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The genes encoding cytokines *IL-2*, *IL-10* and *IL-12B* are primary 1α,25(OH)$_2$D$_3$ target genes☆

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ABSTRACT

A number of studies have described the effects of 1α,25(OH)$_2$D$_3$ in immune system. Most of the known effects of 1α,25(OH)$_2$D$_3$ are indirect since only two functional VDREs that regulate transcription of cytokine gene has been reported until today. In this study we have examined a possibility of direct transcriptional regulation of *IL-2*, *IL-10* and *IL-12B* genes in activated Jurkat or THP-1 cells via liganded VDR by using gene expression analysis and chromatin immunoprecipitation assays. According to our data the *IL-2*, *IL-10* and *IL-12B* genes respond to 1α,25(OH)$_2$D$_3$ treatment by 3–6 h. In addition, all of these genes contain several genomic regions that recruit VDR in a ligand dependent fashion. These data suggest that the above cytokines are under direct transcriptional regulation by 1α,25(OH)$_2$D$_3$.

1. Introduction

The biological effects of the biologically most active form of vitamin D, 1α,25-dihydroxyvitamin D$_3$ (1α,25(OH)$_2$D$_3$) are mediated through the vitamin D receptor (VDR), which is a member of a superfamily of ligand-inducible nuclear receptors that control expression of their primary target genes in response to binding of steroidal or other lipophilic compounds. The binding of 1α,25(OH)$_2$D$_3$ to VDR changes the conformation of the ligand-binding domain of the VDR [1]. The changed conformation promotes the interaction of VDR with its heterodimeric partner, the retinoid X receptor (RXR) and modulates interactions between VDR and a number of different nuclear proteins, such as co-activators and co-repressors [2]. These interactions lead to the association of activating or repressing VDR complexes with specific genomic sequences (VDREs) that are located within the regulatory regions of primary 1α,25(OH)$_2$D$_3$ responding genes and ultimately modulate the expression of these genes.

In addition to the classic functions in controlling the mineral homeostasis and cell growth and differentiation, 1α,25(OH)$_2$D$_3$ has also role in immune response [3,4]. Repression of *IL-2* and *IL-12* cytokines in response to 1α,25(OH)$_2$D$_3$ treatment is well documented in different cells of the immune system as well as *in vivo* [5–11]. The effect of 1α,25(OH)$_2$D$_3$ to the production of cytokine *IL-10* has been reported to be opposite to that of *IL-2* and *IL-12B*, while it is induced by 1α,25(OH)$_2$D$_3$ in T and B cells [12–14]. However, there are also contradictory studies, that suggest that 1α,25(OH)$_2$D$_3$ treatment decreases the amount of IL-10 in cultured cells and living animals [8,10,15]. According to the present understanding, most of the effects of 1α,25(OH)$_2$D$_3$ on cytokine production appear to be indirect, since until today active vitamin D response elements (VDRE) have been identified only within *IL-10* gene [13,16]. In this study we have examined the possibility of direct transcriptional regulation of *IL-2*, *IL-10* and *IL-12B* genes via liganded VDR by using gene expression analysis and chromatin immunoprecipitation assays. According to our data the *IL-2*, *IL-10* and *IL-12B* genes contain several genomic regions that recruit VDR in a ligand dependent fashion.

2. Materials and methods

2.1. Cell culture

THP-1 human acute monocytic leukemia cells and Jurkat human T lymphocyte leukemia cells were maintained in RPMI-1640 medium (Sigma–Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mg/ml streptomycin,

Abbreviations: 1α,25(OH)$_2$D$_3$, 1α,25-dihydroxyvitamin D$_3$; ChIP, chromatin immunoprecipitation; FBS, fetal bovine serum; RE, response element; RXR, retinoid X receptor; TSS, transcription start site; VDR, vitamin D receptor; VDRE, vitamin D response element.

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and 100 U/ml penicillin in a humified 95% air/5% CO_2 incubator. For experiments the cells were maintained in phenol red-free DMEM, supplemented with 5% charcoal-stripped FBS, 2 mM L-glutamine, 0.1 mg/ml streptomycin, and 100 U/ml penicillin. Prior to mRNA or chromatin extraction, THP-1 cells were first treated with 100 ng/ml LPS for 24 h, and Jurkat cells with 2 μg/ml phytohemagglutinin (PHA) (Sigma–Aldrich) and 50 ng/ml 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (Sigma–Aldrich) for 24 h. After that the cells were exposed to either solvent (ethanol, 0.1% final concentration) or to 10 nM 1α,25(OH)$_2$D$_3$ (kindly provided by Milan Uskokovic, Bioxell Inc, Nutley, USA). For mRNA stability studies the cells were first treated as above after which 1 μg/ml actinomycin D (Sigma–Aldrich) was added.

2.2. Total RNA extraction, cDNA synthesis, and real-time quantitative PCR

Total RNA was extracted using High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany), and cDNA synthesis was performed using Transcriptor High Fidelity cDNA Synthesis Kit (Roche) according to the manufacturer's instructions. Real-time quantitative PCR was performed with LightCycler 480 apparatus (Roche) using TaqMan Gene Expression Assays (Applied Biosystems, Carlsbad, CA, USA), with *RPLP0* serving as a control gene. PCR cycling conditions were: 10 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 60 s at 60 °C. Fold changes were calculated using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = \Delta Ct_{(stimulus)} - \Delta Ct_{(solvent)}$, and $\Delta Ct = Ct_{(target\ gene)} - Ct_{(RPLP0)}$. Ct is the cycle at which the threshold line is crossed. Two-tailed Student's *t*-tests were performed to calculate statistical significances between solvent treated and ligand treated samples.

2.3. ChIP assays

Chromatin immunoprecipitation (ChIP) assays were performed as previously described [17]. The recovered chromatin solutions were diluted 1:10 (v/v) in ChIP dilution buffer, and incubated with 1 μg of antibody against VDR (sc-1008) from Santa Cruz Biotechnology (Santa Cruz, CA, USA) at 4 °C overnight. The non-specific IgG (12-370) used as a control was from Upstate Biotechnology (Lake Placid, NY, USA). The immunocomplexes were collected with protein A agarose slurry (Millipore, Billerica, MA, USA) and washed as previously described. The samples were reverse cross-linked with 2 μl of proteinase K (Fermentas, Vilnius, Lithuania) overnight at 64 °C, after which phenol:chloroform extraction and ethanol precipitation were performed. ChIP samples were analyzed with real-time PCR by using SYBR Green dye and 2% agarose gel electrophoresis.

3. Results

3.1. Genes encoding cytokines IL-2, IL-10 and IL-12B respond rapidly to 1α,25(OH)$_2$D$_3$ treatment

We measured ligand-dependent mRNA expression of *IL-2* in Jurkat cells pretreated with PHA/TPA and expression of *IL-10* and *IL-12B* in THP-1 cells pretreated with LPS. According to our data, the expression of *IL-2* (Fig. 1A) and *IL-10* (Fig. 1B) decreased sig-

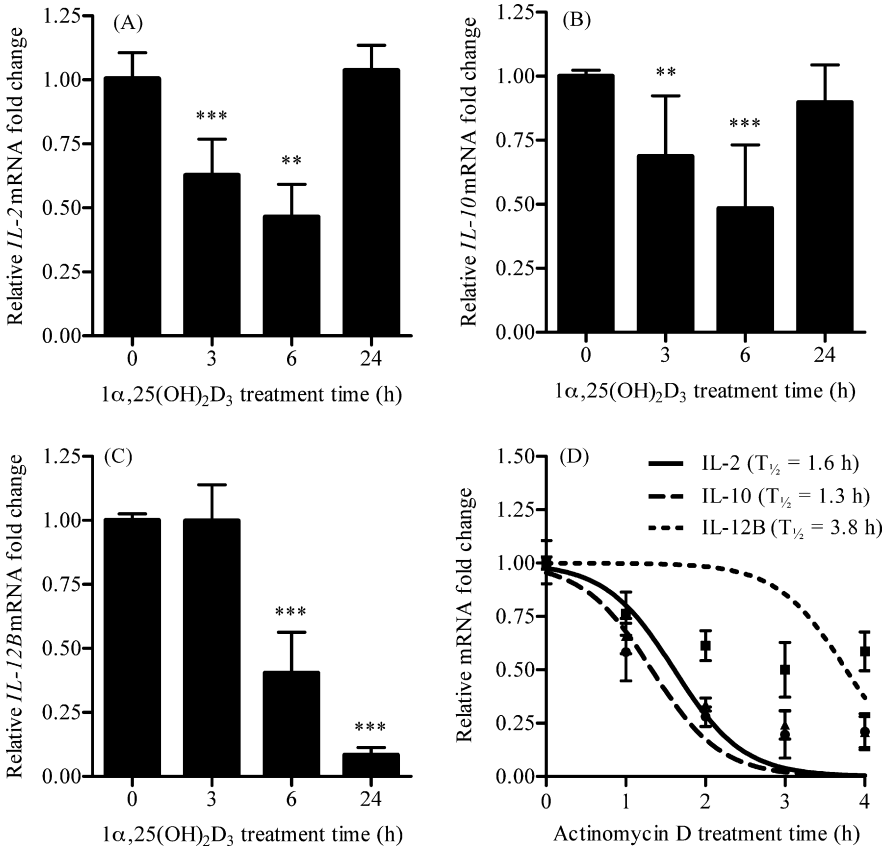

Fig. 1. Expression profiling of the human *IL-2*, *IL-10* and *IL-12B* genes in Jurkat or THP-1 cells. Quantitative real-time PCR was performed in order to study the relative mRNA expression levels of the *IL-2* in activated Jurkat cells (A) and IL-10 (B) and IL-12B (C) in activated THP-1 cells and their responsiveness to 1α,25(OH)$_2$D$_3$ over time. The cells were treated with 10 nM 1α,25(OH)$_2$D$_3$ for indicated times prior to the extraction of RNA. Stabilities of *IL-2*, *IL-10* and *IL-12B* mRNAs in Jurkat or THP-1 cells were studied by treating the cells with actinomycin D (1 μg/ml) prior to extraction of total RNA (D). Two-tailed Student's *t*-tests were performed using Prism4.0c software and *P*-values were calculated in reference to solvent treatments (*P = 0.01 to 0.05, **P = 0.001 to 0.01, ***P < 0.001). In each panel, n is at least 3. Error bars indicate S.D.