Journal of Cellular Biochemistry 98:1450–1456 (2006)

Functional Role of VDR in the Activation of $p27^{Kip1}$ by the VDR/Sp1 Complex

Hsuen-Tsen Cheng,[1] Jing-Yi Chen,[1] Yu-Chun Huang,[2] Hui-Chiu Chang,[2] and Wen-Chun Hung[1]*

[1]Institute of Biomedical Sciences, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
[2]Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Abstract Our previous study demonstrate that vitamin D_3 induces the binding of vitamin D_3 receptor (VDR) to Sp1 transcription factor and stimulates $p27^{Kip1}$ expression via the Sp1 consensus sequences in the promoter. Both VDR and Sp1 are transcriptional activators, it is unclear which protein functions as the transcription component of the VDR/Sp1 complex. To address this issue, we constructed the AF-2 deletion mutant of VDR and tested the effect of vitamin D_3 on $p27^{Kip1}$ expression. In consistent with our previous results, we found that expression of wild-type VDR in SW620 colon cancer cells, which expressed very low level of endogenous VDR, increased vitamin D_3-stimulated $p27^{Kip1}$ promoter activity and protein expression. On the contrary, expression of AF-2 deletion mutant had little effect. DNA affinity precipitation assay (DAPA) showed that both wild-type and deletion mutant of VDR bound to the DNA probe corresponding to the Sp1 binding site in the $p27^{Kip1}$ promoter in a vitamin D_3-dependent manner indicating deletion of AF-2 domain does not affect the interaction between VDR and Sp1. Chromatin immunoprecipitation (CHIP) assay also confirmed that VDR and its AF-2 deletion mutant bound to $p27^{Kip1}$ promoter in vivo. We found that deletion of AF-2 domain abolished the interaction of coactivators SRC-1 and DRIP205 with VDR. Taken together, our results suggest that VDR functions as the transactivation component of the VDR/Sp1 complex to trigger gene expression. J. Cell. Biochem. 98: 1450–1456, 2006.

Key words: vitamin D_3; $p27^{Kip1}$; Sp1; AF-2 domain

The steroid hormone 1α, 25-Dihydroxyvitamin D_3 (vitamin D_3) is involved in the regulation of a numerous cellular processes including cell differentiation, growth, and apoptosis [Deluca and Cantorna, 2001; Gurlek et al., 2002; Johnson et al., 2002]. The biological effect of vitamin D_3 is mediated via the vitamin D_3 receptor (VDR). Binding of vitamin D_3 to its cognate receptor promotes the formation of VDR/retinoid X receptor (RXR) heterodimer and the heterodimer then binds with the vitamin D_3 response element (VDRE) in the promoter of target genes to trigger gene transcription [Kato, 2000; Khorasanizadeh and Rastinejad, 2001]. In addition to classic genomic action, vitamin D_3 can activate several intracellular signaling pathways such as extracellular signal-regulated kinases (ERKs), c-Src kinase, and phosphorlipases in a non-genomic fashion [Buitrago et al., 2000; Boyan et al., 2002; Christakos et al., 2003; Schwartz et al., 2003].

Recent studies demonstrate that nuclear receptors, instead of direct binding to the response element, may interact with other transcription factors to activate gene transcription. For example, 17β-estradiol (E_2) stimulates the formation of estrogen receptor (ER)/Sp1 complex and activates gene expression via the GC-rich Sp1 site [Sun et al., 1998; Safe, 2001]. Similarly, progesterone and androgen receptor may stimulate $p21^{Waf-1}$ via the Sp1 consensus site by interacting with Sp1 [Owen et al., 1998; Lu et al., 2000].

We are interested in the mechanism of the anti-proliferative effect of vitamin D_3. A previous study showed that vitamin D_3 upregulated $p27^{Kip1}$, a typical cyclin-dependent kinase (CDK) inhibitor to inhibit cell cycle progression at the G1/S transition [Inoue et al., 1999]. The

Grant sponsor: MOE Program for Promoting Academic Excellent of Universities, Ministration of Education.

*Correspondence to: Wen-Chun Hung, Institute of Biomedical Sciences, National Sun Yat-Sen University, No. 70, Lien-Hai Road, Kaohsiung 804, Taiwan.
E-mail: hung1228@ms10.hinet.net

Received 20 November 2005; Accepted 29 November 2005
DOI 10.1002/jcb.20780

authors also indicated that activation of p27^{Kip1} by vitamin D_3 is mediated via Sp1 and NF-Y binding sites localized within the −555/−512 region of the promoter. We extended the results of this work and found that vitamin D_3 induced formation of the VDR/Sp1 complex and acted via the Sp1 binding site in the promoter region to stimulate p27^{Kip1} expression [Huang et al., 2004]. Our data suggest that Sp1 constitutively binds p27^{Kip1} promoter and functions as an anchor protein to recruit VDR to stimulate p27^{Kip1} expression after vitamin D_3 treatment. However, whether VDR indeed functions as the transactivation component of the VDR/Sp1 complex has not been clarified. In this study, we address the function of VDR in the VDR/Sp1 complex.

MATERIALS AND METHODS

Expression Vectors, Reporter Plasmids, Reagents, and Cell Culture

CMV–VDR expression vector was obtained from Dr. Freedman [Towers et al., 1993]. Human p27^{Kip1} promoter-luciferase construct were kindly provided by Dr. Sakai [Minami et al., 1997]. Sp1, SRC-1, DRIP205, and p27^{Kip1} antibodies were purchased from Santa Cruz (Santa Cruz, CA). Anti-VDR antibody was obtained from Biomol (Polymouth Meeting, PA). LipofectAMINE was obtained from Invitrogen (Carlsbad, CA) and luciferase assay system was obtained from Promega (Madison, WI). Vitamin D_3 was purchased from Calbiochem. SW620 human colon cancer cells were cultured in DMEM/F12 medium containing 10% charcoal-treated fetal calf serum (FCS) and antibiotics.

Construction of AF-2 Deletion Mutant of VDR

The AF-2 deletion mutant was generated from the CMV–VDR expression vector, which contained full-length VDR (1,281 bp) by deletion the C-terminal 165-bp nucleotides. The sequence of the deletion mutant was verified by direct sequencing.

Promoter Activity Assays

Promoter activity assays were performed as described previously [Huang et al., 2004]. In brief, cells were plated onto 6-well plates at the density of 100,000 cells/well and grown overnight. Cells were co-transfected with 2 μg of p27^{Kip1} promoter-luciferase plasmid and 1 μg of wild-type or AF-2 deletion mutant of VDR expression vectors. After transfection, cells were cultured in 10% charcoal-treated FCS medium for 24 h followed by stimulation with vehicle (0.5% DMSO) or vitamin D_3 (10 nM) for another 48 h. Luciferase activity was determined by using a reporter assay system (Promega) and normalized for protein concentration in cell lysate. Data of three independent experiments were expressed as mean ± SD. Paired results were evaluated by the Student's *t*-test and *P*-value <0.05 was considered significant.

Immunoprecipitation and Immunoblotting

For immunoblotting, cells were harvested in a lysis buffer and equal amount of cellular proteins was subjected to SDS-polyacrylamide gel electrophoresis (SDS–PAGE) as described previously [Pan and Hung, 2002]. Proteins were transferred to nitrocellulose membranes and blots were probed with different primary antibodies followed by horseradish peroxidase-labeled secondary antibodies. Enhanced chemiluminescence reagent was used to depict the protein bands on the blots. For immunoprecipitation, cellular proteins (200 μg) were incubated with anti-VDR antibody at 4°C for 2 h and the immunocomplex was collected by protein A/G agarose. Proteins were released by boiling in SDS–PAGE loading buffer and then subjected to SDS–PAGE analysis. Immunoblotting was performed by probing the blots with anti-SRC-1 or anti-DRIP205 antibody to detect the interaction between VDR and cofactors.

DNA Affinity Precipitation Assay (DAPA)

We identified and quantified the proteins bound to a sequence-specific DNA probe by using DNA–protein binding assays. Biotinated DNA probe was used to interact with nuclear proteins and the DNA–protein complexes were then precipitated by streptavidin-coated beads. After centrifugation, the pelleted beads were collected and washed, and proteins bound to the DNA probe were eluted by SDS–PAGE loading buffer and separated by 7.5% polyacrylamide gels. The separated proteins were analyzed by immunoblotting. The sequence of DNA probe used in this study is: 5′-CGAGCCTCGGCGGGGCGGCTCCCGCCGCCGCAACCAATGGATCT-3′ corresponding to the −555/−512 region of human p27^{Kip1} promoter. Preparation of nuclear extracts and DAPA assays were performed as described previously [Huang

et al., 2004]. Nuclear proteins bound to the DNA probe were separated by SDS–PAGE and immunoblotting was done to investigate the proteins that bound to the DNA probe.

Chromatin Immunoprecipitation (CHIP) Assay

Cells were transfected with wild-type or AF-2 deletion mutant of VDR expression vectors. After transfection, cells were cultured in 10% charcoal-treated FCS medium for 24 h followed by stimulation with vehicle (0.5% DMSO) or vitamin D_3 (10 nM) for another 48 h. Cells were fixed with 1% formaldehyde at 37°C for 10 min, washed with ice-cold PBS with protease inhibitors (1 mM phenylmethylsulphonyl fluoride, 1 μg/ml aprotinin, and 1 μg/ml pepstatin A), scraped and pelleted by centrifugation at 4°C. Cells were resuspended in lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1), incubated for 10 min on ice, and sonicated to shear DNA. After sonication, lysate was centrifuged for 10 min at 13,000 rpm at 4°C. The supernatant was diluted in CHIP dilution buffer (0.01% SDS, 1% Triton X-100, 2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl, and protease inhibitors). Anti-VDR antibody was added to the supernatant and incubated overnight at 4°C with rotation. The immunocomplex was collected by protein A/G agarose and washed sequentially with low salt washing buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 200 mM Tris-HCl, pH 8.1, and 150 mM NaCl), high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 200 mM Tris-HCl, pH 8.1, and 500 mM NaCl), LiCl washing buffer (0.25 M LiCl, 1% NP40, 1% deoxycolate, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.1), and finally 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The immunocomplex was eluted by elution buffer (1% SDS, 0.1 M $NaHCO_3$, and 200 mM NaCl) and the cross-links were reversed by heating at 65°C for 4 h. After reaction, the samples were adjusted to 10 mM EDTA, 20 mM Tris-HCl, pH 6.5, and 40 μg /ml Proteinase K, and incubated at 45°C for 1 h. DNA was recovered and was subjected to PCR amplification by using the primers specific for the detection of the −690/−455 region which contained the Sp1 transcription factor binding sites of human $p27^{Kip1}$ promoter. The sequences for the primers are: sense 5′-CAGGTTTGTTGGCAGCAG TA-3′, antisense 5′-GGAGGCTGACGAAGAA GAAA-3′. The predicted size for the PCR product is 235 bp.

RESULTS

Construction and Expression of VDR AF-2 Deletion Mutant

We first deleted the AF-2 domain of full-length VDR cDNA to create the AF-2 deletion mutant and checked whether the AF-2 deletion mutant could be expressed in SW620 cells. As demonstrated in Figure 1, SW620 cells have very few endogenous VDR. Wild-type or AF-2 deletion mutant of VDR was detected after transfection of expression vectors. However, the protein level of AF-2-deleted VDR was about a half of that of wild-type VDR. The reason for this difference is unknown. Previous studies have indicated that VDR was degraded via the ubiquitin/proteasome pathway [Li et al., 1999]. It is possible that deletion of AF-2 may affect the synthesis or stability of VDR. Transfection of cells with double amount of AF-2 deletion mutant plasmid produced similar protein level of mutant VDR compared to that of wild-type VDR-transfected cells. Therefore, we used double amount of mutant plasmid to transfect cells in our subsequent experiments.

Deletion of AF-2 Domain Reduced Vitamin D_3-Induced $p27^{Kip1}$ Promoter Activity and Protein Expression

We next tested the effect of vitamin D_3 on the $p27^{Kip1}$ promoter activity in cells expressing wild-type or mutant VDR. Our results showed that vitamin D_3 stimulated a 2.4-fold increase of the $p27^{Kip1}$ promoter activity in cells transfected with VDR expression vector. However, no increase was observed in cells transfected with AF-2-deleted mutant expression vector (Fig. 2A). Western blot analysis also confirmed that vitamin D_3-induced increase of $p27^{Kip1}$ protein was not observed in cells transfected with VDR AF-2 deletion mutant (Fig. 2B). These data strongly suggest that VDR functions as the

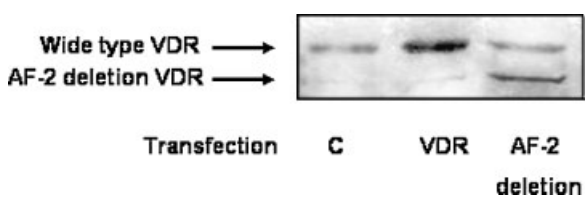

Fig. 1. Expression of VDR and its AF-2 deletion mutant in SW620 cells. Cells were transfected with control vector (C), VDR expression vector (VDR), or AF-2 deletion mutant for 48 h. Cells were harvested and subjected to Western blot analysis to investigate the VDR protein level.

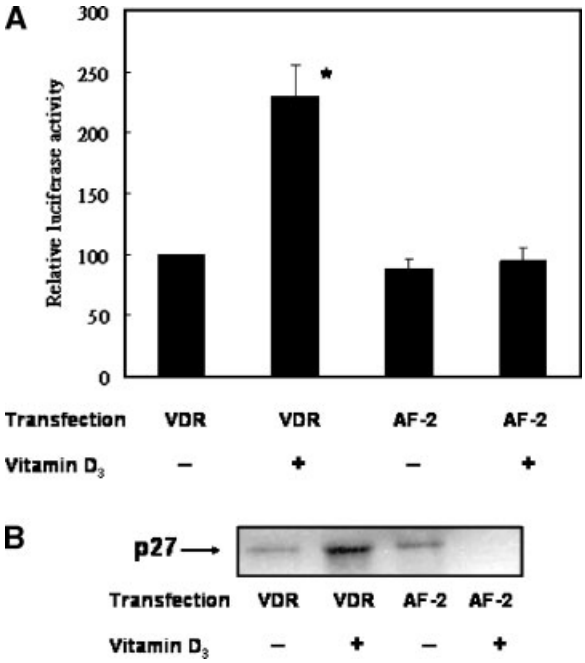

Fig. 2. Effect of AF-2 deletion on vitamin D_3-increased p27^{Kip1} promoter activity and protein expression. **A**: Cells were co-transfected with p27^{Kip1} promoter-luciferase construct and VDR or AF-2 deletion mutant expression vector for 24 h and incubated with vehicle (–) or 10 nM of vitamin D_3 (+) for another 48 h. Promoter activity was assayed and results of three independent experiments were expressed as mean ± SD. Promoter activity of cells treated without or with vitamin D_3 was compared by the Student's *t*-test. *represented *P*-value <0.05. **B**: Cells were transfected with VDR or AF-2 deletion mutant expression vector for 24 h and incubated with vehicle (–) or 10 nM of vitamin D_3 (+) for another 48 h. Cellular proteins were extracted and the expression of p27^{Kip1} protein was examined by Western blot analysis.

transcactivation component of the VDR/Sp1 complex.

Deletion of AF-2 Domain of VDR does not Affect its Interaction With Sp1 In Vitro and In Vivo

Although the aforementioned results suggest that VDR functions as the trans-activation component of the VDR/Sp1 complex. However, we cannot rule out the possibility that deletion of AF-2 may lead to disrupt of the interaction between VDR and Sp1 and reduce vitamin D_3-stimulated p27^{Kip1} promoter activity. Therefore, we investigated the binding of VDR AF-2 deletion mutant with Sp1. We performed DAPA assay to study the binding of VDR and its deletion mutant to the response element localized within the −555/−512 region of the p27^{Kip1} promoter. As shown in Figure 3A, we found that Sp1 bound to the DNA probe as expected since the DNA probe contains binding site for this transcription factors. The binding affinity of Sp1 to the DNA probe was not significantly changed after treatment of vitamin D_3. However, treatment of vitamin D_3 obviously increased a 3–4 fold (expressed as the density ratio of VDR/Sp1) of the binding of wild-type VDR to the DNA probe (Fig. 3A,B). A similar increase of the interaction between AF-2 deletion mutant was also found after incubation of vitamin D_3. These results indicate that deletion of AF-2 domain does not affect the interaction between VDR and Sp1. To verify that VDR and its AF-2 deletion mutant indeed bound to the −555/−512 region of human p27^{Kip1} promoter in vivo, we performed CHIP assay with

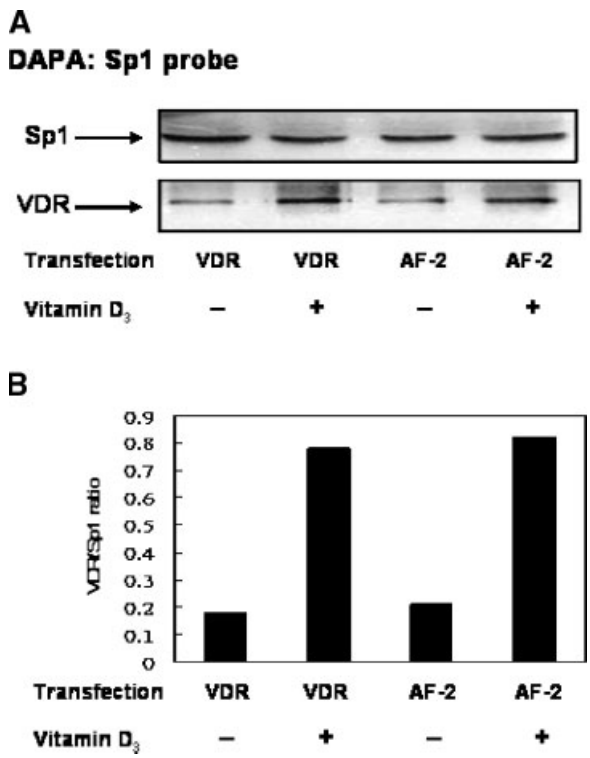

Fig. 3. Binding of VDR and its AF-2 deletion mutant to the DNA probe corresponding to the −555/−512 region of human p27^{Kip1} promoter. **A**: SW620 cells were transfected with VDR or AF-2 deletion mutant expression vector for 24 h and incubated with vehicle (–) or 10 nM of vitamin D_3 (+) for another 48 h. Nuclear proteins were incubated with 6 μg of biotinated DNA probe and 45 μl of 4% streptavidin-coated beads (Sigma) at room temperature for 1 h with shaking. Proteins bound to the beads were eluted and separated by SDS–PAGE. Western blot analysis was performed as described above to investigate the proteins that bound to the DNA probe. **B**: The density of each band in the immunoblot was measured and the VDR/Sp1 ratio of a typical experiment was shown.

anti-VDR antibody to precipitate VDR. As demonstrated in Figure 4A, VDR was found to be physically associated with the promoter region of $p27^{Kip1}$ in cell extracts precipitated by VDR-specific antibody. Density measurement indicated a about threefold increase of VDR/input control ratio was observed after vitamin D_3 treatment (Fig. 4B). A similar increase was also observed in SW620 cells transfected with AF-2 deletion mutant. Precipitation with anti-$p57^{Kip2}$ antibody did not give any specific band [Huang et al., 2004 and data not shown]. Therefore, AF-2 deletion mutant of VDR could bind to the $p27^{Kip1}$ promoter in vivo. Taken together, deletion of AF-2 domain of VDR does not affect its interaction with Sp1.

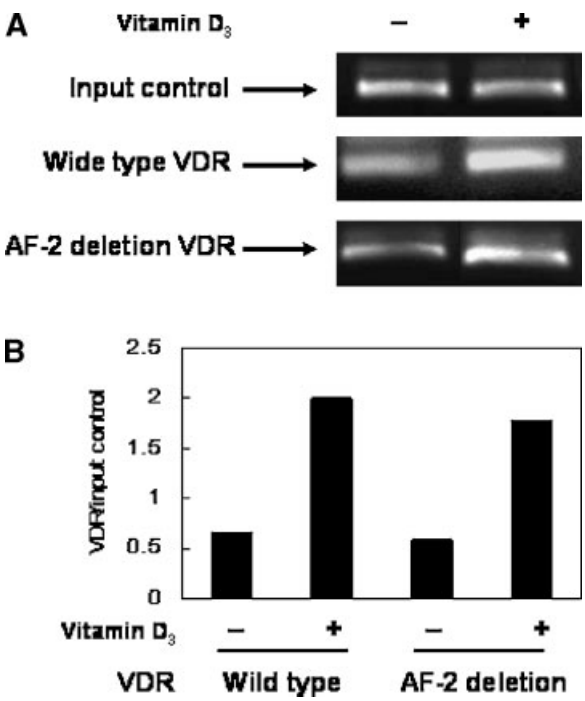

Fig. 4. Association of VDR and its AF-2 deletion with $p27^{Kip1}$ promoter in vivo. **A**: SW620 cells were transfected with VDR or AF-2 deletion mutant expression vector for 24 h and incubated with vehicle (−) or 10 nM of vitamin D_3 (+) for another 24 h. DNA and binding proteins were cross-linked with 1% formaldehyde. Nuclear lysate was prepared and incubated with anti-VDR antibody. The immuno-complex was precipitated with protein A/G agarose and DNA was then recovered and was subjected to PCR amplification by using the primers specific for the detection of the −690/−455 region which contained the Sp1 binding sites of human $p27^{Kip1}$ promoter. The sequences for the primers are: sense 5′-CAGGTTTGTTG GCAGCAGTA-3′, antisense 5′-GGAGGCTGACGAAGAAGAAA-3′ and the predicted size for the PCR product is 235 bp. **B**: The density of each band in the gel was measured and the VDR/input control ratio of a typical experiment was shown.

Deletion of AF-2 Attenuated the Binding of VDR With Coactivators

Nuclear receptors require coactivators to stimulate gene transcription. If VDR indeed functions as the transcription component of the VDR/Sp1 complex, it is reasonable to speculate that reduction of transcriptional activity of the VDR/Sp1 complex might be caused by the disruption of the interaction between VDR and coactivators. Therefore, we investigated the interaction of VDR and coactivators by immunoprecipitation/immunoblotting assay. Indeed, our results indicated that wild-type VDR interacted with SRC-1 and DRIP205, two typical coactivators for VDR. However, these two coactivators could not be co-immunoprecipitated with AF-2 deletion mutant (Fig. 5). Taken together, our data suggest that deletion of AF-2 domain of VDR abolishes its interaction with coactivators and reduces the transcriptional activity of the VDR/Sp1 transcription complex.

DISCUSSION

Steroid hormones exert their biological actions mainly via the nuclear receptor-mediated control of target genes. However, recent studies demonstrate that steroid hormones may act via other transcription factors to activate target genes. Two pioneer studies

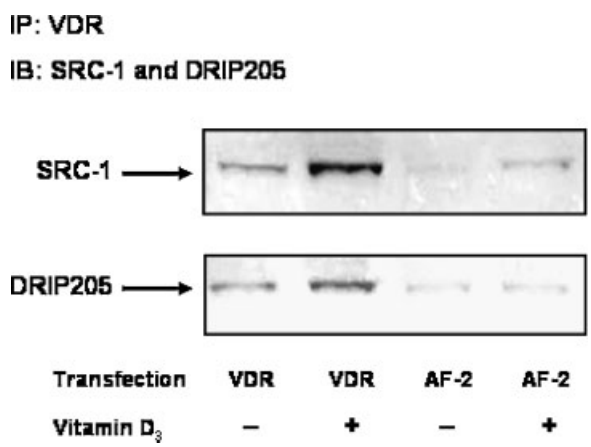

Fig. 5. Deletion of AF-2 abolishes the interaction between VDR and coactivators. SW620 cells were transfected with VDR or AF-2 deletion mutant expression vector for 24 h and incubated with vehicle (−) or 10 nM of vitamin D_3 (+) for another 24 h. Immunoprecipitation was performed by anti-VDR antibody and protein A/G agarose beads were added to pellet the antigen-antibody complex. VDR-associated proteins were eluted and subjected to Western blot analysis by probing the blots with anti-SRC1 or -DRIP205 antibody to detect the interaction between VDR and coactivators.

demonstrated that ER interacted with AP1 transcription factors to activate gene expression [Gaub et al., 1990; Weisz and Rosales, 1990]. Subsequently, ER had also been shown to bind with Sp1 protein to induce cathepsin D expression in MCF-7 breast cancer cells [Krishnan et al., 1994]. More recently, a number of studies confirmed the results of these works and demonstrated that E_2 stimulated a number of target genes including E2F, c-fos, and bcl-2 through one or more GC-rich Sp1 binding sites [Duan et al., 1998; Wang et al., 1998; Dong et al., 1999]. These studies establish a new model by which steroid hormones activate gene expression and provide a new concept that steroid hormone can stimulate the expression of genes in which no consensus hormone response elements are identified in their promoters.

We have provided the first evidence that VDR may physically interact with Sp1 to activate $p27^{Kip1}$ promoter via a GC-rich Sp1 site. Interestingly, two recent studies also demonstrate that VDR interacts with STAT-1 and β-catenin to modulate gene expression [Palmer et al., 2001; Vidal et al., 2002]. These results strongly support the notion that VDR may activate gene transcription via interaction with other transcription factors and the number of target genes, which can be regulated by vitamin D_3 is larger than originally thought. However, the molecular mechanism by which VDR enhances Sp1-mediated transactivation is unclear. Two possible models for the functional interaction between VDR and Sp1 can be suggested. First, VDR may increase the stability of Sp1–GC-rich DNA interaction and trigger the expression of target genes via the Sp1 transactivation domain. Second, Sp1 may serve as an anchor protein for VDR. After binding, VDR may recruit the transcription apparatus by its own transactivation domain to induce gene expression. Our data support the second model by demonstrating that deletion of AF-2 domain of VDR does not affect VDR/Sp1 interaction but obviously reduce the transcriptional activity of the VDR/Sp1 complex on the activation of $p27^{Kip1}$. A recent study also showed that ER is the transcription component of the ER/Sp1 complex and AF-1 and -2 domains are important for the activation of gene expression via the ER/Sp1 complex [Kim et al., 2003]. Results of ours and others studies suggest that nuclear receptor functions as the transcription component of the nuclear receptor/transcription factor complex.

Because vitamin D_3 plays an important role in the regulation of phosphorous and calcium homeostasis, cell growth, differentiation, and apoptosis, it is possible that vitamin D_3 may control some of genes that involved in these physiological processes via the VDR/Sp1 complex and identification of these target genes will be helpful for the understanding of the biological action of vitamin D_3.

ACKNOWLEDGMENTS

We deeply thank Dr. L. P. Freedman and Dr. T. Sakai for providing experimental materials. The study was funded by MOE Program for Promoting Academic Excellent of Universities, Ministration of Education to Wen-Chun Hung, 91-B-FA09-1-4.

REFERENCES

Boyan BD, Sylvia VL, Dean DD, Schwartz Z. 2002. Membrane mediated signaling mechanisms are used differentially by metabolites of vitamin D(3) in musculoskeletal cells. Steroids 67:421–427.

Buitrago C, Vazquez G, De Boland AR, Boland RL. 2000. Activation of Src kinase in skeletal muscle cells by 1, 1,25-(OH(2))-vitamin D(3) correlates with tyrosine phosphorylation of the vitamin D receptor (VDR) and VDR-Src interaction. J Cell Biochem 79:274–281.

Christakos S, Barletta F, Huening M, Dhawan P, Liu Y, Porta A, Peng X. 2003. Vitamin D target proteins: Function and regulation. J Cell Biochem 88:238–244.

Deluca HF, Cantorna MT. 2001. Vitamin D: Its role and uses in immunology. FASEB J 15:2579–2585.

Dong L, Wang W, Wang F, Stoner M, Reed JC, Harigai M, Samudio I, Kladde MP, Vyhlidal C, Safe S. 1999. Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. J Biol Chem 274:32099–32107.

Duan R, Porter W, Safe S. 1998. Estrogen-induced c-fos protooncogene expression in MCF-7 human breast cancer cells: Role of estrogen receptor Sp1 complex formation. Endocrinology 139:1981–1990.

Gaub MP, Bellard M, Scheuer I, Chambon P, Sassone-Corsi P. 1990. Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. Cell 63:1267–1276.

Gurlek A, Pittelkow MR, Kumar R. 2002. Modulation of growth factor/cytokine synthesis and signaling by 1alpha,25-dihydroxyvitamin D(3): Implications in cell growth and differentiation. Endocr Rev 23:763–786.

Huang YC, Chen JY, Hung WC. 2004. Vitamin D3 receptor/Sp1 complex is required for the induction of p27Kip1 expression by vitamin D3. Oncogene 23:4856–4861.

Inoue T, Kamiyama J, Sakai T. 1999. Sp1 and NF-Y synergistically mediate the effect of vitamin D(3) in the p27(Kip1) gene promoter that lacks vitamin D response elements. J Biol Chem 274:32309–32317.

Johnson CS, Hershberger PA, Trump DL. 2002. Vitamin D-related therapies in prostate cancer. Cancer Metastasis Rev 21:147–158.

Kato S. 2000. The function of vitamin D receptor in vitamin D action. J Biochem 127:717–722.

Khorasanizadeh S, Rastinejad F. 2001. Nuclear-receptor interactions on DNA-response elements. Trends Biochem Sci 26:384–390.

Kim K, Thu N, Saville B, Safe S. 2003. Domains of estrogen receptor alpha (ERalpha) required for ERalpha/Sp1-mediated activation of GC-rich promoters by estrogens and antiestrogens in breast cancer cells. Mol Endocrinol 17:804–817.

Krishnan V, Wang X, Safe S. 1994. Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 human breast cancer cells. J Biol Chem 269:15912–15917.

Li XY, Boudjelal M, Xiao JH, Peng ZH, Asuru A, Kang S, Fisher GJ, Voorhees JJ. 1999. 1,25-Dihydroxyvitamin D3 increases nuclear vitamin D3 receptors by blocking ubiquitin/proteasome-mediated degradation in human skin. Mol Endocrinol 13:1686–1694.

Lu S, Jenster G, Epner DE. 2000. Androgen induction of cyclin-dependent kinase inhibitor p21 gene: Role of androgen receptor and transcription factor Sp1 complex. Mol Endocrinol 14:753–760.

Minami S, Ohtani-Fujita N, Igata E, Tamaki T. Sakai T. 1997. Molecular cloning and characterization of the human p27Kip1 gene promoter. FEBS Lett 411:106.

Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. 1998. Progesterone regulates transcription of the p21(WAF1) cyclin-dependent kinase inhibitor gene through Sp1 and CBP/p300. J Biol Chem 273:10696–10701.

Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Munoz A. 2001. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 154:369–387.

Pan MR, Hung WC. 2002. Nonsteroidal anti-inflammatory drugs inhibit matrix metalloproteinase-2 via suppression of the ERK/Sp1-mediated transcription. J Biol Chem 277:32775–32780.

Safe S. 2001. Transcriptional activation of genes by 17 beta-estradiol through estrogen receptor-Sp1 interactions. Vitam Hor 62:231–252.

Schwartz Z, Shaked D, Hardin RR, Gruwell S, Dean DD, Sylvia VL, Boyan BD. 2003. 1alpha,25(OH)2D3 causes a rapid increase in phosphatidylinositol-specific PLC-beta activity via phospholipase A2-dependent production of lysophospholipid. Steroids 68:423–437.

Sun G, Porter W, Safe S. 1998. Estrogen-induced retinoic acid receptor alpha 1 gene expression: Role of estrogen receptor-Sp1 complex. Mol Endocrinol 12:882–890.

Towers TL, Luisi BF, Asianov A, Freedman LP. 1993. DNA target selectivity by the vitamin D3 receptor: Mechanism of dimer binding to an asymmetric repeat element. Proc Natl Acad Sci USA 90:6310–6314.

Vidal M, Ramana CV, Dusso AS. 2002. Stat1-vitamin D receptor interactions antagonize 1,25-dihydroxyvitamin D transcriptional activity and enhance stat1-mediated transcription. Mol Cell Biol 22:2777–2787.

Wang F, Hoivik D, Pollenz R, Safe S. 1998. Functional and physical interactions between the estrogen receptor Sp1 and nuclear aryl hydrocarbon receptor complexes. Nucleic Acids Res 26:3044–3052.

Weisz A, Rosales R. 1990. Identification of an estrogen response element upstream of the human c-fos gene that binds the estrogen receptor and the AP-1 transcription factor. Nucleic Acids Res 18:5097–5106.