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

F.H. Nociti Jr.[1,2], B.L. Foster[1], A.B. Tran[1], D. Dunn[3], R. B. Presland[4], L. Wang[1], N. Bhattacharyya[5], M.T. Collins[5], and M.J. Somerman[1]*

[1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA; [2]Department of Prosthodontics and Periodontics, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil; [3]Department of Periodontics, University of Washington School of Dentistry, Seattle, WA, USA; [4]Department of Oral Health Sciences, University of Washington School of Dentistry, Seattle, WA, USA; and [5]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA; *corresponding author, Martha.somerman@nih.gov

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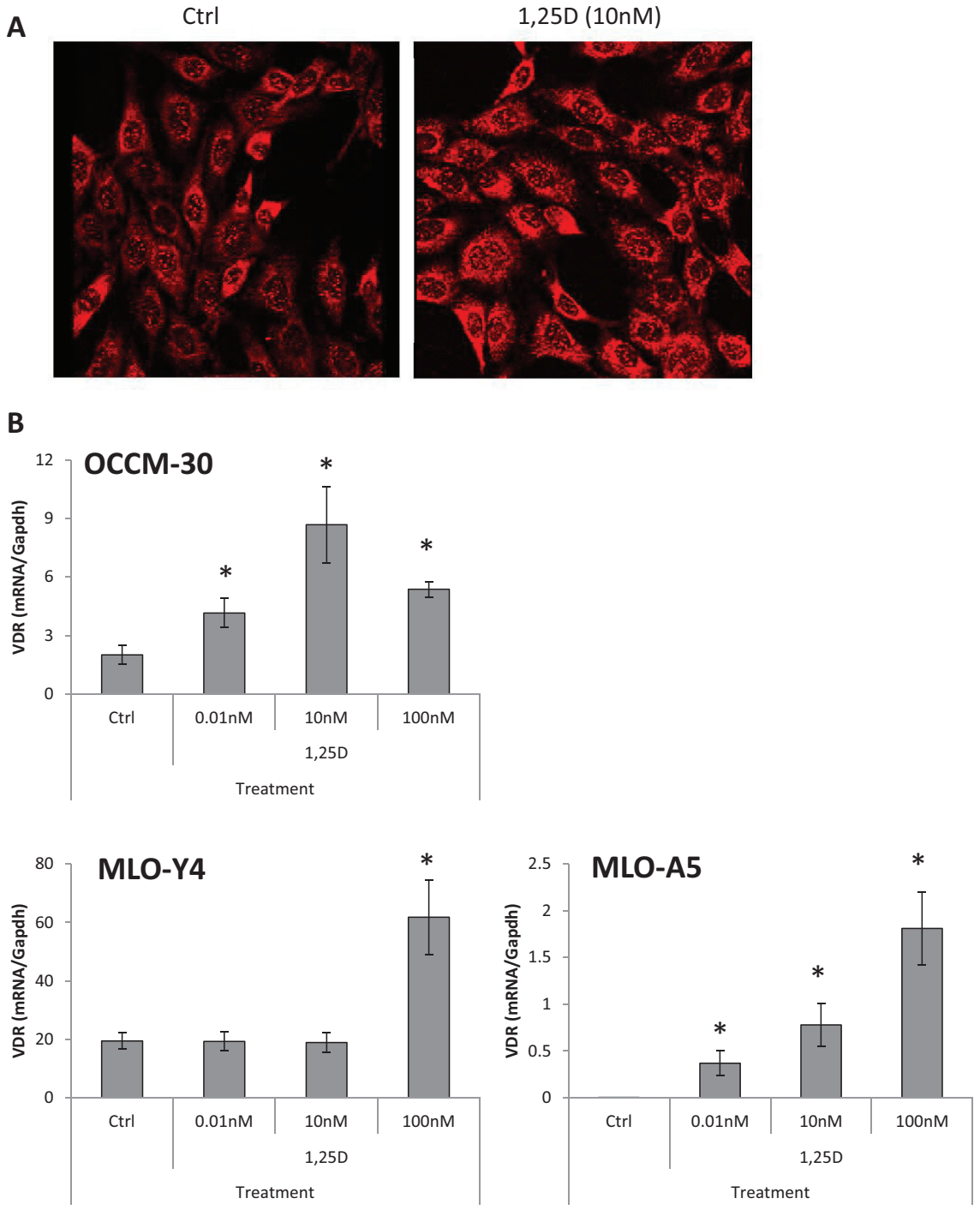
## APPENDIX

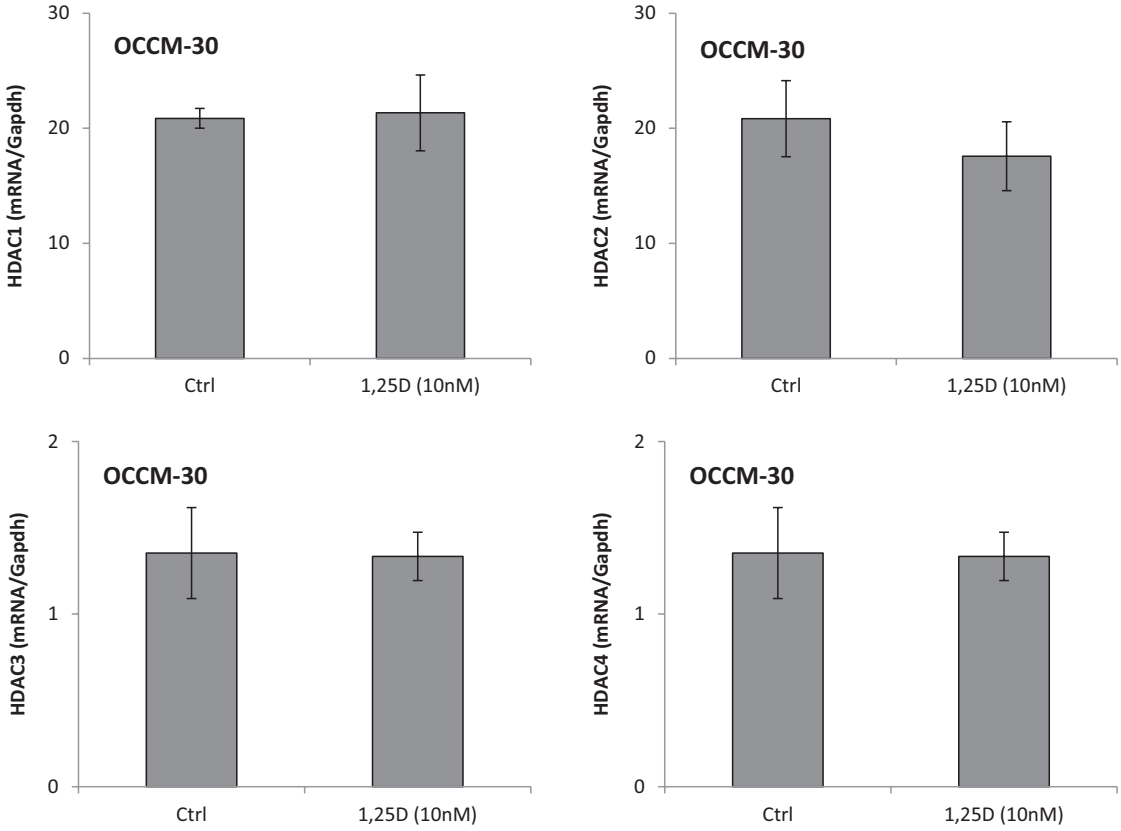
**Appendix Table.** Polymerase Chain Reaction Primer Sequences

| Gene Symbol | Gene Name | Forward (5′-3′) | Reverse (5′-3′) | Gene ID |
|---|---|---|---|---|
| *GAPDH*[a] | Glyceraldehyde-3 phosphate dehydrogenase | ACCACAGTCCATGCCATCAC | TCCACCACCCTGTTGCTGTA | 14433 |
| *DMP-1* | Dentin matrix protein 1 | GCGCGGATAAGGATGA | GTCCCCGTGGCTACTC | 13406 |
| *SPP1* | Secreted phosphoprotein 1 | TTTACAGCCTGCACCC | CTAGCAGTGACGGTCT | 20750 |
| *PHEX* | Phosphate regulating endopeptidase homolog, X-linked | GGCCTTACTACCGTTG | CAGTATCCCGAAGCACA | 18675 |
| *VDR* | Vitamin D receptor | CACCTGGCTGATCTTGTCAGT | CTGGTCATCAGAGGTGAGGTC | 22337 |
| *FGF23* | Fibroblast growth factor 23 | TCACGGGTGTTTGAGT | TCACGGGTGTTTGAGT | 64654 |

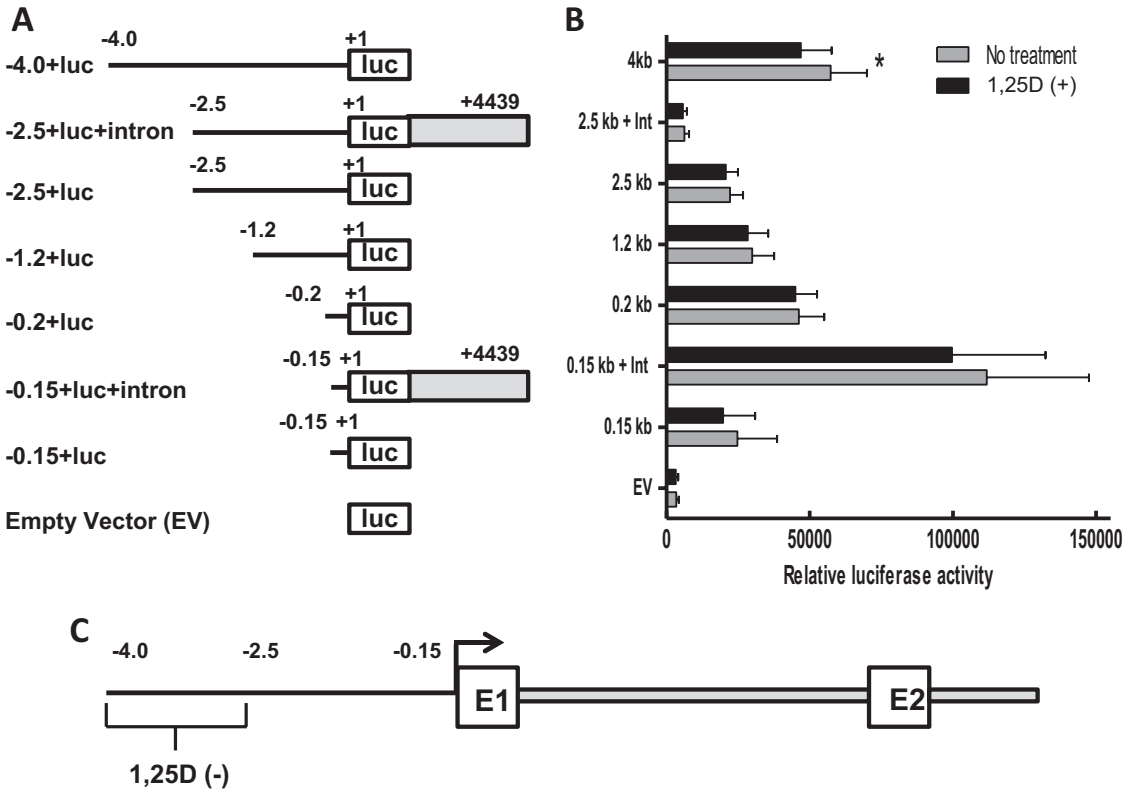
[a]Served as a housekeeping gene.




**Appendix Figure 1.** $1\alpha,25(OH)_2D_3$ (1,25D) regulation of vitamin D receptor (VDR) expression in cementoblasts and osteocyte-like cells. (**A**) Presence of VDR in the perinuclear region of OCCM-30 cells is confirmed by immunocytochemistry and confocal microscopy. Exposure of cells to 10nM 1,25D for 24 hrs results in increased potentiation of immunofluorescence. (**B**) VDR gene expression is noted in OCCM-30, MLO-Y4, and MLO-A5 cells. Treatment of cells for 24 hrs with 0.01nM, 10nM, or 100nM 1,25D increases VDR mRNA. Experiments were repeated at least 2 times in triplicate with comparable results. *Indicates statistical intergroup differences compared with the control group by 1-way analysis of variance, followed by *post hoc* Bonferroni test ($p < .05$).




**Appendix Figure 2.** Histone deacetylase (HDAC) expression in OCCM-30 cells. OCCM-30 cells express mRNA for the most common forms of HDACs, including HDAC 1, 2, 3, and 4. $1\alpha,25(OH)_2D_3$ (1,25D) treatment did not alter mRNA expression of any of these transcripts, as tested by 1-way analysis of variance followed by *post hoc* Bonferroni test ($p < .05$). Experiments were repeated at least 2 times in triplicate with comparable results.




**Appendix Figure 3.** 1$\alpha$,25(OH)$_2$D$_3$ (1,25D) decreases *DMP-1* promoter activity in mouse OCCM-30 cells. (**A**) Truncated upstream murine *DMP-1* promoter-luciferase constructs are shown that were used for transient transfection (Lu *et al.*, *Cells Tissues Organs*, 2005). Briefly, promoter constructs were cloned into the pGL3-Basic vector containing firefly luciferase as a reporter gene (with empty pGL3-Basic plasmid as a control; EV). Cells were cotransfected with pGL3 and Renilla vectors using LipofectAMINE Plus reagent (GIBCO-BRL, Grand Island, NY) or Omni-ultra (DBio, College Park, MD). Twenty-four hours after transfection, cells were rinsed with PBS and treated with 1,25D (10nM) or vehicle (ethanol) for 24 hrs in 2% FBS media. Promoter activity was determined by the ratio of firefly:Renilla luciferase for each construct, and the fold change in the luciferase activity was calculated by dividing the individual value by the control group value. (**B**) Transfected OCCM-30 cells were either treated with the vehicle (gray bars) or 10nM 1,25D (black bars) for 24 hrs, and luciferase activity was determined. Addition of 1,25D decreases luciferase activity in cells that are transfected with the –4-kb-luciferase construct, whereas promoter activity for the other constructs remains unaffected. Data presented are mean ± standard deviation from 3 separate experiments performed in triplicate. Statistical significance was determined by comparing plus *versus* minus 1,25D employing Student *t* test. *Indicates the statistical intergroup differences by Student *t* test ($p < .05$). (**C**) Locations of putative 1,25D negative regulatory regions are indicated within the mouse *DMP-1* promoter upstream region. E1, first exon; E2, second exon; shaded box, first intron.

## APPENDIX REFERENCE

Lu Y, Zhang S, Xie Y, Pi Y, Feng J (2005). Differential regulation of dentin matrix protein 1 expression during odontogenesis. *Cell Tissues Organs* 181(3-4): 241-247.