1,25-Dihydroxyvitamin D3 Regulates Expression of Sex Steroid Receptors in Human Uterine Fibroid Cells

Ayman Al-Hendy, Michael P. Diamond, Ahmed El-Sohemy, and Sunil K. Halder

Department of Obstetrics and Gynecology (A.A.-H., M.P.D., S.K.H.), Georgia Regents University, Medical College of Georgia, Augusta, Georgia 30912; and Department of Nutritional Sciences (A.E.-S.), University of Toronto, Toronto, Canada M5S 3E2

Context: Uterine fibroids (UFs) are the most common benign tumors in premenopausal women. In this study, we evaluated the effects of 1,25-dihydroxyvitamin D3 [1,25(OH)\textsubscript{2}D\textsubscript{3}] for the treatment of UFs.

Objective: To determine the role of 1,25(OH)\textsubscript{2}D\textsubscript{3} on the expression of sex steroid receptors in human UF cells.

Design: Human UFs and their adjacent myometrium were analyzed for expression of estrogen receptor (ER)-\textalpha, progesterone receptor (PR)-A, and PR-B, as well as members of the steroid receptor coactivator (SRC) family. Immortalized human uterine fibroid (human uterine leiomyoma [HuLM]) cells were treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} and assayed for the expression and localization of the aforementioned receptors and SRCs using Western blot, immunohistochemistry, immunofluorescence, and immuno precipitation assays.

Main Outcome Measures: We discovered a correlation between reduced levels of vitamin D receptor (VDR) and increased levels of ER-\textalpha, PR-A, and PR-B in these tissues. We evaluated the effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on the regulation of the aforementioned sex steroid receptors.

Results: We observed an inverse correlation between the up-regulated ER-\textalpha, PR-A, and PR-B and expression of VDR in UFs. Treatment with 1,25(OH)\textsubscript{2}D\textsubscript{3} significantly decreased levels of ER-\textalpha, PR-A, and PR-B, as well as SRCs in HuLM cells (\textit{P} < .05). In contrast, 1,25(OH)\textsubscript{2}D\textsubscript{3} self-induced its own VDR, which resulted in an induction of VDR-retinoid X receptor-\textalpha complex in HuLM cells. Together, these results suggest that 1,25(OH)\textsubscript{2}D\textsubscript{3} functions as an antagonist of sex hormone receptors in HuLM cells.

Conclusions: 1,25(OH)\textsubscript{2}D\textsubscript{3} functions as a potent antiestrogenic/antiprogesteronic agent that may have utility as a novel therapeutic option for UF. (\textit{J Clin Endocrinol Metab} 100: E572–E582, 2015)

Uterine fibroids (UFs; or leiomyomas) are the leading cause of hysterectomy in women of reproductive age (1–3). Increasing evidence supports the theory that ovarian steroids such as estrogen and progesterone play key roles in the growth of UFs (4–6). UFs are three to four times more likely to occur in African American women, who also suffer from vitamin D deficiency, compared to their Caucasian counterparts (7, 8). The exact cause of this markedly high occurrence of UFs is not yet fully understood, but such association may suggest a role for vitamin D in fibroid biology. We and others have recently reported that women with UFs have lower levels of serum vitamin D3 compared to women who do not have UFs (9–11). Moreover, we also demonstrated a direct association of lower levels of serum vitamin D3 with increased size of UFs within different ethnic groups (9).

Abbreviations: Co-IP, coimmunoprecipitation; DAPI, 4',6 diamidino-2-phenylindole; ER, estrogen receptor; FITC, fluorescein isothiocyanate; HuLM, human uterine leiomyoma; 1,25(OH)\textsubscript{2}D\textsubscript{3}, 1,25-dihydroxyvitamin D3; PARP, poly (ADP-ribose) polymerase; PR, progesterone receptor; RhoGDI, rho GDP-dissociation inhibitor; RXR, retinoid X receptor; SRC, steroid receptor coactivator; UF, uterine fibroid; VDR, vitamin D receptor; VDRE, vitamin D response element.
1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] is a member of the steroid hormone family and serves as the major regulator of calcium and phosphate homeostasis in the body system (12). Studies have shown that 1,25(OH)2D3 can induce growth arrest, differentiation, and apoptosis in a wide variety of cancer cells (13, 14). Recent studies have demonstrated that 1,25(OH)2D3 or its noncalcemic analog, paricalcitol, inhibits fibroid tumor growth in vivo and can inhibit proliferation of human UF cells in vitro (15–17). Furthermore, we also demonstrated that 1,25(OH)2D3 can inhibit the expression and activities of matrix metalloproteinases and reduce the expression of extracellular matrix proteins in cultured UF cells (18, 19).

In a 2004 report of Andersen et al (20), it is clearly illustrated that fibroid primary cultures have an elevated response to 17β-estradiol compared with myometrial cultures. UF cells are responsive to sex steroid hormones, and such responsiveness is enhanced in rodent and human tumors as well as tumor-derived cell lines as compared to their myometrial counterparts (2, 21, 22). The effects of estrogen are mediated primarily via estrogen receptor (ER)-α, the nuclear receptor that belongs to a superfamily of ligand-regulated transcription factors (23). Subsequent to estradiol binding to ER-α, the receptor undergoes a conformational change, dimerizes, and binds either directly to DNA via estrogen response elements or indirectly via interactions with other DNA-bound transcription factors such as Sp1 or AP-1 (23–25). Estradiol exerts its biological effect in estrogen-responsive tissues by binding to ER-α and modulating the transcription of target genes including growth factors and proto-oncogenes, among others (26). Moreover, nongenomic effects of estrogen can be induced by cytoplasmic signaling pathways, which include the activation of the MAPK pathway transduced from growth factor receptors or plasma membrane localized ER (27, 28). Because estrogen functions via binding with ER-α (29), the factors and mechanisms that regulate the level of ER-α are important in determining the amplitude of estrogen-mediated actions in UF cells. This knowledge will augment our efforts in the development of new therapeutic approaches for the treatment of UF cells.

Recent studies demonstrated that 1,25(OH)2D3 can reduce gene expression of ER-α in human breast cancer cells (30). We have recently reported that lower levels of vitamin D receptor (VDR) are associated with a higher risk of UF pathogenesis (19). Published literature also demonstrates up-regulation of steroid receptors, particularly ER-α in UF cells as compared with the adjacent myometrium, yet nothing is known as to whether there is an association between the expression status of these sex steroid receptors and lower levels of VDR. It has also not been determined whether 1,25(OH)2D3 plays an important role in the regulation of sex steroid hormone receptors in UF cells. The aim of this study is to evaluate whether a possible correlation exists between the higher expression of steroid receptors and lower levels of VDR in human UF and to determine whether 1,25(OH)2D3 has the potential to suppress the expression of those receptors in cultured human UF cells, thus implicating a potential nonsurgical therapeutic utility for the treatment of UF.

Materials and Methods

Cell lines and cultures

Human uterine leiomyoma (HuLM) cells were a kind gift from Dr Darlene Dixon (National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina) (31). These cells were grown in smooth muscle basal medium at 37°C in a humidified atmosphere of 5% CO2 as previously described (32). Primary human UF cells used in this study were described in our prior publications (18).

Reagents and antibodies

1,25(OH)2D3, estrogen, protein G- sepharose, fluorescein isothiocyanate (FITC)-conjugated phalloidin, and anti-β-actin antibodies were purchased from Sigma Biochemicals. NE-PER nuclear and cytoplasmic extraction reagents were purchased from Pierce Biotechnology. Anti-ER-α, anti-PR-A, anti-PR-B, anti-VDR, anti-retinoid X receptor (RXR)-α, anti-poly (ADP-ribose) polymerase (PARP), and anti-rho GDP-dissociation inhibitor (RhoGDI) antibodies were purchased from Santa Cruz Biotechnology. CY3- and FITC-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. Anti-SRC1, anti-SRC2, and anti-SRC3 antibodies were purchased from Thermo Scientific.

Protein extraction from human tissue samples

Human UF and the adjacent myometrium tissue samples were collected from consenting individuals undergoing surgery (hysterectomy or myomectomy) for UF removal (abdominal, vaginal, and laparoscopic). Tissue samples were collected at various locations within Texas and Tennessee under approved Institutional Review Board protocols (Protocol no. 090630WJR246 11). Methodology for the extraction of proteins from stored tissue samples has been previously described (19). Using Western blot analyses, protein lysates were examined for the expression of ER-α, PR-A, PR-B, VDR, and members of the steroid receptor coactivator (SRC) family.

Cell proliferation assay

Cell counts were conducted to determine the effect of 1,25(OH)2D3 on estrogen-induced proliferation of HuLM cells. HuLM cells were seeded onto 12-well tissue culture plates (Becton Dickinson) and treated with estrogen in the absence or presence of increasing doses of 1,25(OH)2D3 at different time points; then cells were counted at each time point as described in the figure legend (see Figure 2A). Averaged cell numbers from triplicate wells were used in preparing the graph. Each data point represents the mean ± SD of triplicate wells (n = 3).
Western blot analyses

For analyses of protein expression, 0.7 × 10⁶ HuLM cells were cultured in 60-mm tissue culture dishes, serum starved for 20 hours, and subsequently treated with increasing doses of 1,25(OH)₂D₃, as described in figure legends (see Figures 3, 5, and 6). Preparation of protein lysates from 1,25(OH)₂D₃-treated and untreated cells as well as Western blot analyses were performed as previously described (19). A 0 nm concentration of 1,25(OH)₂D₃ in each individual experiment served as the control.

Coimmunoprecipitation (Co-IP) analyses

To determine the physical interaction between VDR and RXR-α in HuLM cells, we performed a Co-IP assay as previously described (15). We seeded 2 × 10⁶ HuLM cells in 10-cm tissue culture dishes and incubated them overnight. Cells were preincubated in serum-free medium for 20 hours and treated with increasing doses of 1,25(OH)₂D₃ for 48 hours; cell lysates were prepared as previously described (15). For Co-IP assay, equal amounts (1 mg) of clear lysates were incubated with 2.5 μg polyclonal anti-RXR-α antibody for 2.5 hours at 4°C, followed by incubation with 25 μL of protein G-Sepharose for an additional hour. Immunoprecipitates were assayed by Western blot analyses using polyclonal anti-VDR antibody. Similarly, Co-IP was performed with anti-VDR antibody, and then Western blot analyses were performed with anti-RXR-α antibody. Western blot analyses were also performed to verify expression of VDR and RXR-α in the above lysates.

Nuclear and cytoplasmic fractions

HuLM cells (2 × 10⁶) were seeded in 10-cm tissue culture dishes and incubated overnight. Cells were preincubated in serum-free medium for 20 hours and treated with increasing doses of 1,25(OH)₂D₃ for 48 hours. Thereafter, nuclear and cytoplasmic fractions were prepared from pelleted cells using NE-PER nuclear and cytoplasmic extraction reagents; 15 μg of each cytoplasmic and nuclear fraction was subjected to Western blot analyses using antibodies as indicated in figure legends (see Figures 3D and SD).

Immunofluorescence analyses

Immunofluorescence analyses were conducted as previously described (15). Briefly, HuLM cells were cultured, serum starved, and subsequently treated with 1,25(OH)₂D₃ for 48 hours. After fixation/permeabilization steps, cells were incubated with either mouse monoclonal or rabbit polyclonal primary antibodies for 1 hour at room temperature, followed by an additional 1-hour incubation with CY3- or FITC-conjugated mouse monoclonal or rabbit polyclonal secondary antibodies. Fluorescent images were taken using an Axiovert 100 M inverted microscope (Nikon TE2000-E fluorescence microscope). Signal intensities were visually compared between untreated control and 1,25(OH)₂D₃-treated cells.

Statistical analysis

Student’s t test was used in assessing significant differences in the expression levels of ER-α, PR-A, and PR-B in human UF vs adjacent myometrium samples. Student’s t test was also used to assess any significant differences between untreated control vs 1,25(OH)₂D₃-treated data points and within the 1,25(OH)₂D₃-treated data points. Values were considered statistically significant at a 95% confidence level when P < .05. Data were presented as the mean ± SD.

Results

Up-regulation of ER-α and PRs correlated with reduced levels of VDR in human UF tumors

To determine whether up-regulation of sex steroid receptors correlates with lower levels of VDR in human UFs, we performed Western blot analyses using protein lysates prepared from several independent human UF lesions and adjacent myometrium. Our results demonstrated that at least 70% of UF lesions (10 of 14 patients) expressed an induced level of ER-α. Similarly, expression levels of PR-A and PR-B were also up-regulated in UFs (Figure 1A). We observed that VDR expression was lower in 60% of cases (eight of 14) in UFs compared to adjacent myometrium. Correlation between up-regulation of ER-α and reduced expression of VDR was observed in five of 10 cases of UF (Figure 1A). To further verify whether human UF expressed deregulated CYP27B1 (an enzyme responsible for synthesizing the active form of vitamin D3), we applied immunohistochemical analyses. We found lower levels of both CYP27B1 and VDR in UF when compared with the adjacent myometrium (Figure 1B), suggesting that both vitamin D synthesis and signaling apparatus are diminished in UF. Thus, the above results suggest an inverse correlation between higher levels of ER-α, PR-A, and PR-B and lower levels of VDR in human UF.

1,25(OH)₂D₃ inhibits estrogen-induced proliferation of cultured HuLM cells

To determine whether estrogen can induce proliferation of HuLM cells, whereas treatment with 1,25(OH)₂D₃ suppresses estrogen-induced proliferation, we performed a cell proliferation assay. We found that estrogen at 10 nm concentration slightly induced HuLM cell proliferation at 48 hours and that induction was further enhanced in a time-dependent manner. Estrogen significantly induced the proliferation of HuLM cells at 96 hours (1.3-fold; P < .05) and 144 hours (1.5-fold; P < .05) (Figure 2A). On the other hand, 1,25(OH)₂D₃ significantly inhibited estrogen-induced proliferation of HuLM cells in a dose-dependent manner (Figure 2A; P < .05). To examine whether estrogen induces HuLM cell proliferation by affecting the expression of VDR, we performed Western blot analyses (Figure 2B). We found that estrogen reduced the expression of VDR in a dose-dependent manner in HuLM cells (Figure 2B). We further observed that estrogen or progesterone treatment affects the expression of steroid receptors by performing Western blot analyses. We found that either estrogen or progesterone significantly in-
duced the levels of PR-A and PR-B in HuLM cells, whereas the levels of ER-α did not show significant changes (Figure 2, C and D). Additionally, the levels of SRC1, SRC2, and SRC3 were not affected by estrogen or progesterone (Figure 2C). These results suggest that estrogen induces growth of HuLM cells, whereas 1,25(OH)2D3 function by inhibiting the growth-promoting effects of estrogen on cultured HuLM cells.

1,25(OH)2D3 treatment reduces the expression of ER-α, PR-A, and PR-B proteins in cultured HuLM cells

To examine whether 1,25(OH)2D3 reduces the expression of sex steroid hormone receptors, we performed Western blot analyses. We found that 1,25(OH)2D3 reduced the levels of ER-α in a dose- and time-dependent manner when compared with untreated controls (Figure 3A). 1,25(OH)2D3 at 10 nM concentration significantly reduced the levels of ER-α protein, which was further reduced by higher concentrations of 1,25(OH)2D3 (Figure 3A). On the other hand, 1,25(OH)2D3 induced the levels of VDR in a dose- and time-dependent manner in HuLM cells. In parallel, we also found that 1,25(OH)2D3 at 10 nM concentration significantly reduced levels of PR-A and PR-B in HuLM cells, which was also further reduced by higher concentrations (Figure 3B). Using immunofluorescence analyses, we further observed that HuLM cells showed clear structural smooth muscle F-actin staining, whereas 1,25(OH)2D3 reduced staining in a dose-dependent manner (Supplemental Figure 1). Using immunofluorescence analyses, we found that ER-α, PR-A, and PR-B staining signals (red) were primarily localized in the nuclei of HuLM cells (Figure 3C). Treatment of 1,25(OH)2D3 at 10 nM concentration notably reduced nuclear staining, which was further reduced by higher concentrations (Figure 3C). On the other hand, the staining signals for VDR (green) were also present in the nuclei of HuLM cells, whereas 1,25(OH)2D3 induced nuclear VDR staining in a dose-dependent manner (Figure 3C, top panel).

Human UFs express higher levels of sex SRC family proteins, SRC2 and SRC3, when compared with adjacent myometrium

To test whether SRCs are up-regulated in human UFs, we performed Western blot analyses using protein samples isolated from human UFs and their adjacent myometrium. We found that SRC2 was overexpressed in 50% of the cases (seven of 14) of UFs, whereas the SRC3 was overexpressed in 40% of cases (six of 14) as compared with adjacent myometrium (Figure 4). These results indicate that human UFs express higher levels of SRC2 and SRC3 proteins and that up-regulation may have an impact in the tumorigenicity of UFs.
1,25(OH)₂D₃ reduces the expression of SRC family proteins in cultured HuLM cells

To examine the effect of 1,25(OH)₂D₃ on the expression of SRCs, we first performed Western blot analyses. We found that at 10 nM concentration, 1,25(OH)₂D₃ significantly reduced the levels of SRC1, SRC2, and SRC3, which were further reduced in a dose-dependent manner (Figure 5, A and B; P < .05). Treatment of 1,25(OH)₂D₃ did not affect the expression of RXR-α in HuLM cells (Figure 5A). Next, to examine the effect of 1,25(OH)₂D₃ on expression and subcellular localization of SRCs in HuLM cells, we performed immunofluorescence analyses. We found that staining signals for both SRC2 and SRC3 (red) were mainly localized in the nuclei of HuLM cells, whereas 1,25(OH)₂D₃ reduced those staining signals in a dose-dependent manner (Figure 5C, middle and bottom panels). In contrast, staining signals for SRC1 (red) were somewhat localized in both chambers with more intensity in the nuclei and wherein signals were also reduced by 1,25(OH)₂D₃ in a dose-dependent manner (Figure 5C,
We further quantitatively determined the effects of 1,25(OH)₂D₃ on nuclear SRC2 and SRC3 by performing Western blot analysis using cytoplasmic and nuclear fractions, as described in Figure 3D. We found that both SRC2 and SRC3 were present in the nuclei of HuLM cells, whereas 1,25(OH)₂D₃ reduced the levels of those proteins in a dose-dependent manner (Figure 5D). As expected, we noticed that 1,25(OH)₂D₃ consistently induced the levels of VDR in a dose-dependent manner in HuLM cells (Figure 5D). To further mimic these findings.
in human UFs, we performed similar immunofluorescence and Western blot analyses and showed that 1,25(OH)₂D₃ reduced SRC2 and SRC3 in primary fibroid cells (Supplemental Figure 2). These results suggest that 1,25(OH)₂D₃ reduces the expression levels of nuclear SRC family members in cultured human HuLM cells.

**1,25(OH)₂D₃ treatment induces physical association between VDR and RXR-α in cultured HuLM cells**

To determine whether VDR and RXR-α interact to form VDR-RXR-α complex in HuLM cells, we performed a Co-IP assay. Using anti-RXR-α antibody for Co-IP, the presence of VDR in the complex was verified by Western blot with anti-VDR antibody. Unlike the VDR findings above, we found that similar levels of RXR-α were present in the complex, and such levels were not altered by 1,25(OH)₂D₃ (Figure 6A, bottom panel). Consistent with Western blot data, our immunofluorescence analyses also confirmed that 1,25(OH)₂D₃ induced the expression levels of VDR (green, top panel) in HuLM cells, whereas the expression levels of nuclear RXR-α (red, bottom panel) were unaffected by 1,25(OH)₂D₃ (Figure 6C). These results suggest that VDR and RXR-α physically interact to form the VDR-RXR-α complex, which is further induced by 1,25(OH)₂D₃ treatment in HuLM cells.

**Discussion**

The dysregulation of steroid hormones and their receptors is thought to be a primary factor for UF growth (33). Although few reports have shown minimum differences in ER and PR mRNA levels between UFs, the vast majority of previous articles have demonstrated an increased level of ER-α and PR-A/B in fibroid lesions as compared to adjacent myometrium (34–37). These discordant data may reflect intrinsic tumor-to-tumor variations that are unavoidable and, indeed, expected in such a common disease and may reflect some anthropometric factors (weight, race, age, etc), environmental influences (diet, exposure, etc), or genetics, eg, mutation status of MED12, HMGA2, epigenetic, or other factors yet to be discovered (38–40). Our group, along with several others, has previously shown that both 25-hydroxyvitamin D3 deficiency and 1,25(OH)₂D₃ deficiency are risk factors for the occurrence of UFs (9–11). We have also demonstrated that fibroid tumors express reduced levels of VDR as compared with the adjacent myometrium (19). These findings suggest that both 1,25(OH)₂D₃ deficiency and reduced expression of VDR, and therefore cumulative attenuation of vitamin D signaling, may be contributing factors in UF pathogenesis.

To date, no study has examined the correlation of ER-α, PR-A, and PR-B with VDR in human UFs. Moreover, the effects of 1,25(OH)₂D₃ on these nuclear receptors in human UF cells has not yet been determined. Herein, we showed higher levels of ER-α in 70% of cases of UFs as compared with adjacent myometrium (Figure 1). We also observed that these specific fibroid tumors expressed higher levels of ER-α, PR-A, and PR-B; and concurrently expressed reduced levels of VDR. Published studies have demonstrated the up-regulation of progesterone receptors (PR-A and PR-B) in UF as compared to adjacent myometrium (41, 42). Consistent with previous findings, we also observed up-regulation of PR-A and PR-B in UF as compared to adjacent myometrium, and this up-regulation was associated with reduced levels of VDR as shown in Figure 1. A recent study has demonstrated that deregulation of VDR and CYP27B1 occurs during breast cancer development and contributes to abrogation of tumor suppressive effects triggered by vitamin D3 (43). Our results in Figure 1B, show reduced levels of CYP27B1 and
VDR in UF and further confirm earlier VDR findings by Western blot analyses, suggesting overall cumulative attenuated vitamin D signaling in fibroid tumor lesions. Therefore, it is possible that higher estrogenic function may suppress 1,25(OH)2D3 function by both reducing the synthesis of bioactive vitamin D3 and limiting available VDR in UF cells.

A number of studies have shown that estrogen is a major player in the growth of UFs. We first confirmed that estrogen induces the proliferation of HuLM cells in a dose- and time-dependent manner (Figure 2A). Interestingly, we found that 1,25(OH)2D3 effectively suppressed estrogen-induced proliferation of HuLM cells (Figure 2A). We also observed that estrogen inhibits VDR, whereas it induced the expression of PRs in HuLM cells (Figure 2B, C, and D). Importantly, these results demonstrate that estrogen induces the proliferation of HuLM cells by inducing PRs and by inhibiting VDR, whereas 1,25(OH)2D3 functions as an antiestrogenic agent in these cells.

We further examined the effect of 1,25(OH)2D3 on the expression of ER-α, PR-A, and PR-B in HuLM cells. Our results demonstrate that 1,25(OH)2D3 has the potential to suppress nuclear ER-α, PR-A, and PR-B by inducing VDR, which seems to function as an antiestrogen in human UF cells. Previous studies demonstrated that 1,25(OH)2D3 is capable of transcriptionally repressing ER-α gene expression, leading to a decreased estrogenic response and reduction of the proliferative stimulus for human breast tumor (44, 45). Thus, the mechanism by which 1,25(OH)2D3 regulates ER-α in HuLM cells appears to be the direct repression of ER-α expression by 1,25(OH)2D3. Our immunofluorescence and Western blot results in Figure 3, C and D, indicate that ER-α, PR-A, and PR-B are mostly localized in the nuclei of HuLM cells, whereas 1,25(OH)2D3 effi-
1,25(OH)₂D₃ functions through its own nuclear SRC family members. In a study conducted by Minghetti and Norman (49), it was shown that 1,25(OH)₂D₃ reduced nuclear expression in a dose-dependent manner (Figure 5, C and D). These results further demonstrate that 1,25(OH)₂D₃ has an important role in reducing the expression of nuclear SRC family members.

In summary, we observed an association of higher levels of ER-α, PR-A, or PR-B with reduced levels of VDR in human UFs. Estrogen suppressed the levels of VDR and induced the proliferation of HuLM cells, whereas 1,25(OH)₂D₃ significantly reduced the estrogen-induced proliferation of HuLM cells. Treatment of 1,25(OH)₂D₃ reduced the expression of nuclear ER-α, PR-A, PR-B, and the nuclear SRC family members in HuLM cells. In contrast, 1,25(OH)₂D₃ induced its own VDR in a dose- and time-dependent manner in HuLM cells. Moreover, we also demonstrated a potential negative VDRE in the proximal promoter of the ER-α gene (44, 50). We next performed a Co-IP assay to verify the physical interaction between VDR and RXR-α in HuLM cells. Our results in Figure 6A clearly demonstrate that VDR can physically interact with RXR-α in HuLM cells to form the VDR-RXR-α complex, which is further induced by 1,25(OH)₂D₃ due to induced levels of VDR. Thus, these results demonstrated that 1,25(OH)₂D₃ induced nuclear VDR and can form elevated levels of VDR-RXR-α complex, which may further bind to the VDRE on ER-α promoter and ultimately suppress the expression of gene expression of ER-α.

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showed that VDR can form a heterodimeric VDR-RXR-α complex in HuLM cells, whereas 1,25(OH)2D3 induced that complex due to the induction of VDR, suggesting that vitamin D-mediated reduction of steroid receptor gene expression requires VDR-RXR-α complex in HuLM cells. Future research study designs will seek to determine the detailed molecular mechanisms by which 1,25(OH)2D3 regulates ER-α gene expression in human UF cells.

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Address all correspondence and requests for reprints to: Sunil K Halder, PhD, Senior Research Scientist, Department of Obstetrics and Gynecology, Georgia Regents University, 1120 15th Street, Room CB-2915B, Augusta, GA 30912. E-mail: shalder@gru.edu.

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