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## ABSTRACT

Calcium and phosphorus homeostasis is achieved by interplay among hormones, including 1,25(OH)<sub>2</sub>D<sub>3</sub> (1,25D), parathyroid hormone, and fibroblast growth factor 23 (FGF23), and their interactions with other proteins. For example, mutations in dentin matrix protein 1 (DMP-1) result in increased FGF23 and hypophosphatemic rickets. 1,25D is reported to modulate FGF23; thus, we hypothesized that 1,25D may be involved in modulating DMP-1 in an intermediary step. Murine cementoblasts (OCCM-30) and osteocyte-like cells (MLO-Y4 and MLO-A5), known to express DMP-1, were used to analyze effects of 1,25D on *DMP-1* expression *in vitro*. *DMP-1* mRNA levels decreased by 50% ( $p < .05$ ) in the presence of 1,25D in all cell types, while use of a vitamin D receptor (VDR) agonist (EB1089) and antagonist (23S,25S)-DLAM-2P confirmed that VDR pathway activation was required for this response. Further analysis showed that histone deacetylase recruitment was necessary, but neither protein kinase A nor C pathways were required. In conclusion, our results support the hypothesis that 1,25D regulates *DMP-1* expression through a VDR-dependent mechanism, possibly contributing to local changes in bone/tooth mineral homeostasis.

**KEY WORDS:** Vitamin D receptor, phosphate homeostasis, osteogenesis, SIBLING family, fibroblast growth factor 23, phosphate regulating endopeptidase homolog.

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# Vitamin D Represses Dentin Matrix Protein 1 in Cementoblasts and Osteocytes

## INTRODUCTION

The active form of vitamin D, 1,25(OH)<sub>2</sub>D<sub>3</sub> (1,25D), plays a central role in regulating mineralization of skeletal and dental tissues, signaling through the vitamin D receptor (VDR). VDR and its binding partner, retinoid X receptor, recognize vitamin D response elements (VDREs) in the promoter region of 1,25D-responsive genes, modulating numerous biological pathways. 1,25D can also produce rapid, nongenomic (*i.e.*, VDRE-independent) responses linked to calcium (Ca<sup>2+</sup>) fluxes and activation of protein kinase C (PKC). Several mineralized tissue genes are 1,25D targets, including *Spp1* (gene for osteopontin), *Bglap* (osteocalcin), *Lrp5* (low-density lipoprotein-related protein 5), *Tnfrsf11* (receptor activator of NF-κB ligand), and *Tnfrsf11b* (osteoprotegerin) (Haussler *et al.*, 2012; Pike *et al.*, 2012).

1,25D and 2 other hormones—parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23)—control circulating Ca<sup>2+</sup> and phosphate (P<sub>i</sub>) levels and, subsequently, mineralization (Haussler *et al.*, 2012; Kolek *et al.*, 2005). FGF23 directly antagonizes 1,25D by decreasing its activating hydroxylation step in the kidney and increasing its catabolism, secondarily restricting absorption/reabsorption of Ca<sup>2+</sup> and P<sub>i</sub> (Haussler *et al.*, 2012; Shimada *et al.*, 2004). 1,25D upregulates FGF23 expression in osteocytes, providing a feedback loop between these factors (Barthel *et al.*, 2007; Haussler *et al.*, 2012). PTH stimulation of FGF23 expression closes the loop of the bone-kidney-parathyroid axis of Ca<sup>2+</sup> and P<sub>i</sub> regulation. However, several aspects of the interactions among 1,25D, PTH, and FGF23 remain unclear. For example, increased levels of circulating FGF23 are observed in hereditary hypophosphatemic rickets, despite causative mutations in disparate genes, including phosphate-regulating endopeptidase homolog, X-linked (*PHEX*), and dentin matrix protein 1 (*DMP-1*). Inactivating mutations in *PHEX* cause X-linked hypophosphatemia (MIM 307800). Mutations in *DMP-1* cause type 1 autosomal recessive hypophosphatemic rickets (ARHR1; MIM 241520), remarkably similar to X-linked hypophosphatemia. While it remains unclear how increased FGF23 and hypophosphatemia result from loss of an endopeptidase or matrix protein, proposed mechanisms include a common FGFR1 signaling pathway (Martin *et al.*, 2011) and/or controlling levels of circulating aspartate-rich motif peptides (David *et al.*, 2011).

PHEX and *DMP-1* represent potential regulatory links between 1,25D and FGF23. PHEX is downregulated by 1,25D (Ecarot and Desbarats, 1999; Hines *et al.*, 2004); however, attempts to define mechanisms have not been fruitful. Parallel roles for PHEX and *DMP-1* in controlling FGF23 suggest that 1,25D may also downregulate *DMP-1*; yet, the limited studies on this topic are conflicting (Farrow *et al.*, 2009; Ito *et al.*, 2013). We hypothesized that 1,25D-mediated regulation of *DMP-1* may provide a link between 1,25D and FGF23, and we tested this using *DMP-1* expressing murine cementoblasts (OCCM-30) and osteocyte-like cells (MLO-Y4 and MLO-A5).

## MATERIALS & METHODS

### Cell Lines

An established immortalized murine cementoblast cell line (OCCM-30) (D'Errico *et al.*, 2000) and 2 osteocyte-like cell lines—MLO-A5 preosteocytes and MLO-Y4 mature osteocytes (Kato *et al.*, 1997; Kato *et al.*, 2001)—were used for these studies.

### Cell Culture

Cell culture reagents were obtained from Life Technologies (Gaithersburg, MD, USA). Fetal bovine serum (FBS) and calf serum were purchased from Hyclone (Logan, UT, USA). Cell seeding concentration was adjusted for approximately 100% confluence at RNA isolation: OCCM-30 cells at  $1 \times 10^4$ , MLO-Y4 cells at  $1.3 \times 10^4$ , and MLO-A5 cells at  $5.3 \times 10^4$  cells/cm<sup>2</sup>. After 24 hrs, medium was changed to DMEM with 2% FBS plus antibiotics for OCCM-30 cells or  $\alpha$ -MEM with heat-inactivated 1% FBS and 1% fetal calf serum for osteocyte-like cells. Medium was changed at days 3 and 5, and 1,25D (10 nM) or vehicle (ethanol) plus ascorbic acid (50  $\mu$ g/mL) was added on day 5. Total RNA was harvested 24 hrs after addition of 1,25D, unless otherwise stated. For time course, RNA was harvested at 3, 6, 18, 24, 48, and 72 hrs after treatment.

To determine if effects of 1,25D on *DMP-1* expression were transcription dependent, cells were pretreated for 30 mins with transcription inhibitor actinomycin D (2  $\mu$ g/mL) before addition of 1,25D. To determine whether effects required *de novo* protein synthesis, cells were pretreated with cycloheximide (1/10/100  $\mu$ M) before addition of 1,25D, with RNA harvested 24 hrs later.

To determine involvement of signaling pathways, dose-response experiments were performed with inhibitors GF109203X, U73122, H-89, MDL12,330A, and PD98059 at 1/20/100  $\mu$ M and SB203580 at 1/15/100  $\mu$ M, with doses based on previous reports and confirmation of cell viability in our experimental system (Lochner and Moolman, 2006; Tada *et al.*, 2011; Bai *et al.*, 2013; Wu-Zhang and Newton, 2013). Cells were then pretreated for 30 mins with GF109203X (1  $\mu$ M) or U73122 (20  $\mu$ M), H-89 (20  $\mu$ M) or MDL12,330A (20  $\mu$ M), SB203580 (15  $\mu$ M), PD98059 (1  $\mu$ M); PKC/PLC, PKA/AC, MAPK, and MEK/ERK inhibitors, respectively; followed by addition of 1,25D. RNA was harvested 24 hrs later.

To determine involvement of VDR, cells were treated with VDR agonist EB-1089 (10 nM) or antagonist (23S,25S)-DLAM-2P (Ishizuka *et al.*, 2008), with/without 1,25D. Cells

were pretreated with (23S,25S)-DLAM-2P for 1 hr before addition of 1,25D. RNA was harvested 24 hrs later.

To determine involvement of histone deacetylases (HDACs), cells were treated with suberoylanilide hydroxamic acid (SAHA) (2  $\mu$ M; inhibitor of class I and II HDACs), MGCD0103 (0.1  $\mu$ M; inhibitor of class I HDACs), MC1568 (1  $\mu$ M; inhibitor of class IIa HDACs), and tubastatin (2  $\mu$ M; inhibitor of class IIb, HDAC 6). Cells were pretreated with HDAC inhibitors for 30 mins before addition of 1,25D.

1,25D, cycloheximide, actinomycin D, and SAHA were purchased from Sigma Aldrich (St. Louis, MO, USA). EB1089, PD98059, H-89, SB203580, U73122, GF109203X, MDL 12,330A, and MC1568 were purchased from Tocris Bioscience (Bristol, UK). MGCD0103 was obtained from SelleckChem (Houston, TX, USA).

Immunocytochemistry was performed with rat anti-VDR and GAPDH antibodies (Abcam, Cambridge, MA, USA) and anti-rat secondary antibody (Molecular Probes, Eugene, OR, USA).

### Real-Time Quantitative Polymerase Chain Reaction

Isolation of total RNA, cDNA synthesis, and quantitative polymerase chain reaction were performed for gene expression analysis on the Roche Lightcycler 480 system (Roche Applied Science, Indianapolis, IN, USA) using intron-spanning primers (Appendix Table) and relative expression analysis from Cp values (the Efficiency method, Roche Applied Science software). One-way analysis of variance and *post hoc* Tukey or Bonferroni test were used to detect intra- and intergroup gene expression differences. Student *t* test was used for comparisons between 2 groups (SigmaStat, SSI, San Jose, CA, USA).

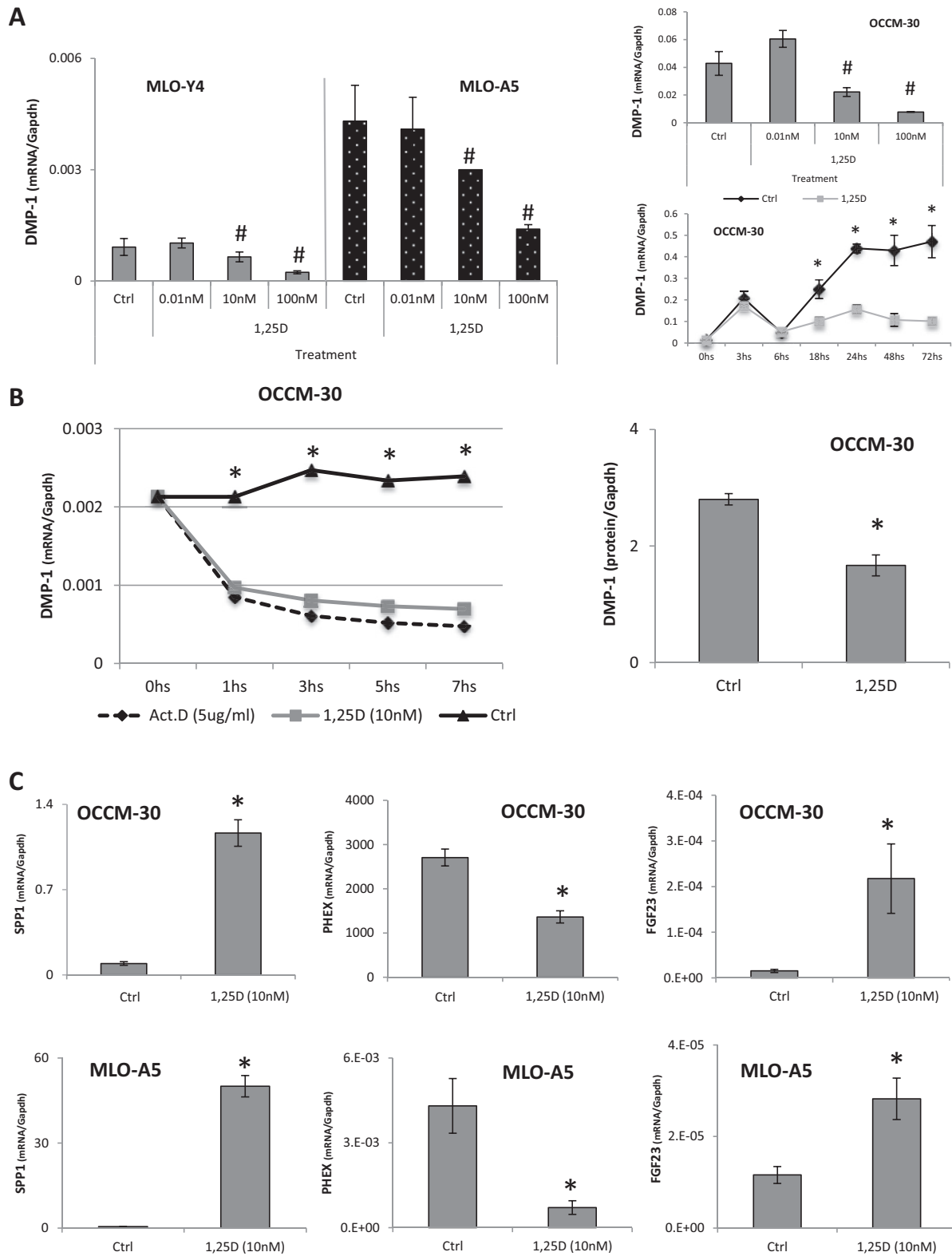
### Western Blot Analysis

Cells were exposed to 1,25D under serum-free conditions for 48 hrs; 10  $\mu$ g of total protein was used for SDS-PAGE in a 4% to 12% (w/v) polyacrylamide stacking gel (Invitrogen, Grand Island, NY, USA), then transferred to a nitrocellulose membrane. Rabbit anti-*DMP-1* antibody (Huang *et al.*, 2008) and IRDye secondary antibodies (LI-COR, Lincoln, NE, USA) were used. An imaging system (ODYSSEY CLx-LI-COR) with Image Studio Software (LI-COR) was used to quantify proteins.

## RESULTS

### 1,25D Downregulates *DMP-1* Expression in Cementoblasts and Osteocyte-like Cells

For all cells types used, 1,25D downregulated *DMP-1* gene expression in dose-dependent fashion (0.01, 10, 100 nM) by 24 hrs (Fig. 1A). Time course analysis based on OCCM-30 cells treated with 1,25D (10 nM) indicated suppression of *DMP-1* mRNA (approximately 3-fold decrease,  $p < .05$ ) by 18 hrs, which was maintained up to 72 hrs, when the experiment was terminated. Pretreatment of OCCM-30 cells for 30 mins with actinomycin D indicated that 1,25D-mediated decrease of *DMP-1* mRNA expression was regulated at the transcriptional level (Fig. 1B) and *de novo* protein synthesis was not required (determined with cycloheximide; data not shown). 1,25D decreased *DMP-1* protein levels by 48 hrs.



**Figure 1.**  $1\alpha,25(\text{OH})_2\text{D}_3$  (1,25D) decreases the expression of *DMP-1* mRNA and protein. **(A)** 1,25D downregulates *DMP-1* mRNA expression in a dose-dependent manner (0.01, 10, 100 nM) by 24 hrs following treatment of MLO-Y4 and MLO-A5 osteocyte-like cells and OCCM-30 cementoblasts. In addition, 1,25D at 10 nM suppresses *DMP-1* mRNA by 18 hrs in OCCM-30 cells, and suppression is maintained up to 72 hrs. **(B)** 1,25D treatment does not affect *DMP-1* mRNA stability in actinomycin D (Act.D)-treated cells compared with the control group, and Western blot data demonstrate significant reduction in *DMP-1* protein expression in media obtained from OCCM-30 cells at 48 hrs after 1,25D treatment. **(C)** Treatment of OCCM-30 and MLO-A5 cells with 10 nM 1,25D significantly regulates vitamin D-responsive genes, including *SPP1* (*OPN*), *PHEX*, and *FGF23* mRNA levels. Experiments were repeated at least 2 times in triplicate with comparable results. #Indicates statistical intergroup differences compared with the control by 1-way analysis of variance, followed by *post hoc* Bonferroni test ( $p < .05$ ). \*Indicates statistical intergroup difference by the Student *t* test ( $p < .05$ ).

1,25D (10 nM) significantly increased *OPN/SPP1* (15-fold/100-fold), decreased *PHEX* (2-fold/6-fold), and increased *FGF23* (14-fold/2.6-fold) mRNA levels in OCCM-30 and MLO-A5 cells, respectively (Fig. 1C), as previously reported for other cell types (Ecarot and Desbarats, 1999; Hines *et al.*, 2004; Nagata *et al.*, 1994), validating the use of OCCM-30 cells in further experiments.

### PKC, PKA, MAPK, and MEK/ERK Pathways are Not Required for 1,25D Effect on *DMP-1*

OCCM-30 cells were pretreated with pathway-specific inhibitors before addition of 1,25D (10 nM). Inhibition of PKC, PKA, MAPK, or MEK/ERK signaling failed to block 1,25D-mediated downregulation of *DMP-1* (Fig. 2), suggesting that these pathways are not required for observed changes.

### Class I HDAC Activity is Required for VDR-mediated Regulation of *DMP-1*

Increased VDR transcripts were noted in 1,25D-treated OCCM-30, MLO-Y4, and MLO-A5 cells (Appendix Fig. 1). Cells exposed to 10 nM EB1089, a VDR agonist, exhibited reduced *DMP-1* mRNA and increased *FGF23* mRNA at 24 hrs, similar to effects of 1,25D (Fig. 3A).

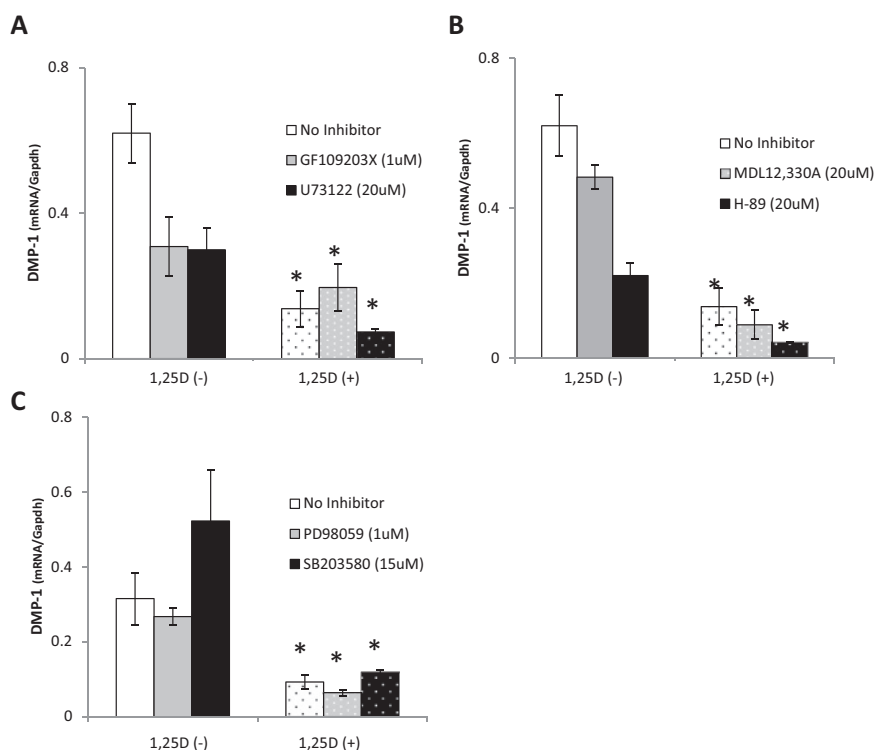
Conversely, addition of (23S,25S)-DLAM-2P, a VDR antagonist, rescued both *DMP-1* and *FGF23* gene expression in the presence of 1,25D in OCCM-30 and MLO-A5 cells (Fig. 3B).

VDR-dependent transrepression has been shown to require HDAC activity via a heterodimer complex. We determined whether HDAC was required for 1,25D-mediated downregulation of *DMP-1* expression. OCCM-30 cells expressed transcripts for the most common forms of HDACs (1-4), and 1,25D did not alter their expression (Appendix Fig. 2).

Application of a broad-spectrum HDAC inhibitor, SAHA, rescued 1,25D-mediated reduction of *DMP-1* mRNA levels in OCCM-30 cells ( $p > .05$ ) (Fig. 3C). Next, OCCM-30 cells were pretreated with class-specific HDAC inhibitors. Class I (HDAC 1/2/3) inhibitor MGCD0103 reproduced SAHA-mediated recovery of *DMP-1*, while MC1568 (class IIa inhibitor), as well as TH-C106 and Tubastatin (class I, HDAC 1/3 inhibitor and class IIb, HDAC 6 inhibitor, respectively; data not shown), did not block 1,25 D reduction of *DMP-1* mRNA. The results suggest that a class I HDAC, likely HDAC 2, is involved in the regulation of *DMP-1* expression by 1,25D.

## DISCUSSION

Discovery of new factors, hormones, and pathways controlling calcium and phosphate and directing physiologic and pathologic



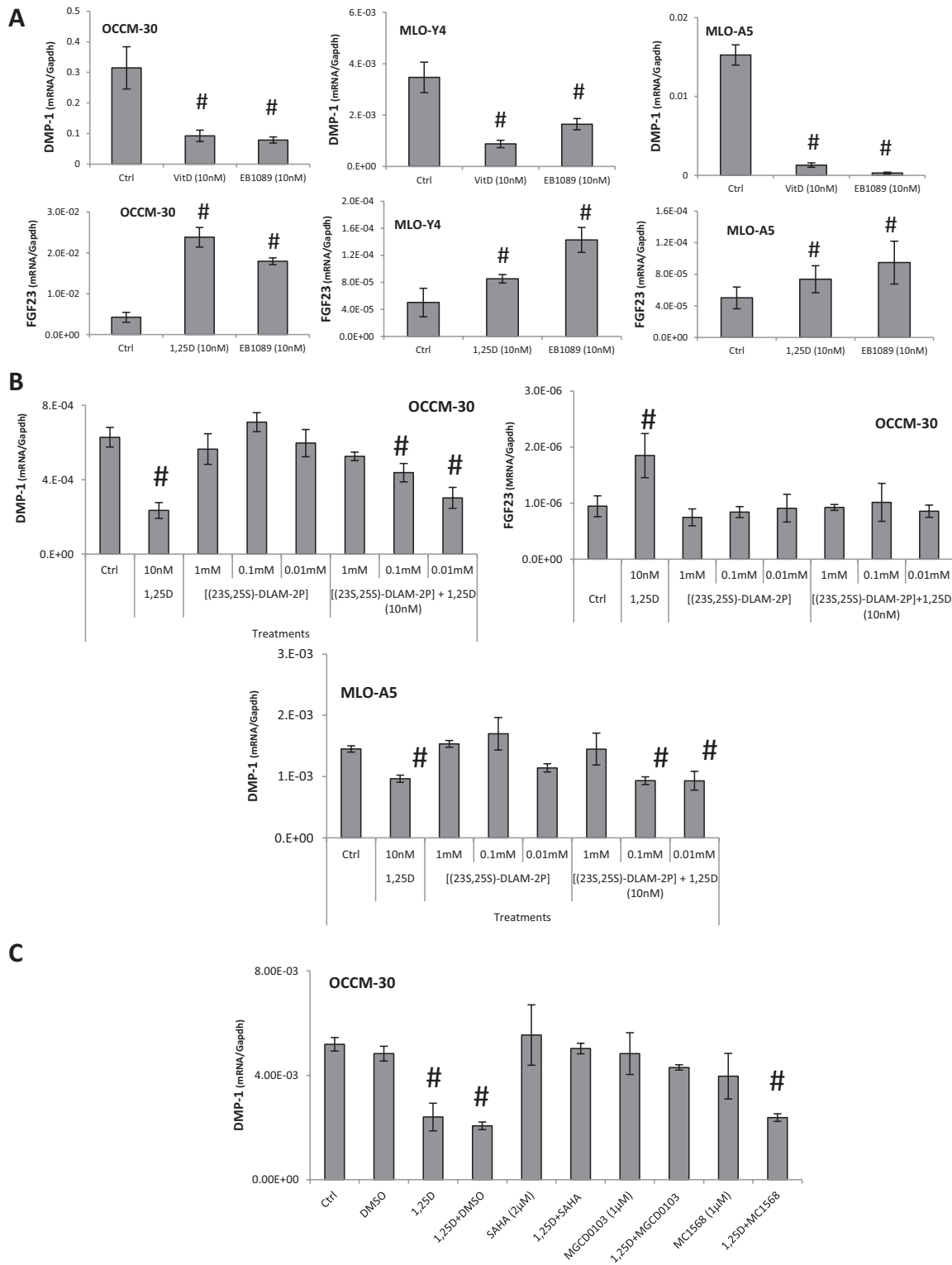
**Figure 2.** PKC, PKA, MAPK, or MEK/ERK pathways are not required for  $1\alpha,25(\text{OH})_2\text{D}_3$  (1,25D) effect on *DMP-1*. OCCM-30 cells were pretreated for 30 mins with (A) 1  $\mu\text{M}$  GF109203X or 20  $\mu\text{M}$  U73122 (PKC/PLC inhibitors), (B) 20  $\mu\text{M}$  H-89 or 20  $\mu\text{M}$  MDL12,330A (PKA/AC inhibitors), (C) 1  $\mu\text{M}$  PD98059 (MEK/ERK inhibitor) or 15  $\mu\text{M}$  SB203580 (MAPK inhibitor). Cells were then treated with 10 nM 1,25D or vehicle (ethanol) and RNA harvested 24 hrs later. 1,25D-mediated downregulation of *DMP-1* expression is not blocked by any of the signaling inhibitors evaluated, indicating that these pathways are not required. Experiments were repeated at least 2 times in triplicate with comparable results. \*Indicates statistical intergroup difference by the Student *t* test ( $p < .05$ ).

mineralization not only make us appreciate these complex processes but create opportunities for new therapeutic approaches. Our findings here demonstrate that 1,25D decreases *DMP-1* levels in murine cementoblasts and osteocyte-like cells via the VDR pathway, possibly contributing to local changes in bone and tooth matrix mineralization and identifying a potential new mechanism for 1,25D regulation of FGF23 and systemic mineral homeostasis.

### *DMP-1* is a Target Gene Regulated by 1,25D

*DMP-1* is an extracellular matrix protein first discovered in dentin, though later studies identified a broader range of expression in bone, with high levels in osteocytes (Huang *et al.*, 2008). Further studies revealed that *DMP-1*-null mice exhibit severe hypophosphatemia, and biochemical aspects of hypophosphatemic rickets, notably high levels of circulating FGF23 (Liu *et al.*, 2008). Mutations in the human *DMP-1* gene were identified as causative for ARHR1, which features elevated FGF23, hypophosphatemia, low (or inappropriately normal) 1,25D, hyperparathyroidism, osteomalacia, and tooth defects.

1,25D-induced transactivation by the VDR is well documented, and positive VDREs in gene promoters are known (Pike *et al.*, 2012). In contrast, mechanisms involved in negative gene regulation by VDR remain poorly understood. DR3-like and E-box-type negative VDRE sequences (nVDREs) have been



**Figure 3.** Class I histone deacetylase (HDAC) activity is required for vitamin D receptor (VDR)-mediated regulation of DMP-1. **(A)** Participation of the VDR signaling pathway in  $1,25(\text{OH})_2\text{D}_3$  (1,25D)-mediated inhibition of DMP-1 and increase in FGF23 expression was analyzed with a VDR agonist, EB1089, and antagonist, [(23S,25S)-N-benzyl-1 $\alpha$ ,25-dihydroxyvitaminD(3)-26,23-lactam [(23S,25S)-DLAM-2P]. Activation of the VDR pathway by EB1089 mimics the effect of 1,25D on DMP-1 and FGF23 expression in OCCM-30, MLO-Y4, and MLO-A5 cells. **(B)** Inhibition of VDR signaling by (23S,25S)-DLAM-2P blocks the 1,25D-mediated inhibition of DMP-1 expression in MLO-A5 and OCCM-30 cells and FGF23 upregulation in OCCM-30 cells. **(C)** Application of 2  $\mu\text{M}$  suberoylanilide hydroxamic acid (SAHA), a broad-spectrum HDAC class I and II inhibitor, rescues 1,25D-mediated reduction of DMP-1 mRNA levels in OCCM-30 cells. Treatment with 1  $\mu\text{M}$  MGCD0103 (HDAC class I 1/2/3 inhibitor) mimics the rescue effect of SAHA. However, treatment with 1  $\mu\text{M}$  MC1568 (HDAC class IIa inhibitor) does not inhibit 1,25D-mediated downregulation of DMP-1. Experiments were repeated at least 2 times in triplicate with comparable results. #Indicates intergroup statistical differences compared with the control group by 1-way analysis of variance, followed by the Bonferroni test ( $p < .05$ ).

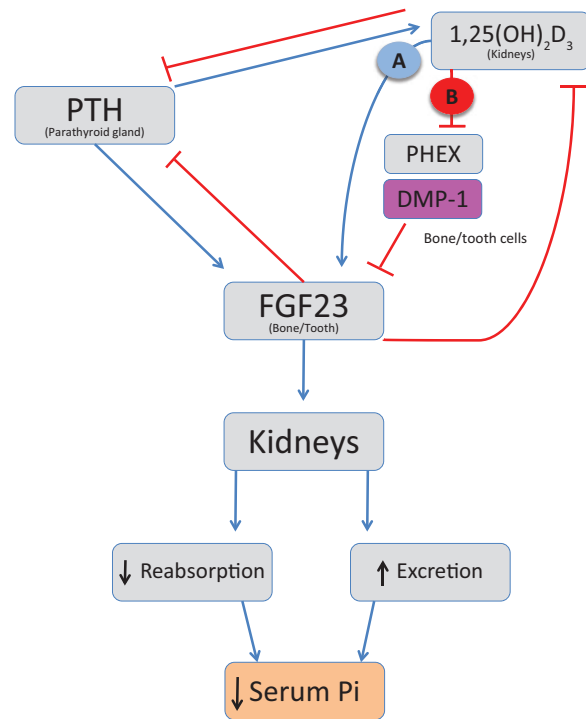


characterized in the promoter regions of the *PTH*, *PTHrP*, and *CYP27B1* genes, directly or indirectly binding the VDR as a monomer or the VDR–retinoid X receptor heterodimer, driving transcriptional repression (Murayama *et al.*, 1998; Okazaki *et al.*, 2003). The association of the VDR–retinoid X receptor heterodimer with nVDRE sequences may occur via a bHLH transcription factor referred to as VDIR (Murayama *et al.*, 2004). Existing data support an essential role of HDACs in promoting conformational chromatin changes allowing for transcription to occur (Pike *et al.*, 2012). After demonstrating 1,25D repression of *DMP-1* gene and protein expression in cementoblasts and osteocyte-like cells, we confirmed expression of VDR in OCCM-30 cells and demonstrated expression of common HDAC isoforms. Next we demonstrated that the VDR agonist EB-1089 replicated the effects of 1,25D by downregulating *DMP-1* transcripts, while the VDR antagonist (23S,25S)-DLAM-2P rescued *DMP-1* expression in the presence of 1,25D. Use of broad-spectrum and selective HDAC inhibitors revealed that recruitment of class I HDACs (1/2/3) were required for downregulation of *DMP-1*. These data provide strong evidence that 1,25D-mediated regulation of *DMP-1* is dependent on VDR activation, and further, that gene repression is dependent on HDAC function, as for many genes that are negatively regulated by nuclear hormone receptors (Zhang and Dufau, 2003).

Having established that *DMP-1* expression was modulated by a VDR/HDAC-containing complex, we also examined the murine *DMP-1* promoter for the presence of nVDRE sequences. Four putative nVDRE sequences were previously identified (Murayama *et al.*, 2004; Tovar Sepulveda and Falzon, 2003), and by gel shift assay, none were found to be involved in the 1,25D-mediated effect on *DMP-1* (data not shown). Moreover, promoter studies were inconclusive, similar to studies on 1,25D and the *PHEX* promoter (Hines *et al.*, 2004). Promoter analysis demonstrated that the only 1,25D-responsive *DMP-1* promoter construct contained 4 kb of 5' flanking DNA, suggesting that the 1,25D-responsive region is located between 2.5 and 4 kb upstream of the transcription start site (Appendix Fig. 3). In the future, we would like to further investigate the role that the promoter region plays in the 1,25D-mediated negative regulation of the *DMP-1* gene. Based on these findings, it is possible that 1,25D may indirectly affect *DMP-1* expression by regulating the expression of other genes. Future studies using RNAseq approaches will help determine the specific DNA sequences and transcription factor(s) involved in *DMP-1* regulation by 1,25D, and *ex vivo* and *in vivo* studies may assist us determining direct and indirect mechanisms involved.

### Role of *DMP-1* in 1,25D-FGF23 Regulation

The results here provide potential new insights on interactions between hormones directing mineral metabolism, 1,25D and FGF23, summarized in a hypothesized model in Fig. 4. While it is established that FGF23 is upregulated by 1,25D, the molecular mechanisms remain to be clarified. A recent report highlighting the ability of combined  $P_i$  and 1,25D to increase FGF23 expression *in vitro* did not evaluate 1,25D alone, leaving its role in the induction unclear (Ito *et al.*, 2013). A direct effect of 1,25D on FGF23 expression has been suggested to be via VDR,



**Figure 4.** Model for hypothesized  $1\alpha,25(\text{OH})_2\text{D}_3$  (1,25D) regulation of FGF23 expression, in part mediated through its effects on *DMP-1* expression. 1,25D and parathyroid hormone (PTH) have been reported to stimulate FGF23 secretion (blue arrows) controlling inorganic phosphate ( $P_i$ ) reuptake and excretion by the kidneys. FGF23 suppresses 1,25D and PTH production (red capped lines). Although controversial, 1,25D has been suggested to directly regulate FGF23 by activation of the VDR pathway, leading to gene transactivation through a VDRE sequence in the promoter region of the FGF23 gene (pathway A), and indirectly through its suppression of *PHEX* (pathway B). We now provide evidence suggesting that the ability of 1,25D to increase FGF23 expression may additionally be linked to its ability to downregulate *DMP-1* expression (pathway B), consistent with excess FGF23 observed in *ARHR1* resulting from mutations in the *DMP1* gene. Effects of 1,25D on *DMP1* would also be expected to influence local mineralized tissue properties, such as mineralization, which can affect systemic Ca and  $P_i$  homeostasis.

with the identification of a putative VDRE sequence in the promoter (Haussler *et al.*, 2012). However, because ongoing protein synthesis seems to be required for the action of 1,25D on FGF23 expression, an intermediary transfactor may participate in the mechanism (Kolek *et al.*, 2005). It has also been suggested that 1,25D may control FGF23 expression by its repressor action on *PHEX*, and results here suggest that *DMP-1* may be involved in parallel fashion. *PHEX* represses FGF23 expression (Yuan *et al.*, 2008), possibly via FGF receptor signaling (Martin *et al.*, 2011) and/or control of aspartate-rich motif serum levels (David *et al.*, 2011). Hypophosphatemic rickets, caused by increased levels of FGF23, results from mutations in both *DMP-1* and *PHEX*. The mechanisms by which *DMP-1* and *PHEX* affect FGF23 expression remain unclear; however, possibilities include (1) FGF receptor 1 signaling (Martin *et al.*, 2011), (2) potential for *DMP-1* to act as a transcription factor (Narayanan *et al.*, 2003; Siyam *et al.*, 2012), (3) alterations in

local extracellular matrix that trigger cells to increase FGF23 production, or (4) interactions between PHEX/DMP-1 and yet unknown genes and proteins.

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