et al.*, 2000), and so on. In this study, the effect of $1,25(OH)_2D_3$ on transcription factors was analyzed in MoDC with the result showing that they were differentially regulated, with transcription factor ETR101 and cyclin-dependent kinase inhibitor 1A being down-regulated and transcription factor 1 mitochondrial and transcription factor ETR103 being up- regulated. It can be inferred from the above data that a multiple molecular network is involved in the inhibitory role of $1,25(OH)_2D_3$ on DC's differentiation, maturation and function. The result of cDNA microarray may facilitate the elucidation of complex pharmacological process underlying $1,25(OH)_2D_3$ on DC.

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