0090-9556/04/3207-685–688$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 32, No. 7
1206/1157672
DMD 32:685–688, 2004
Printed in U.S.A.

Short Communication

C-JUN N-TERMINAL KINASE MODULATES 1,25-DIHYDROXYVITAMIN D_3-INDUCED CYTOCHROME P450 3A4 GENE EXPRESSION

Received June 23, 2003; accepted March 26, 2004

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

1,25-Dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) is known to induce the expression of cytochrome P450 3A4 (CYP3A4) in human colon carcinoma Caco-2 cells. Recently, it was demonstrated that the vitamin D receptor (VDR) regulates 1,25$(OH)_2D_3$-induced *CYP3A4* gene expression through the xenobiotic-responsive element and the vitamin D-responsive element located on the 5′-flanking region of the *CYP3A4* gene. On the other hand, we previously reported that protein kinases such as protein kinase C and tyrosine kinases contribute to the induction of CYP3A4 mRNA by 1,25$(OH)_2D_3$. In the present study, we examined the involvement of mitogen-activated protein kinases (MAPKs) in the 1,25$(OH)_2D_3$-induced *CYP3A4* gene expression using MAPK inhibitors. Curcumin, a c-Jun N-terminal kinase (JNK) pathway inhibitor, and anthra[1,9-cd]pyrazole-6(2*H*)-one (SP600125), a JNK inhibitor, suppressed the induction of CYP3A4 mRNA by 1,25$(OH)_2D_3$, but not 2′-amino-3′-methoxyflavone (PD098059), a mitogen-activated protein kinase kinase-extracellular signal-regulated kinase (ERK) pathway inhibitor, or 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole (SB203580), a p38 inhibitor. In addition, we demonstrated that SP600125 dose-dependently inhibited the *CYP3A4* promoter activity induced by 1,25$(OH)_2D_3$ using the reporter plasmid of the *CYP3A4* promoter. However, SP600125 did not affect 1,25$(OH)_2D_3$-induced transactivation of the DR3 via VDR. These results indicate that JNK, but not ERK or p38, is required for the optimal activation of the *CYP3A4* gene induced by 1,25$(OH)_2D_3$.

Cytochrome P450 (P450) plays an important role in the oxidative metabolism of numerous endogenous and exogenous compounds. In humans, cytochrome P450 3A4 (CYP3A4) is the predominant P450 isoform in the liver and small intestinal epithelial cells (Watkins et al., 1987; Paine et al., 1997) and is responsible for the metabolism of more than 60% of therapeutic drugs. Intestinal CYP3A4 is thought to contribute to the first-pass metabolism of orally administered drugs (Paine et al., 1997). The *CYP3A4* gene is inducible by many xenobiotics, including rifampicin, dexamethasone, and phenobarbital (Pichard et al., 1990). The nuclear receptor, pregnane X receptor, is known to contribute to the *CYP3A4* gene induction by these drugs (Kliewer et al., 1998; Lehmann et al., 1998; Goodwin et al., 1999). It has recently been demonstrated that 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) is a potent inducer of the *CYP3A4* gene in the human colon carcinoma cell line Caco-2, which has been extensively used as an experimental model of small intestinal cells (Schmiedlin-Ren et al., 1997; Hara et al., 2000).

1,25$(OH)_2D_3$, the most active metabolite of vitamin D, functions to regulate cellular proliferation and differentiation and calcium homeostasis in the intestine, bone, and kidney (Christakos et al., 1996). Most of these physiological activations are mediated by the vitamin D receptor (VDR), which belongs to the nuclear hormone receptor superfamily. The VDR acts as a ligand-inducible transcription factor through heterodimerization with the retinoid X receptor and binding to the vitamin D response element (VDRE) within the vitamin D-inducible genes (Christakos et al., 1996).

Recently, a direct repeat separated by three nucleotides (DR3) and an everted repeat separated by six nucleotides (ER6) within the 5′-flanking region of the *CYP3A4* gene were identified as VDRE (Thummel et al., 2001; Drocourt et al., 2002). We and others demonstrated that the VDR is an essential factor for 1,25$(OH)_2D_3$-induced CYP3A4 expression (Thummel et al., 2001; Drocourt et al., 2002; Hara et al., 2002). On the other hand, several reports have demonstrated that the phosphorylation step is critical for the expression of xenobiotic-induced P450 genes such as *CYP1A1* and *CYP3A* (Chen and Tukey, 1996; Galisteo et al., 1999). We also showed that alteration of the cellular phosphorylation state mediated by protein kinase C (PKC) and protein tyrosine kinases affects the 1,25$(OH)_2D_3$-mediated induction of CYP3A4 mRNA in Caco-2 cells (Hara et al., 2002). In addition, the inhibition of the phosphorylation reduced the VDR-mediated enhancement of osteocalcin gene transcription (Desai et al., 1995). These results suggest that the phosphorylation step is critical for the complete CYP3A4 induction by 1,25$(OH)_2D_3$ via VDR. In the present study, we examined whether MAPKs are involved in the 1,25$(OH)_2D_3$-induced expression of the *CYP3A4* gene via VDR.

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

ABBREVIATIONS: P450, cytochrome P450; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; VDR, vitamin D receptor; VDRE, vitamin D response element; DR3, direct repeat separated by three nucleotides; ER6, everted repeat separated by six nucleotides; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PD098059, 2′-amino-3′-methoxyflavone; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; SP600125, anthra[1,9-cd]pyrazole-6(2*H*)-one; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; RT-PCR, reverse transcription-polymerase chain reaction; TK, thymidine kinase.

Materials and Methods

Chemicals. $1,25(OH)_2D_3$ (purity; 99%) was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). PD098059 was purchased from Wako Pure Chemicals (Osaka, Japan). Curcumin was purchased from Nacalai Tesque (Kyoto, Japan). SB203580 was purchased from Calbiochem (San Diego, CA). SP600125 was synthesized as previously described (Möhlau, 1912a,b).

Cell Culture and Treatment. Caco-2 cells were grown and were treated with reagents as previously described (Hara et al., 2002). In brief, after subconfluent cells were maintained for 24 h in serum-free medium, the cells were pretreated with the mitogen-activated protein kinase kinase-ERK pathway inhibitor PD098059 (Davies et al., 2000), the JNK pathway inhibitor curcumin (Chen and Tan, 1998), the JNK inhibitor SP600125 (Bennett et al., 2001), and the p38 inhibitor SB203580 (Davies et al., 2000) for 30 min at the concentrations indicated in the figure legends, followed by incubation for 24 h in the presence of 100 nM $1,25(OH)_2D_3$.

RT-PCR Reaction. RT-PCR was performed as previously described (Hara et al., 2002). The CYP3A4 and VDR mRNA levels were normalized to the glyceraldehydes-3-phosphate dehydrogenase mRNA level in each sample.

Construction of Plasmids. The VDR expression plasmid was constructed as follows. Human VDR cDNA was generated by PCR amplification of human small intestine cDNA (BD Biosciences Clontech, Palo Alto, CA) using VDR-specific primers (forward, 5′-AGGGATCCATGGAGGCAATGGCGGC-CAGCA-3′; reverse, 5′-CAGAATTCAGGAGATCTCATTGCCAAA-3′). The VDR cDNA obtained was inserted into the pcDNA FLAG vector (previously constructed in our laboratory) digested with BamHI/XhoI. The CYP3A4 promoter-reporter plasmids were constructed as follows: the 5′-flanking region of the *CYP3A4* gene between −315 and +22 was generated by PCR amplification of human genomic DNA. The fragment was inserted into the XhoI/HindIII sites of the pGL3 basic vector (Promega, Madison, WI). This construct was designated as 3A4(−315)-pGL3. It was reported that VDR binds to two sites within the *CYP3A4* gene 5′-flanking region and regulates the induction by $1,25(OH)_2D_3$ (Thummel et al., 2001; Drocourt et al., 2002). One site containing one ER6 element is located at approximately −160, and the other site containing one ER6 element and two DR3 elements is located from −8000 to −7000 (Sueyoshi and Negishi, 2001). Therefore, we used the reporter plasmid, 3A4(−7836/−315)-pGL3, to which the distal enhancer from −7836 to −7200 was connected upstream of the proximal promoter from −315 to +22, as the *CYP3A4* promoter. The plasmid was constructed by inserting the fragment between −7836 and −7200 of the *CYP3A4* gene amplified by PCR of human genomic DNA into the MluI/XhoI sites of the plasmid 3A4(−315)-pGL3. The primers used were as follows: the −315/+22 upstream primer 5′-CACTC-GAGGACAGCCATAGAGACAAGGGCA-3′, the −315/+22 downstream primer 5′-CATGAAGCTTTCCTGCCCTGCACA-3′, the −7836/−7200 upstream primer 5′-CTACGCGTTCTAGAGAGATGGTTCATTCCT-3′, and the −7836/−7200 downstream primer 5′-GTCTCGAGAATGATCTCGT-CAACAGGTT-3′. The $(DR3)_3$-TK-pGL3 reporter plasmid was generated by inserting the fragment containing three copies of the DR3 element (AGGT-CAAGGAGGTCA), which is known as a typical VDRE, upstream of the luciferase gene driven by the thymidine kinase promoter in the pGL3 basic vector. The fragment was obtained by annealing two oligonucleotides (sense oligonucleotide 5′-CAGGTCAAGGAGGTCAAGGAGGTCAAGGAGGTCAA-GGAGGTCAAGGAGGTCAGA-3′, and antisense oligonucleotide 5′-CGCG-TCTGACCTCCTTGACCTCCTTGACCTCCTTGACCTCCTTGACCTCCT-TGACCTGAGCT-3′).

Transfection Study. Caco-2 cells were cultured to 80 to 90% confluence in 35-mm dishes and transfected using LipofectAMINE 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's directions with FLAG-VDR expression vector or the empty expression vector and luciferase-reporter plasmids. Three hours later, the mixture was replaced with Dulbecco's modified Eagle's medium containing 10% fetal calf serum, and the cells were maintained for 21 h, followed by treatment with $1,25(OH)_2D_3$ for 24 h. Then luciferase activities of *Photinus* and *Renilla* in the cell lysates were measured by a dual luciferase reporter assay system (Promega). *Renilla* luciferase activity was used to normalize all *Photinus* luciferase values of transfection efficiency.

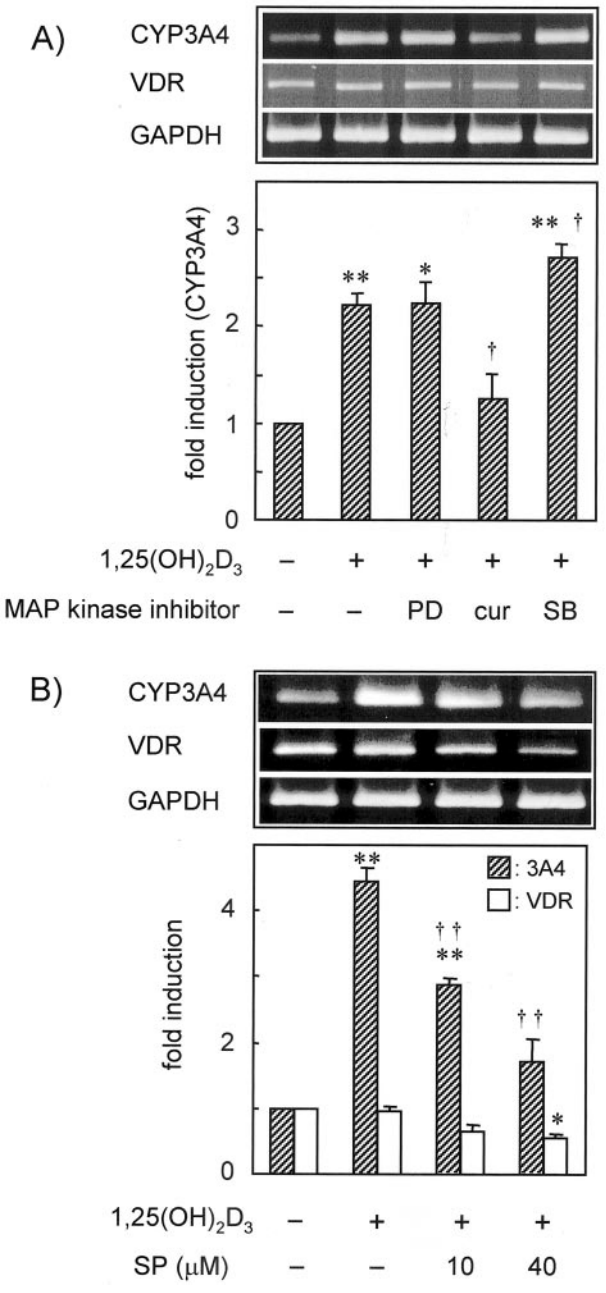

FIG. 1. Effects of MAPK inhibitors on the induction of CYP3A4 mRNA by $1,25(OH)_2D_3$. A, effects of PD098059 (PD), curcumin (cur), and SB203580 (SB) on $1,25(OH)_2D_3$-induced CYP3A4 mRNA. Caco-2 cells were pretreated with PD098059 (25 μM), curcumin (15 μM), or SB203580 (10 μM) for 30 min, followed by incubation with $1,25(OH)_2D_3$ (100 nM) for 24 h. B, dose-dependent inhibition of $1,25(OH)_2D_3$-induced CYP3A4 mRNA expression by SP600125 (SP). Caco-2 cells were pretreated with the indicated concentrations of SP600125 for 30 min, followed by incubation with $1,25(OH)_2D_3$ (100 nM) for 24 h. RT-PCR was performed according to the procedure described under *Materials and Methods*. The two photos show the results of RT-PCR analysis. Values (mean ± S.E., $n = 3$ or 4) are expressed as the fold induction relative to untreated cells. * and **, significant differences ($P < 0.05$ and $P < 0.01$, respectively) compared with untreated cells. † and ††, significant differences ($P < 0.05$ and $P < 0.01$, respectively) compared with $1,25(OH)_2D_3$-treated cells.

Statistical Analysis. Data were analyzed using the Student's *t* test. A *P* value less than 0.05 was considered significant.

Results and Discussion

In the present experiments, we first examined the effects of MAPK inhibitors on the induction of CYP3A4 mRNA by $1,25(OH)_2D_3$. The

CYP3A4 mRNA expression in Caco-2 cells increased 2.2-fold in the presence of 100 nM $1,25(OH)_2D_3$ as shown in Fig. 1A. The CYP3A4 mRNA induction was inhibited by treatment with curcumin, but not by PD098059 or SB203580. Curcumin, a JNK pathway inhibitor, is known to partly inhibit ERK at the concentration used (Chen and Tan, 1998). However, the possibility of ERK also being involved is eliminated from the results that PD098059, a specific mitogen-activated protein kinase kinase-ERK pathway inhibitor, had no effect on $1,25(OH)_2D_3$-induced CYP3A4 mRNA expression. To confirm the involvement of JNK in the CYP3A4 mRNA induction, we used SP600125, a specific JNK inhibitor. As shown in Fig. 1B, SP600125 significantly inhibited the induction in a dose-dependent manner.

As shown in our previous study (Hara et al., 2002), VDR plays an important role in $1,25(OH)_2D_3$-induced CYP3A4 mRNA expression. Therefore, we confirmed whether the MAP kinase inhibitors used affect the constitutive expression of VDR mRNA. Treatment with PD098059, curcumin, or SB203580 had no effect on the constitutive expression of VDR mRNA, whereas SP600125 reduced VDR mRNA expression at high concentration (Fig. 1). These results indicate that JNK inhibition by SP600125 not only suppresses the induction of CYP3A4 mRNA by $1,25(OH)_2D_3$, but it also reduces the basal expression of VDR. Thus, it is possible that the reduction of VDR expression by SP600125 might be involved in the suppression of CYP3A4 mRNA induction in part.

To verify the mechanism involved in the transcriptional activation of the *CYP3A4* gene via VDR, we constructed the CYP3A4 reporter plasmid, 3A4(−7836/−315)-pGL3. The 3A4(−7836/−315)-pGL3 was transfected into Caco-2 cells with the VDR expression vector or the empty expression vector. After the cells had been treated with 100 nM $1,25(OH)_2D_3$ for 24 h, the *CYP3A4* promoter activity was measured. As shown in Fig. 2A, the treatment with $1,25(OH)_2D_3$ markedly increased the *CYP3A4* promoter activity in cells transfected with the VDR expression vector (18.7 ± 2.4-fold), whereas the same treatment hardly increased the promoter activity in cells transfected with the empty vector. These results indicate that $1,25(OH)_2D_3$ via VDR activates the chimera CYP3A4 promoter.

To determine whether MAPKs regulate $1,25(OH)_2D_3$-induced *CYP3A4* gene expression at the transcriptional level, we examined the effect of MAP kinase inhibitors on the activation of the *CYP3A4* promoter using 3A4(−7836/−315)-pGL3. As shown in Fig. 2, B and C, treatment with PD098059 or SB203580 did not suppress $1,25(OH)_2D_3$-induced activation of 3A4(−7836/−315)-pGL3, whereas with SP600125, it dose-dependently inhibited the activation. These results were consistent with the results shown in Fig. 1, and indicate that JNK contributes to the induction of the *CYP3A4* gene by $1,25(OH)_2D_3$. The treatment with SP600125 reduced the basal levels of *CYP3A4* promoter activity by approximately 40% (Fig. 2, B and C), whereas it reduced the induced *CYP3A4* promoter activity by over 80%. These results suggested that JNK is involved especially in *CYP3A4* promoter activation induced by $1,25(OH)_2D_3$.

Recent studies reveal that the DR3 and the ER6 in the 5′-flanking region of the *CYP3A4* gene are functional VDREs (Thummel et al., 2001; Drocourt et al., 2002), and VDR plays a key role in $1,25(OH)_2D_3$-induced *CYP3A4* gene expression (Thummel et al., 2001; Drocourt et al., 2002; Hara et al., 2002). On the other hand, it was demonstrated that the phosphorylation of VDR itself by PKC and casein kinase II specifically modulates its transcriptional ability (Hsieh et al., 1991; Jurutka et al., 1996). Therefore, it is possible that the phosphorylation of VDR itself by JNK would modulate the transcriptional ability of VDR. To verify this possibility, we investigated whether SP600125 suppresses the activation of the promoter driven by VDR through the transactivation of the DR3 using $(DR3)_3$-TK-pGL3. As shown in Fig. 2A, $(DR3)_3$-TK-pGL3 was markedly activated by $1,25(OH)_2D_3$ in cells cotransfected with the VDR expression vector, but not in empty expression vector-cotransfected cells, indicating that the transactivation of this construct is VDR-dependent. As shown in Fig. 2D, the activity of $(DR3)_3$-TK-pGL3 increased 8.8-fold in the presence of 100 nM of $1,25(OH)_2D_3$, and treatment with SP600125 did not inhibit the activity of $(DR3)_3$-TK-pGL3 induced by $1,25(OH)_2D_3$. Taken together, SP600125 decreased the induction of 3A4(−7836/−315)-pGL3 activity by $1,25(OH)_2D_3$, whereas it did not affect the transactivation of the DR3 element by $1,25(OH)_2D_3$ via VDR. These results suggest that JNK does not modulate the transcriptional activity of VDR.

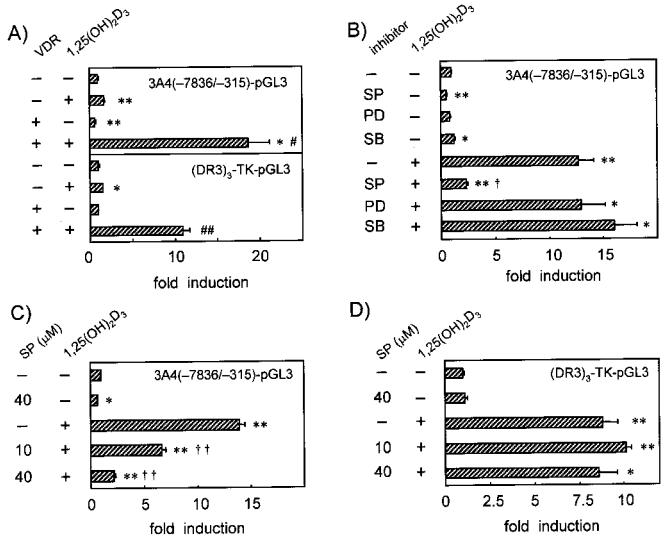

FIG. 2. Reporter analysis of the CYP3A4 promoter and the DR3 element. A, $1,25(OH)_2D_3$ activates the *CYP3A4* promoter and the DR3 via VDR. Caco-2 cells were cotransfected with the VDR expression vector (1.5 μg) or the empty expression vector (1.5 μg), the 3A4(−7836/−315)-pGL3 (1 μg) or the $(DR3)_3$-TK-pGL3 (1 μg), and the pRL-TK vector (0.1 μg), and then treated with $1,25(OH)_2D_3$ (100 nM) for 24 h. B, effects of MAPK inhibitors on the transcriptional activation of the *CYP3A4* promoter by $1,25(OH)_2D_3$. Caco-2 cells were cotransfected with the VDR expression vector (1.5 μg), the 3A4(−7836/−315)-pGL3 (1 μg), and the pRL-CMV vector (0.02 μg). Cells were pretreated with SP600125 (SP, 40 μM), PD098059 (PD, 25 μM), and SB203580 (SB, 10 μM) for 30 min, followed by incubation with or without $1,25(OH)_2D_3$ (100 nM) for 24 h. C, SP600125 dose-dependently decreases transcriptional activation of the *CYP3A4* promoter by $1,25(OH)_2D_3$. Caco-2 cells were cotransfected with the VDR expression vector (1.5 μg), the 3A4(−7836/−315)-pGL3 (1 μg), and the pRL-CMV vector (0.02 μg). Cells were pretreated with SP600125 (SP, 10 or 40 μM) for 30 min, followed by incubation with $1,25(OH)_2D_3$ (100 nM) for 24 h. D, effect of SP600125 on the transcriptional activation of the DR3 element by $1,25(OH)_2D_3$. Caco-2 cells were cotransfected with the VDR expression vector (0.5 μg), the $(DR3)_3$-TK-pGL3 (1 μg), and the pRL-CMV vector (0.02 μg). The cells were then pretreated with SP600125 (SP, 10 or 40 μM) for 30 min, followed by incubation with $1,25(OH)_2D_3$ (100 nM) for 24 h. Values (mean ± S.E., $n = 3$) are expressed as the fold induction relative to untreated cells. * and **, significant differences ($P < 0.05$ and $P < 0.01$, respectively) compared with untreated cells. # and ##, significant differences ($P < 0.05$ and $P < 0.01$, respectively) compared with vehicle-treated cells transfected the VDR expression vector. † and ††, significant differences ($P < 0.05$ and $P < 0.01$, respectively) compared with $1,25(OH)_2D_3$-treated cells.

The detailed mechanism by which JNK regulates $1,25(OH)_2D_3$-induced CYP3A4 expression remains unknown from this study. However, previous reports demonstrated that the xenobiotic-induced *CYP2B1* expression mediated by the constitutive androstane receptor requires the transcription factor Sp1 for optimal expression (Muangmoonchai et al., 2001). In addition, it has been reported that the synergism between VDR and other transcription factors, including AP-1 and Sp1, activates the induction of VDR-mediated transactivation (Liu and Freedman, 1994). These observations indicate that the synergism between nuclear receptors and other transcription factors

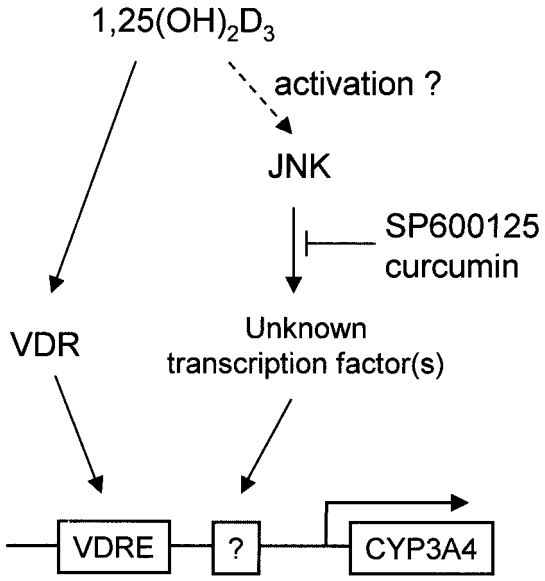

FIG. 3. *Schema of regulatory mechanism of CYP3A4 promoter activation caused by 1,25(OH)$_2$D$_3$. 1,25(OH)$_2$D$_3$-induced* CYP3A4 *promoter activation might be regulated by the synergism between VDR and transcription factor(s) that is modulated by JNK.*

coordinates nuclear receptor-mediated responses to xenobiotics. On the other hand, 1,25(OH)$_2$D$_3$ is known to rapidly activate some protein kinases, including PKC, ERK, and JNK, through a non-genomic signaling pathway in various cell types, including Caco-2 cells (Chen et al., 1999). Therefore, it is likely that JNK activation caused by 1,25(OH)$_2$D$_3$ might be involved in the induction of the *CYP3A4* gene. Taken together, the mechanism appears to be that 1,25(OH)$_2$D$_3$-induced *CYP3A4* gene activation is synergistically regulated by VDR and transcription factor(s) such as AP-1 and Sp1 that are modulated by JNK (Fig. 3).

In summary, our data indicate that JNK, but not ERK or p38, is an important regulator involved in 1,25(OH)$_2$D$_3$-induced *CYP3A4* gene activation. MAPKs are activated by various extracellular stimuli and regulate many gene expressions through phosphorylation of transcription factors. Therefore, elucidation of the involvement of MAPKs in the xenobiotic-induced expression of *P450* genes may be instrumental in understanding the induction of P450s under disease conditions such as inflammatory disorders and cancer.

Laboratory of Clinical Pharmaceutics (Y.Y., H.H., T.A.) and Department of Organic Chemistry (T.I., T.K.), Gifu Pharmaceutical University, Gifu, Japan

YOKO YASUNAMI
HIROKAZU HARA
TATSUNORI IWAMURA
TADASHI KATAOKA
TETSUO ADACHI

References

Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, et al. (2001) SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci USA* **98:**13681–13686.

Chen A, Davis BH, Bissonnette M, Scaglione-Sewell B, and Brasitus TA (1999) 1,25-dihydroxyvitamin D$_3$ stimulates activator protein-1-dependent Caco-2 cell differentiation. *J Biol Chem* **274:**35505–35513.

Chen YH and Tukey RH (1996) Protein kinase C modulates regulation of the *CYP1A1* gene by the aryl hydrocarbon receptor. *J Biol Chem* **271:**26261–26266.

Chen YR and Tan TH (1998) Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene* **17:**173–178.

Christakos S, Raval-Pandya M, Wernyj RP, and Yang W (1996) Genomic mechanisms involved in the pleiotropic actions of 1,25-dihydroxyvitamin D$_3$. *Biochem J* **316:**361–371.

Davies SP, Reddy H, Caivano M, and Cohen P (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* **351:**95–105.

Desai RK, van Wijnen AJ, Stein JL, Stein GS, and Lian JB (1995) Control of 1,25-dihydroxyvitamin D$_3$ receptor-mediated enhancement of osteocalcin gene transcription: effects of perturbing phosphorylation pathways by okadaic acid and staurosporine. *Endocrinology* **136:**5685–5693.

Drocourt L, Ourlin J-C, Pascussi J-M, Maurel P, and Vilarem M-J (2002) Expression of *CYP3A4*, *CYP2B6* and *CYP2C9* is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J Biol Chem* **277:**25125–25132.

Galisteo M, Marc N, Fautrel A, Guillouzo A, Corcos L, and Lagadic-Gossmann D (1999) Involvement of cyclic nucleotide- and calcium-regulated pathways in phenobarbital-induced cytochrome P-450 3A expression in mouse primary hepatocytes. *J Pharmacol Exp Ther* **290:**1270–1277.

Goodwin B, Hodgson E, and Liddle C (1999) The orphan human pregnane X receptor mediates the transcriptional activation of *CYP3A4* by rifampicin through a distal enhancer module. *Mol Pharmacol* **56:**1329–1339.

Hara H, Mitani N, and Adachi T (2000) Inhibitory effect of nitric oxide on the induction of cytochrome P450 3A4 mRNA by 1,25-dihydroxyvitamin D$_3$ in Caco-2 cells. *Free Radic Res* **33:**279–285.

Hara H, Yasunami Y, and Adachi T (2002) Alteration of cellular phosphorylation state affects vitamin D receptor-mediated CYP3A4 mRNA induction in Caco-2 cells. *Biochem Biophys Res Commun* **296:**182–188.

Hsieh JC, Jurutka PW, Galligan MA, Terpening CM, Haussler CA, Samuels DS, Shimizu Y, Shimizu N, and Haussler MR (1991) Human vitamin D receptor is selectively phosphorylated by protein kinase C on serine 51, a residue crucial to its trans-activation function. *Proc Natl Acad Sci USA* **88:**9315–9319.

Jurutka PW, Hsieh J-C, Nakajima S, Haussler CA, Whitfield GK, and Haussler MR (1996) Human vitamin D receptor phosphorylation by casein kinase II at Ser-208 potentiates transcriptional activation. *Proc Natl Acad Sci USA* **93:**3519–3524.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstörm RH, et al. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92:**73–82.

Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J Clin Investig* **102:**1016–1023.

Liu M and Freedman LP (1994) Transcriptional synergism between the vitamin D$_3$ receptor and other nonreceptor transcription factors. *Mol Endocrinol* **8:**1593–1604.

Möhlau R (1912a) Über Anthrachinonyl-Monohydrazine. *Chem Berl* **45:**2233–2244.

Möhlau R (1912b) Über eine neue Synthese der Anthrachinonyl-Hydrazine. *Chem Berl* **45:**2244–2248.

Muangmoonchai R, Smirlis D, Wong SC, Edwards M, Phillips IR, and Shephard EA (2001) Xenobiotic induction of cytochrome P450 2B1 (CYP2B1) is mediated by the orphan nuclear receptor constitutive androstane receptor (CAR) and requires steroid co-activator 1 (SRC-1) and the transcription factor Sp1. *Biochem J* **355:**71–78.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, and Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* **283:**1552–1562.

Pichard L, Fabre I, Fabre G, Domergue J, Saint Aubert B, Mourad G, and Maurel P (1990) Cyclosporin A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab Dispos* **18:**595–606.

Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, Lown KS, and Watkins PB (1997) Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1α,25-dihydroxyvitamin D$_3$. *Mol Pharmacol* **51:**741–754.

Sueyoshi T and Negishi M (2001) Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu Rev Pharmacol Toxicol* **41:**123–143.

Thummel KE, Brimer C, Yasuda K, Thottassery J, Senn T, Lin Y, Ishizuka H, Kharasch E, Schuetz J, and Schuetz E (2001) Transcriptional control of intestinal cytochrome P-4503A by 1α,25-dihydroxy vitamin D$_3$. *Mol Pharmacol* **60:**1399–1406.

Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, and Guzelian PS (1987) Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J Clin Investig* **80:**1029–1036.

Address correspondence to: Dr. Tetsuo Adachi, Laboratory of Clinical Pharmaceutics, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan. E-mail: adachi@gifu-pu.ac.jp