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THE 1α,25-DIHYDROXY VITAMIN D3 RECEPTOR PREFERENTIALLY RECRUITS THE COACTIVATOR SRC-1 DURING UP-REGULATION OF THE OSTEOCALCIN GENE

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Abstract

Binding of 1α,25-dihydroxy vitamin D3 to the C-terminal domain (LBD) of its receptor (VDR), induces a conformational change that enables interaction of VDR with transcriptional coactivators such as the members of the p160/SRC family or the DRIP (Vitamin D Interacting Complex)/ Mediator complex. These interactions are critical for VDR-mediated transcriptional enhancement of target genes. Recent reports indicate that nuclear receptors, including VDR, interact with p160/ SRC members and the DRIP/Mediator complex in a sequential, cyclical, and mutually exclusive manner when bound to a target promoter, exhibiting also a high exchange rate. Here, we present an overview of how these coactivators are recruited to the bone-specific osteocalcin (OC) gene in response to short and long exposures to 1α,25-dihydroxy vitamin D3. We find that in intact osteoblastic cells VDR and SRC-1 rapidly bind to the OC promoter in response to the ligand. This recruitment correlates with transcriptional enhancement of the OC gene and with increased histone acetylation at the OC promoter. In contrast, binding of the DRIP205 subunit, which anchors the DRIP/Mediator complex to the VDR, is detected at the OC promoter after several hours of incubation with 1α,25-dihydroxy vitamin D3. Together, our results indicate that VDR preferentially recruits SRC-1 to enhance basal bone-specific OC gene transcription. We propose a model where specific protein-DNA and protein-protein interactions that occur within the context of the OC gene promoter in osteoblastic cells stabilize the preferential association of the VDR-SRC-1 complex.

Keywords

1α,25-dihydroxy vitamin D3; VDR; SRC-1; DRIP; Osteocalcin transcription

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1. Introduction

1α,25-dihydroxy vitamin D3 plays an important role in bone metabolism by directly regulating the expression of bone-related genes [1]. 1α,25-dihydroxy vitamin D3 exerts its genomic effects through the 1α,25-dihydroxy vitamin D3 receptor (VDR) which is a member of the super family of nuclear receptors [2]. As for other nuclear receptors, binding of the ligand induces conformational changes in the C-terminal ligand binding domain (LBD) of VDR that enable it to interact with co-activators of the p160/SRC family such as SRC-1/NCoA-1, SRC-2/NCoA-2/GRIP/TIF2, and SRC-3/ACTR that are critical for transcriptional activation [1,2]. p160/SRC coactivators form high molecular weight complexes by interacting with other co-activator proteins such as p300, its related homologue CBP and P/CAF [3]. Moreover, p160/SRC coactivators have been shown to recruit CBP/p300 and P/CAF to ligand-bound nuclear receptors. These findings indicate that multiprotein complexes containing different activities are functionally linked to ligand-dependent transcriptional regulation [2]. It has been reported that coactivators such as SRC-3/ACTR, SRC-1/NCoA-1, CBP/p300 and P/CAF contain intrinsic histone acetyl transferase (HAT) activity. Therefore, protein complexes including independent HAT activities can be recruited to gene promoters by nuclear receptors in a ligand-dependent manner [2]. Once bound to these promoters, the HAT activities may contribute to chromatin remodelling events that further increase access of additional regulatory factors to their cognate elements [4].

The multisubunit DRIP (VDR-Interacting Protein) complex also binds to VDR in response to the ligand 1α,25-dihydroxy vitamin D3 [5,6]. This interaction occurs through the LBD of VDR in much the same manner as for the p160/SRC co-activators, resulting also in transcriptional enhancement [7]. In contrast to p160/SRC co-activators DRIP is devoid of HAT or another chromatin remodelling activity and interacts with nuclear receptors through a single subunit called DRIP205, which anchors the other subunits to the receptor LBD. Several of these subunits are also present in the Mediator complex, which interacts with the C-terminal domain (CTD) of the RNA polymerase II, forming the holoenzyme complex [8].

Therefore, the DRIP complex appears to function as a transcriptional co-activator by forming a molecular bridge between the VDR and the basal transcriptional machinery.

2. The osteocalcin gene as a model to study 1α,25-dihydroxy vitamin D3-mediated responsiveness

The rat osteocalcin (OC) gene encodes a 10 kDa bone-specific protein that is induced in osteoblasts with the onset of mineralization at late stages of differentiation [9]. Transcription of the OC gene is controlled by modularly organized basal and hormone-responsive elements (see Figure 2B), located within a distal (−600 to −400) and proximal (−170 to −70) promoter regions [10]. A key regulatory element that controls OC gene expression is recognized by the 1α,25-dihydroxy vitamin D3 receptor (VDR) complex upon ligand stimulation. This 1α,25-dihydroxy vitamin D3 responsive element (VDRE) is located in the distal region (Figure 2B) of the OC promoter (positions −465 to −437) and functions as an enhancer to increase OC gene transcription [11]. Another key regulator of the OC gene expression is the transcription factor Runx2, a member of the Runt homology family of proteins [12]. The rat OC gene promoter contains three recognition sites for Runx2 interactions, site A (−605 to −595), site B (−438 to −430), and site C (−138 to −130). Mutation of all three Runx2 sites results in significantly reduced OC expression in bone-derived cells [13].

We have recently shown that within the OC gene promoter context there is a tight functional relationship between Runx2 and the 1α,25-dihydroxy vitamin D3-dependent pathways [14]. Runx2 and VDR are components of the same nuclear complexes and colocalized at punctuate foci within the nucleus of osteoblastic cells [14]. Additionally, mutation of the distal Runx2 sites A and B (which flank the VDRE, see Figure 2B) abolishes 1α,25-dihydroxy vitamin D3-enhanced OC promoter activity [14]. In contrast to most nuclear receptors, VDR does not contain an AF-1 transactivation domain at the N-terminal end. Therefore, VDR is unable to interact with coactivators through this end [2]. We have recently shown that the protein-protein interaction between Runx2 and VDR requires a domain located at the N-terminal end of VDR [14], indicating that Runx2 plays a key role in the 1α,25-dihydroxy vitamin D3-dependent stimulation of the OC gene promoter in osteoblastic cells by directly stabilizing the binding of VDR to the VDRE. Runx2 also allows recruitment of the coactivator p300 to the OC promoter, which results in upregulation of both basal and 1α,25-dihydroxy vitamin D3-enhanced OC gene transcription [15]. Based on these results, we have postulated that Runx2-mediated recruitment of p300 may be facilitating the subsequent interaction of p300 with VDR upon ligand sitimulation [14 and see Figure 2B].

3. SRC-1 and DRIP are recruited to the osteocalcin promoter in a sequential manner

Recent studies have shown that different co-activators are recruited by nuclear receptors to hormone-regulated promoters in a sequential and cyclic manner [16,17,18,19,20]. In particular, it has been reported that in osteoblastic cells there is rapid and cyclic association of VDR with the 24-hydroxylase and osteopontin genes in response to 1α,25-dihydroxy vitamin D3 [20]. This interaction is accompanied by an also rapid, cyclic, sequential and mutually exclusive recruitment of the coactivator complexes SRC-1/p300/CBP and DRIP. Binding of SRC-1/p300/CBP is found correlated with an increase of histone H4 acetylation at the 24-hydroxylase promoter but not at the osteopontin promoter, suggesting that in osteoblastic cells different promoters are modulated by distinct mechanisms in response to 1α,25-dihydroxy vitamin D3.

We have determined that both DRIP205 and SRC-1 coactivators can also contribute to the 1α,25-dihydroxy vitamin D3-mediated enhancement of the OC gene promoter activity (Figure 1). We transiently transfected ROS 17/2.8 osteoblastic cells with a construct containing the full length (1.1 kb) OC promoter controlling the expression of the reporter gene luciferase. This construct contains all the basal tissue-specific and 1α,25-dihydroxy vitamin D3-dependent regulatory elements [15]. The effect of DRIP205 (Figures 1A and 1B) or SRC-1 (Figures 1C and 1D) was assessed by cotransfecting CMV-driven DRIP205 or SRC-1 expression plasmids, respectively. Overexpression of both DRIP205 and SRC-1 results in upregulation of the 1α,25-dihydroxy vitamin D3-dependent enhancement of the OC promoter activity without affecting basal activity. This stimulatory effect requires an intact and functional VDRE, as a mutated version of the OC promoter-Luciferase construct (pmSHE-OC-Luc) that is not recognized by VDR [15] can not be up-regulated by either DRIP205 (Figure 1B) or SRC-1 (Figure 1D) in cells incubated with 1α,25-dihydroxy vitamin D3.

To assess whether DRIP205 and SRC-1 are recruited to the OC gene promoter in ROS 17/2.8 cells exposed to 1α,25-dihydroxy vitamin D3, chromatin immunoprecipitation (ChIP) analyses were carried out [14]. ROS 17/2.8 cells were incubated with 1α,25-dihydroxy vitamin D3 for different periods (0 to 12 hours) and the extend to which these coactivators are associated with the distal OC promoter region was quantified by combining ChIP and real time PCR. We find that VDR binds to the OC promoter shortly (one hour, not shown)

after the cells are exposed to 1α,25-dihydroxy vitamin D3, reaching a peak in binding after 4 hours of incubation with the ligand (Figure 2A). Interaction of VDR is accompanied by recruitment of SRC-1 but not of DRIP205, indicating that SRC-1 is the primary coactivator that is associated with the OC promoter upon 1α,25-dihydroxy vitamin D3 stimulation (Figure 2A and data not shown). RT-PCR and nuclear run off analyses ([10] and not shown) demonstrate that 1α,25-dihydroxy vitamin D3-mediated transcriptional up-regulation is highest after 4 hours of treatment with 1α,25-dihydroxy vitamin D3. In addition, we find that a 1α,25-dihydroxy vitamin D3-mediated increase in histone acetylation at the OC gene promoter occurs within the first 2 hours of incubation with this hormone [21 and data not shown], therefore accompanying 1α,25-dihydroxy vitamin D3-dependent transcriptional enhancement and binding of the VDR-SRC-1 complex to the distal OC promoter.

Interestingly, DRIP205 is only found at the OC promoter of ROS17/2.8 cells after several hours of exposure to 1α,25-dihydroxy vitamin D3 (e.g. 8 hours), when SRC-1 is not longer bound (Figure 2A). Interaction of DRIP205 with the OC gene promoter is tightly associated with increased binding of DRIP130, a key component of the mediator complex (Figure 2A). This may be an indication that a direct interaction between the VDR-associated complex bound to the distal OC promoter and the RNA pol II holoenzyme complex takes place after DRIP205 is recruited to the promoter (see Figure 2B).

4. Conclusions

In conclusion, our results indicate that in osteoblastic ROS 17/2.8 cells exposed to 1α,25-dihydroxy vitamin D3, VDR preferentially recruits the coactivator SRC-1 to upregulate OC gene transcription (see Figure 2B). DRIP205 is found interacting at the OC promoter after several hours of 1α,25-dihydroxy vitamin D3 treatment, concomitant with the release of SRC-1. This is in contrast to previous reports indicating that SRC-1 and DRIP205 are recruited to steroid hormone-regulated promoters in a sequential, mutually exclusive and cyclic manner [16,17,18,19,20]. Hence, a general mechanism including rapid and cyclic coactivator recruitment may not apply to all nuclear receptor-regulated genes and therefore extensive analyses at the different 1α,25-dihydroxy vitamin D3-responsive promoters should be carried out.

Taking together these and previous results, we propose that the preferential recruitment of SRC-1 at the OC gene relies on the specific organization of regulatory elements at the distal region of the promoter (Figure 2B). This organization has been shown to facilitate protein-protein interactions that stabilize binding of Runx2-p300 and VDR-Runx2 to this promoter region [14,15]. As SRC-1 can also form stable complexes with p300, this may provide the basis for the formation of a stable complex at the distal OC promoter region that includes Runx2, p300, VDR, and SRC-1 (see Figure 2B).

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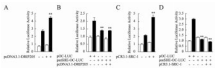

Figure 1.

ROS 17/2.8 cells were transiently transfected with either DRIP205 (pcDNA3.1-DRIP205, A and B) or SRC-1 (pCR3.1-SRC-1, C and D) expression plasmids and the rat OC gene promoter-luciferase reporter construct carrying a wild type (pOC-Luc) or mutated (pmSHE-OC-Luc) VDRE. Cells were incubated in the absence (white bars) or presence (black bars) of 1α,25-dihydroxy vitamin D3 for 24 hours, harvested, and the luciferase activity measured. The combination of plasmids used in each experiment is explained below the graphs. The luciferase activity data were normalized to values for pCMV-β-galactosidase activity as an internal control. Each bar represents the mean +/− standard error of the mean (n=6; $p<0.05$).

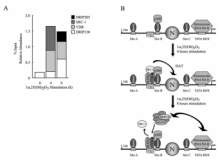

Figure 2.

A) ChIP assays on formaldehyde-cross-linked chromatin isolated from ROS 17/2.8 cells cultured in the presence or absence of 1α,25-dihydroxy vitamin D3. After immunoprecipitation the OC distal promoter region was amplified by specific primers [14] and quantified by real time PCR. The positive signal over the control (non-specific IgG) is represented as percentage of the input chromatin material and indicates the relative abundance of each specific protein (defined at the right of the graph) at the distal OC promoter region. The treatment with 1α,25-dihydroxy vitamin D3 is indicated below the graph. B) Schematic representation of the OC promoter including key regulatory elements and the cognate transcription factors that modulate basal tissue-specific and 1α,25-dihydroxy vitamin D3-enhanced OC transcription in osteoblastic cells. N marks the presence of a positioned nucleosome between the distal and proximal OC promoter regions. VDRE, 1α,25-dihydroxy vitamin D3 responsive element; HAT, histone acetyl transferase activity.