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Chronic kidney disease and vitamin D metabolism in human bone marrow–derived MSCs

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Abstract

Vitamin D that is synthesized in the skin or is ingested undergoes sequential steps of metabolic activation with a cascade of cytochrome P450 enzymatic hydroxylations in liver and kidney to produce 1 α ,25-dihydroxyvitamin D [1 α ,25(OH) $_2$ D]. There are many tissues that are able to synthesize 1 α ,25(OH) $_2$ D, but the biological significance of extrarenal hydroxylases is unresolved. Human marrow-derived mesenchymal stem cells (marrow stromal cells, hMSCs) give rise to osteoblasts, and their differentiation is stimulated by 1 α ,25(OH) $_2$ D. In addition to being targets of 1 α ,25(OH) $_2$ D, hMSCs can synthesize it; from those observations, we further examined the local autocrine/paracrine role of vitamin D metabolism in osteoblast differentiation. Research with hMSCs from well-characterized subjects provides an innovative opportunity to evaluate effects of clinical attributes on regulation of hMSCs. Like the renal 1 α -hydroxylase, the enzyme in hMSCs is constitutively decreased with age and chronic kidney disease (CKD); both are regulated by PTH1-34, IGF-1, calcium, 1 α ,25(OH) $_2$ D, 25(OH)D, and FGF23. CKD is associated with impaired renal biosynthesis of 1 α ,25(OH) $_2$ D, low bone mass, and increased fracture risk. Studies with hMSCs from CKD patients or aged subjects indicate that circulating 25(OH)D may have an important role in osteoblast differentiation on vitamin D metabolism and action in hMSCs.

Keywords

MSCs; vitamin D metabolism; CYP27B1; 25(OH)D; 1 α ,25(OH) $_2$ D; CKD; bone

Introduction

Vitamin D deficiency, a global health problem, is associated with increased risk of fracture and many musculoskeletal disorders in children and adults, including rickets, osteomalacia, osteoporosis, and muscle weakness, and non-skeletal illnesses, including cancer, diabetes, autoimmune diseases, infectious diseases, and cardiovascular disease.^{1–6} Numerous scientific organizations have developed guidelines for preventing and treating vitamin D deficiency and insufficiency, but not all intervention studies show significant effects on all those disorders.^{2–6}

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Competing interests

The authors declare no competing interests.

There are many circulating metabolites of vitamin D, and, because it can be synthesized in human skin and requires activation by two hydroxylation steps, it is more properly considered a hormone than a vitamin. Cholecalciferol (vitamin D₃) is synthesized from 7-dehydrocholesterol in the skin by exposure to ultraviolet sunlight with spectrum 280–320 nm (UVB). Vitamin D, in the form of ergocalciferol (vitamin D₂) from plants or vitamin D₃ from fish, can be obtained from dietary supplements or food sources. Nearly all vitamin D metabolites in serum (~ 88%) circulate bound to the D-binding protein (DBP), with a smaller proportion bound to albumin, and 0.03% circulating freely.⁷ Vitamins D₂ and D₃ undergo sequential steps of metabolic activation and degradation with a cascade of cytochrome P450 enzymatic reactions in different organs (Fig. 1). In the liver, the first enzymatic reaction hydroxylates them to 25-hydroxyvitamin D [25(OH)D] and is catalyzed by the mitochondrial enzyme CYP27A1 and the microsomal enzyme CYP2R1. The 25(OH)D metabolites are the most abundant forms in the serum and are used as a measure of vitamin D status. In the kidney, the active hormone, 1 α ,25-dihydroxyvitamin D (1 α ,25(OH)₂D) is produced by the mitochondrial 1 α -hydroxylase enzyme CYP27B1. In many tissues, CYP24A1 catalyzes 24-hydroxylation of 25(OH)D and 1 α ,25(OH)₂D, resulting in 24,25(OH)₂D and 1 α ,24,25(OH)₃D. Other functions for 24R,25(OH)₂D have been identified in the intestine and in osteoblasts.^{51,89,90}

Besides renal proximal tubular cells, biosynthesis of the activated 1 α ,25(OH)₂D occurs in many cell types, such as human monocyte–derived dendritic cells,^{8,9} monocyte–macrophage cell lines,¹⁰ preosteoclastic monocytes,¹¹ and human prostate and other cancer cells.¹² The discovery that osteoblasts¹³ and hMSCs¹⁵ synthesize 1 α ,25(OH)₂D raises new questions about the mechanisms by which 25(OH)D may regulate bone formation locally. Anephric patients have very low serum levels of 1 α ,25(OH)₂D, a fact that is taken to mean that its extrarenal synthesis contributes very little to the maintenance of normal serum levels.¹⁴ CYP27B1 enzymatic activity, however, is an important source of local 1 α ,25(OH)₂D for autocrine/paracrine actions in those extrarenal tissues.

Bone, kidney, and intestine are considered classic target tissues of 1 α ,25(OH)₂D, and those organ systems interact to regulate mineral homeostasis, but data now indicate that all cells may be targets of 1 α ,25(OH)₂D action.^{15–20} Genomic actions of 1 α ,25(OH)₂D entail its direct binding to a heterodimer of the nuclear vitamin D receptor (VDR) and RXR receptor complex, with subsequent binding to specific DNA sequences, the vitamin D response elements. There is widespread distribution of the VDR that mediates 1 α ,25(OH)₂D effects on cellular proliferation and differentiation.^{21,22} Non-genomic effects of 1 α ,25(OH)₂D are rapid and involve activation of a range of intracellular signaling molecules.

Vitamin D metabolism in hMSCs

Human marrow stromal cells (i.e., mesenchymal stem cells (hMSCs)) give rise to osteoblasts,^{23–28} and *in vitro* differentiation to osteoblasts is stimulated by the activated hormone 1 α ,25(OH)₂D.^{28,29} We have studied hMSCs obtained as tissue discarded during orthopedic procedures in projects aimed at identifying mechanisms of human bone cell differentiation and the influence of clinical factors on those mechanisms.¹⁵ We observed that, in addition to 1 α ,25(OH)₂D, both vitamin D₃ (D₃) and 25(OH)D were capable of

stimulating osteoblastogenesis of hMSCs.^{15,30} Those observations led to the discovery of vitamin D hydroxylases in hMSCs at different constitutive levels, found to be correlated with serum 25(OH)D, 1 α ,25(OH)₂D, and PTH of the subject from whom the MSCs were isolated.¹⁵ We provided evidence that antiproliferative and prodifferentiation effects of 25(OH)D depended on CYP27B1 in hMSCs.²⁴ Although there is no information that marrow synthesis of 1 α ,25(OH)₂D contributes to the circulation, the amount of *in vitro* biosynthesis of 1 α ,25(OH)₂D from added 25(OH)D amounts to >16 pg/mL, which is more than the level of added 1 α ,25(OH)₂D that stimulates *in vitro* osteoblastogenesis.¹⁵ In addition, that level of biosynthesis corresponds to normal serum 1 α ,25(OH)₂D (range of 15–75 pg/mL). Those calculations suggest that local biosynthesis of 1 α ,25(OH)₂D by hMSCs is at biologically meaningful levels and may play an autocrine/paracrine role in bone formation.

In vitro treatment with 25(OH)D upregulated CYP27B1 and insulin-like growth factor 1 (IGF-1) in hMSCs and stimulated osteoblast differentiation as monitored by the rise in alkaline phosphatase (ALP) enzymatic activity.¹⁵ The positive correlation between serum 25(OH)D and 1 α -hydroxylase/CYP27B1 (1 α OHase) gene expression in hMSCs from subjects with low serum 25(OH)D and *in vitro* dose-dependent increase in CYP27B1 gene expression with low doses of 25(OH)D may signify substrate feedforward induction¹⁵ (Fig. 2A). When hydroxylation of 25(OH)D was blocked by ketoconazole,¹⁵ a cytochrome P450 inhibitor, or by CYP27B1 siRNA,²⁴ biosynthesis of 1 α ,25(OH)₂D was reduced, and 25(OH)D was no longer able to stimulate osteoblastogenesis. There was a dose-dependent downregulation of CYP27B1 in MSCs by 1 α ,25(OH)₂D, associated with upregulation of CYP24A1. The downregulation of CYP27B1 by 1 α ,25(OH)₂D illustrates a feedback repression at the level of gene expression (Fig. 2B). Greater amounts of substrate, 25(OH)D, and increased CYP27B1 would result in increased synthesis of 1 α ,25(OH)₂D, which, in turn, would downregulate the enzyme, as suggested by the lower levels of CYP27B1 with higher levels of serum or added 25(OH)D.¹⁵ Further evidence of regulation of vitamin D hydroxylases in hMSCs is that the higher levels of added 25(OH)D or 1 α ,25(OH)₂D upregulated 24-hydroxylase (CYP24A1)¹⁵ (Fig. 2B). 24-Hydroxylation reduces the amount of 1 α ,25(OH)₂D and reduces risk of hypercalcemia.

Skeletal IGF-1 may play multiple roles in skeletal growth and homeostasis. Circulating 25(OH)D, by virtue of its local conversion to 1 α ,25(OH)₂D catalyzed by CYP27B1 in hMSCs, amplifies vitamin D signaling through IGF-1 upregulation, which in turn induces CYP27B1 to potentiate osteoblast differentiation and skeletal homeostasis initiated by IGF-1.¹⁵ Not only do both 25(OH)D and 1 α ,25(OH)₂D stimulate IGF-1 gene expression and osteoblast differentiation with hMSCs, other pro-osteoblastogenic agents, such as estradiol,³¹ the adrenal hormone dehydroepiandrosterone (DHEA),³¹ and parathyroid hormone (PTH),²⁶ upregulate IGF-1 and osteoblast differentiation in hMSCs. IGF-1 itself stimulates *in vitro* biosynthesis of 1 α ,25(OH)₂D and osteoblast differentiation in hMSCs.¹⁵ Thus, IGF-1 is a common intracellular mediator for many osteoanabolic agents (Fig. 3).

Effects of age on vitamin D metabolism and osteoblast differentiation in hMSCs

There are many properties of hMSCs that are dramatically affected by the age of the subject from whom they were isolated.¹⁶ We have found reproducible age-related declines in hMSC potential for proliferation and osteoblast differentiation^{25,26,91} and declines in the magnitude of stimulation of osteoblast differentiation by 25(OH)D,²⁶ 1 α ,25(OH)₂D,²⁸ and PTH.²⁷ There are conflicting reports from the 1990s and early 2000s about the effect of age on osteoblast potential of human MSCs that have been explained by differences in marrow sources (necropsy, biopsy, surgical), anatomical sites, isolation procedures, and ambiguous terminology.⁹² Studies that used 3-dimensional colony size and number to assess osteoblast differentiation often contradicted each other because of differences in thresholds for enumeration, subjective practices, and different supplements in the culture media; those assays have also been criticized for inappropriate use of parametric statistical methods.⁹³

The age-related decline in hMSC stimulation by 25(OH)D was attributed to age-related declines in constitutive expression of CYP27B1.²⁶ There were also age-related declines in expression of the PTH receptor²⁷ and in PTH signaling of the cAMP response element binding protein (CREB) and β -catenin.²⁷ For example, the age-related resistance to 25(OH)D was explained by constitutive expression of CYP27B1 in MSCs from subjects older than 55 years being 56% of that in hMSCs from subjects younger than 50 years.²⁶

Experiments using PTH1-34 to upregulate CYP27B1 expression and enzymatic activity in hMSCs from older patients led to the rejuvenation of osteoblast differentiation, with synergy between PTH1-34 and 25(OH)D.^{26,45} Thus, PTH1-34 stimulated hMSCs from older subjects with responsiveness to 25(OH)D by upregulating CYP27B1 expression and activity and did so through the CREB and IGF-1 signaling pathways, as shown by *CREB* siRNA and by inhibitors of IGF-1R kinase.²⁶ This IGF-1 requirement for PTH anabolic action on bone was demonstrated *in vivo* with mice.⁹⁴ It is useful to remember that the anabolic actions of PTH upon intermittent exposure to low doses are not similar to catabolic skeletal effects, as seen in situations of chronic high concentrations of circulating PTH, such as in CKD.⁹⁵

Comparison of CYP27B1 regulation in renal cells and in MSCs

The kidney is the primary (if not the only) source of circulating 1 α ,25(OH)₂D under normal conditions. The multiple effects of 1 α ,25(OH)₂D on bone, kidney, intestine, and parathyroid glands are strictly coordinated and regulated to ensure mineral homeostasis without risk of hypercalcemia. There appears to be only one renal 1 α -hydroxylase gene—*CYP27B1*—and it is tightly regulated by three hormones, PTH, 1 α ,25(OH)₂D, and fibroblast growth factor 23 (FGF23). A primary stimulus of renal CYP27B1 is the initial elevation of PTH due to hypocalcemia. PTH stimulates the transcription of renal CYP27B1,³² and, in turn, 1 α ,25(OH)₂D suppresses PTH production at the transcriptional level.³³ Depending upon concentrations, 1 α ,25(OH)₂D and 25(OH)D also modulate CYP27B1 expression. Thus, 1 α ,25(OH)₂D inhibits renal CYP27B1 by two major means: direct inhibition of CYP27B1³⁴ and indirectly by suppression of PTH. FGF23 is a bone-derived hormone that dampens elevations in serum phosphate by inhibiting renal tubular reabsorption of phosphate and

reduces circulating $1\alpha,25(\text{OH})_2\text{D}$ through inhibition of renal *CYP27B1* transcription and upregulation of *CYP24A1*.³⁵

There is great interest in regulation of extrarenal *CYP27B1*, especially in keratinocytes³⁶ and macrophages.³⁷ Human macrophage *CYP27B1* plays an important role in the vitamin D-dependent antimycobacterial response and mediates the effect of $25(\text{OH})\text{D}$ to induce antibacterial activity.³⁷ Expression of *CYP27B1* in keratinocytes and in macrophages is stimulated by tumor necrosis factor α , interferon γ , and other cytokines.³⁶ Neither keratinocytes nor macrophages show effects of PTH on *CYP27B1* transcription, as do renal cells, but it has been shown that FGF23 inhibits *CYP27B1* expression in normal peripheral monocytes and in monocytes from peritoneal dialysates.³⁸ Studies with *Fgf23*^{-/-} *1 α -Luc*⁺ mice demonstrate FGF23-dependent regulation of *CYP27B1* in heart, aorta, spleen, lung, skin, brain, and testis.³⁹

There are many ways in which regulation of *CYP27B1* expression in hMSCs is similar to that in kidney cells (Table 1), including upregulation by PTH^{26,32,40} and downregulation by FGF23.^{41,42} Another similarity between MSC and renal regulation of *CYP27B1* is that IGF-1 upregulates both.^{15,26,43} This is especially critical in that exogenous IGF-1 stimulates both osteoblast differentiation and $1\alpha,25(\text{OH})_2\text{D}$ biosynthesis in MSCs and that $25(\text{OH})\text{D}$, $1\alpha,25(\text{OH})_2\text{D}$, and PTH stimulate osteoblast differentiation through endogenous IGF-1 signaling, as shown by our reports.^{15,26,27} Age is associated with decreased constitutive expression of *CYP27B1* in both kidney cells and in MSCs.^{26,44} It is possible to rejuvenate *CYP27B1* and osteoblast differentiation with MSCs from elders by synergistic action of PTH and $25(\text{OH})\text{D}$, a combination shown to require histone deacetylation.^{26,45} Calcium and phosphate are other two regulators of *CYP27B1*. Calcium downregulates *CYP27B1* in both kidney⁴⁶ and MSCs.⁴⁷ Phosphate decreases *CYP27B1* in kidney,^{42,48} but phosphate's effect on *CYP27B1* in human MSCs is as yet unknown. Most of this information on regulation of *CYP27B1* comes from studies with human MSCs, but some information is from porcine MSCs.^{47,50}

In vivo, $1\alpha,25(\text{OH})_2\text{D}$ limits renal *CYP27B1* activity by inhibiting serum PTH and increasing serum FGF23, as well as reducing $1\alpha,25(\text{OH})_2\text{D}$ levels by inducing the catalytic enzyme *CYP24A1*;³⁶ it has also been reported that $1\alpha,25(\text{OH})_2\text{D}$ inhibits *CYP27B1* expression via VDR in the kidney.⁴⁹ The regulation of the extrarenal *CYP27B1* may differ from that of the renal *CYP27B1*.⁹⁶ However, as we summarized in Table 1, the regulation of *CYP27B1* in human MSCs is very similar to that of renal *CYP27B1*. Our data¹⁵ showed that $1\alpha,25(\text{OH})_2\text{D}$ downregulated *CYP27B1* at > 0.01 nM and upregulated *CYP24A1* at > 1 nM in human MSCs, suggesting that, at low concentration, the major effect of $1\alpha,25(\text{OH})_2\text{D}$ alone on its own levels occurs through inhibition of *CYP27B1*, and not induction of *CYP24A1*; at high concentrations, the effect of $1\alpha,25(\text{OH})_2\text{D}$ on its own levels occurs through both inhibition of *CYP27B1* and induction of *CYP24A1*. Our other data showed that *CYP27B1* is upregulated by PTH and downregulated by FGF23 in hMSCs;^{26, 41} FGF23 is also regulated *CYP24A1*.⁴¹ It is unknown whether the major effect of $1\alpha,25(\text{OH})_2\text{D}$ on its own levels is through inhibition of *CYP27B1* or induction of *CYP24A1* in *in vivo* cases where $1\alpha,25(\text{OH})_2\text{D}$, PTH, and FGF23 are together.

Chronic kidney disease and vitamin D metabolism

Chronic kidney disease (CKD) is associated with progressive reductions in the renal synthesis of $1\alpha,25(\text{OH})_2\text{D}$,^{57–61} development of secondary hyperparathyroidism,^{62,63} low bone mass, and increased risk of fractures.^{64–68} According to the National Health and Nutrition Examination Surveys (NHANES), 13% of the U.S. population has CKD, defined as persistent albuminuria or impaired estimated glomerular filtration rate (eGFR).⁶⁹ More than 45% of adults > 70 years have an eGFR < 60 mL/min per 1.73 m² or an albumin-to-creatinine ratio of > 30 mg/g.⁶⁹ For individuals with advanced CKD, the risk of fracture is 4- to 17-fold greater than in the normal population.^{64–66} Analysis of NHANES data showed a 2-fold greater hip fracture risk in patients with an eGFR of < 60.⁶⁷ The Women's Health Initiative-Observational (WHI-OS) case-control analysis of 397 incident hip fractures and 397 age-matched controls showed that elevated cystatin-C, a measure of renal function not affected by muscle mass, was associated with a 2.5 odds ratio for hip fracture in women with an eGFR < 60 compared with non-CKD women.⁶⁸ In a cohort of 35 men and women that we studied,²⁸ whole-body bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DXA) was lower in subjects with low eGFR, with a correlation coefficient of 0.37 ($P = 0.032$) (Fig. 4). Those subjects did not have known renal disease, and most had eGFR values in the normal range. There is an urgent need to define the myriad of mechanisms that may contribute to the increased fracture risk in CKD and to ascertain how to modify that risk.

CKD mineral and bone disorder (CKD-MBD) is a systemic condition that links disorders of mineral and bone metabolism due to CKD to either one or all of the following: abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism; abnormalities in bone turnover, mineralization, volume, linear growth or strength; and extraskeletal calcification.^{70,71} Vitamin D deficiency and insufficiency are common among patients with CKD or who are undergoing dialysis.⁷² Among 988 dialysis patients analyzed by Thadhani *et al.*, 79% had 25(OH)D levels < 30 ng/mL, and 57% had deficient levels < 20 ng/mL.⁵⁷ Other analyses show that vitamin D deficiency is more common in those with CKD (14% for CKD stage 1 and 27% for CKD stage 5), compared with non-CKD individuals (8%).⁷³ Overall, CKD was associated with 39% increased odds for vitamin D deficiency even after multivariable adjustment for demographic variables and factors known to influence vitamin D levels, including dietary vitamin D intake. Stage 5 CKD-MBD is associated with profound vitamin D deficiency, alterations in mineral metabolism, and markedly increased fracture risk. For example, in a U.S. population-based study, the mean 25(OH)D level was 12.6 ± 9.1 ng/mL in individuals with stage 5 CKD.⁶¹ Bone biopsies in subjects with 25(OH)D levels < 15 ng/mL showed reduced bone formation and mineralizing surfaces compared with those with higher vitamin D levels.⁷⁴ Reductions in $1\alpha,25(\text{OH})_2\text{D}$ levels are seen well before the development of end-stage renal disease (ESRD); a cross-sectional study of 1814 individuals with CKD showed a decrease in $1\alpha,25(\text{OH})_2\text{D}$ for individuals with lower eGFR ($R^2 = 0.38$, $P < 0.0001$).⁷⁵

Recent emerging data on nutritional vitamin D supplementation in patients with CKD are potentially very important because 25(OH)D serves as substrate for extrarenal effects of vitamin D in target tissues that express CYP27B1, including hMSCs.^{15,24} The results from

small or not randomized clinical trials suggest that nutritional vitamin D supplementation may be needed in patients with kidney disease,⁸⁶ and that correction of 25(OH)D levels in individuals with CKD may benefit bone metabolism and bone quality.⁷⁶ The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines currently recommend vitamin D supplementation in patients with stage 3 to 4 CKD with 25(OH)D levels < 30 ng/mL.^{77,78} In addition, KDIGO clinical practice guideline suggests that vitamin D deficiency and insufficiency in CKD patients should be corrected using treatment strategies recommended for the general population.⁷⁸ Vitamin D supplementation (ergocalciferol or cholecalciferol) in CKD patients aims to prevent secondary hyperparathyroidism;^{72,79} randomized controlled trials, however, especially with newer vitamin D analogs and nutritional vitamin D compounds, have demonstrated serum PTH reductions but with a possible increased risk of hypercalcemia and/or hyperphosphatemia in CKD-MBD patients.⁸⁵

Impact of renal function and CKD on vitamin D metabolism in hMSCs

Little is known about vitamin D biosynthesis in non-renal cells in patients with CKD. Our recent reports provide new information about the mechanisms of and biological significance of the combined presence of vitamin D hydroxylases and VDR in hMSCs that support an autocrine/paracrine role for vitamin D metabolism in hMSCs.²⁸ We showed, for example, that the VDR is required for the prodifferentiation and antiproliferative actions of $1\alpha, 25(\text{OH})_2\text{D}$ in hMSCs.⁴⁵ We provided some new information about vitamin D metabolism in hMSCs from subjects with CKD.^{30,41} FGF23 downregulates the VDR in hMSCs; this raises the possibility of skeletal vitamin D resistance in CKD.⁴¹ A VDR deficiency in the parathyroid glands is believed to cause, at least in part, the resistance of parathyroid cells to $1\alpha, 25(\text{OH})_2\text{D}$ in uremic hyperparathyroidism.⁸¹ There was a significant correlation between *in vitro* osteoblastogenic stimulation (alkaline phosphatase activity) by $1\alpha, 25(\text{OH})_2\text{D}$ and eGFR.^{28,30} We were fortunate to obtain deidentified discarded marrow from a 57-year-old male orthopedic patient with ESRD who had been undergoing hemodialysis for more than 2 years; although serum 25(OH)D level was not available, this subject had secondary hyperparathyroidism and was being treated with cinacalcet, calcium acetate, and active D, commonly used medications in this population.³⁰ There was 50% lower constitutive expression of CYP27B1 in hMSCs from the dialysis subject, compared with an age/gender-matched control (Fig. 5); the about 600-fold greater expression of 24-hydroxylase in those hMSCs may contribute to low serum 25(OH)D in CKD.³⁰ This is also consistent with the subject being treated with active D.¹⁵ This is in accord with a recent report of unexpected elevations in renal CYP24A1 mRNA and protein in uremic rats, as well as greater immunolocalization of CYP24A1 protein in kidney biopsies from CKD relative to control subjects.⁸² We also cultured hMSCs in osteoblastogenic medium $\pm 1\alpha, 25(\text{OH})_2\text{D}$, 25(OH)D, or D₃.³⁰ That all vitamin D metabolites stimulated osteoblastogenesis in hMSCs from this dialysis subject as well as the control suggests that 25(OH)D sufficiency may be important for skeletal health in older populations and in CKD.⁴⁷ Emerging data on nutritional vitamin D supplementation in patients with CKD-MBD are critically important because 25(OH)D serves as substrate for the many extra-renal effects of vitamin D in target

tissues, including hMSCs.^{15,28} It is as yet unknown whether correction of 25(OH)D levels in individuals with ESRD will stimulate bone formation *in vivo* and reduce fracture risk.

The fact that the vitamin D 1 α -hydroxylase enzyme has been found in parts of the body outside the kidney suggests that there may be a role for nutritional vitamin D in patients with kidney disease.⁸⁶ A recent study in hemodialysis patients showed that 1 α ,25-dihydroxyvitamin D levels increased after supplementation with nutritional vitamin D; this was taken as evidence that, even in ESRD, there is enough extrarenal 1 α -hydroxylase activity to influence serum levels.⁸⁷ Extrarenal synthesis and activity of 1 α ,25-dihydroxyvitamin D may also be of benefit in those settings. A nonrandomized study of 158 hemodialysis patients who received cholecalciferol supplementation showed higher 25(OH)D, 1 α ,25-dihydroxyvitamin D, and albumin levels, while at the same time reduced serum calcium, PTH, brain natriuretic peptide, left ventricular mass index, and erythropoietin-stimulating agent and active vitamin D doses.⁸⁸ The limitations of trials temper conclusions regarding their effects on patient-level skeletal outcomes. Larger, high-quality randomized trials focused on skeletal outcomes are needed.⁸⁶

CKD is associated with increased levels of FGF23 and a deficiency of its co-receptor Klotho.^{30,77,78,80,83} FGF23 has extrarenal effects on the cardiovascular, central nervous, and immune systems⁸³ and non-renal cells, such as MSCs⁸⁴ and human monocytes.³⁸ The main physiological function of Klotho for *in vivo* mineral homeostasis is achieved by its role as co-receptor mediating FGF23 action.⁸⁰ Our new data with hMSCs showed that there was a significant correlation for constitutive expression of membrane-bound klotho ($r = 0.52$, $P = 0.019$) and a statistical trend of positive correlation for expression of secreted klotho ($r = 0.42$, $P = 0.064$) with eGFR in a cohort of subjects without kidney disease.⁴¹ There was virtually undetectable constitutive expression of klotho in MSCs from CKD subjects. This relationship was exhibited in control hMSCs by finding that *in vitro* treatment with rhFGF23 downregulated klotho expression.⁴¹ Furthermore, rhFGF23 downregulated CYP27B1 and VDR gene expression and inhibited osteoblastogenesis that was stimulated by either 25(OH)D or 1 α ,25(OH)₂D in hMSCs.⁴¹ Dysregulated extrarenal vitamin D metabolism in human MSCs may contribute to impaired osteoblastogenesis and altered bone and mineral metabolism in CKD subjects owing to elevated FGF23. It is not known whether there is equivalent inhibition of CYP27B1 in renal cells and in hMSCs by FGF23.

Summary and conclusions

Vitamin D that is synthesized in the skin or ingested undergoes sequential steps of metabolic activation and degradation via a cascade of cytochrome P450 enzymatic hydroxylations in liver and kidney. The first hydroxylation in the liver to 25(OH)D is mediated by CYP27A1 and/or CYP2R1; the second hydroxylation in the kidney to the active hormone 1 α ,25(OH)₂D is mediated by CYP27B1. Our recent data demonstrate that not only are hMSCs a target of vitamin D action via the VDR, they also have the machinery to hydroxylate vitamin D metabolites. Osteoblast differentiation is stimulated by 25(OH)D, but that stimulation is dependent on activity of CYP27B1 in hMSCs.²⁴ Stimulation of osteoblast differentiation by either 25(OH)D or 1 α ,25(OH)₂D requires VDR, as shown by knockdown studies with *VDR* siRNA.⁴⁵ These findings on mechanisms of osteoblast differentiation with

hMSCs support the hypotheses that local vitamin D metabolism serves an autocrine/paracrine role in human osteoblastogenesis. *In vitro* studies with hMSCs show the many similar ways by which MSC and renal CYP27B1 are regulated (Fig. 6 and Table 1). Our *in vivo/in vitro* approach demonstrates that hMSCs retain clinical characteristics of the subjects from whom the cells were obtained.^{15,28} Correlation analyses revealed that there are significantly reduced *in vitro* effects of $1\alpha,25(\text{OH})_2\text{D}$ to stimulate osteoblast differentiation in hMSCs obtained from subjects who were older than 65 years of age or who had impaired renal function, assessed by estimated glomerular filtration rate.²⁸ Moreover, $25(\text{OH})\text{D}$ does not stimulate MSCs from elders as well as cells from young subjects and that there is an age-related decline in CYP27B1 expression in hMSCs.^{26,28} That resistance to $25(\text{OH})\text{D}$ is reversible by *in vitro* treatment with PTH1-34, which upregulates CYP27B1.²⁶ Studies on the interactions between PTH1-34 and $25(\text{OH})\text{D}$ revealed that PTH1-34 upregulates VDR gene expression and that $25(\text{OH})\text{D}$ upregulates PTHR1 gene expression.⁴⁵ Data about interactions of $25(\text{OH})\text{D}$ and PTH1-34 with their mutual receptors add information about the mechanisms of synergy in promoting osteoblast differentiation in hMSCs from elders. In addition, our studies show that $25(\text{OH})\text{D}$, by virtue of its local conversion to $1\alpha,25(\text{OH})_2\text{D}$ catalyzed by CYP27B1 in hMSCs, amplifies vitamin D signaling through IGF-1 upregulation, which in turn induces CYP27B1 in a feedforward mechanism to potentiate osteoblast differentiation.¹⁵ The expression and 1α -hydroxylase enzyme activity of CYP27B1 are upregulated by $25(\text{OH})\text{D}$,¹⁵ E_2 ,⁵³ and PTH²⁶, and are downregulated by $1\alpha,25(\text{OH})_2\text{D}$.¹⁵ CKD⁴¹ and FGF23⁴¹ downregulate CYP27B1 gene expression in hMSCs (summarized in Fig. 6). These data with hMSCs indicate that vitamin D metabolism in marrow is regulated as in kidney, and that vitamin D metabolism in hMSCs may promote osteoblastogenesis in an autocrine/paracrine manner.

Because of the possibility that extrarenal vitamin D activation is essential for skeletal health, more information is needed about the biological, physiological and clinical significance of vitamin D metabolism in hMSCs and the effects of CKD on vitamin D metabolism, regulation, and action in marrow and the roles of 1α -hydroxylation and the VDR in osteoblast differentiation of human marrow progenitors/stem cells. That information is needed to advance our understanding of the role of extrarenal vitamin D synthesis and potential impacts on bone health and may provide novel evidence of the importance of vitamin D sufficiency on maintenance of skeletal mass in individuals with CKD.

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Figure 1.

Classical vitamin D metabolism. Vitamin D undergoes sequential steps of metabolic activation and degradation with a cascade of cytochrome P450 enzymatic reactions. The first hydroxylation in the liver produces 25(OH)D, and the second hydroxylation in the kidney produces the active hormone, 1α,25(OH)₂D. The 25-hydroxylation is catalyzed by CYP27A1 and/or CYP2R1 and the 1α-hydroxylation by CYP27B1. CYP24A1 catalyzes 24-hydroxylation to 24,25(OH)₂D and 1α,24,25(OH)₃D. Summarized from Refs. 1–6.

Figure 2. Regulation of vitamin D hydroxylases in hMSCs from *in vivo* and *in vitro* studies.^{15, 24} (A) The positive correlation between serum 25(OH)D and 1 α -hydroxylase/CYP27B1 (1 α OHase) gene expression in hMSCs from subjects with low serum 25(OH)D and *in vitro* dose-dependent increase in CYP27B1 (1 α OHase) and IGF-1 gene expression with low doses of 25(OH)D (10 nM) may signify substrate feedforward induction. (B) The constitutive expression of CYP27A1/25OHase was lower in hMSCs from subjects with higher levels of serum 25(OH)D. Constitutive expression of CYP27B1 (1 α OHase) was lower in hMSCs from subjects with elevated serum 25(OH)D or 1 α ,25(OH)₂D. There was *in*

vitro reduction of CYP27B1 (1 α OHase) with higher doses of 25(OH)D (> 10nM) or 1 α , 25(OH)₂D (0.01 nM). These data suggest product feedback repression of vitamin D 25- and 1 α -hydroxylases in hMSCs. Both higher levels of 25(OH)D (at 1000 nM) and 1 α , 25(OH)₂D (1 nM) *in vitro* stimulated CYP24A1 gene expression in hMSCs.

Figure 3.

Role of insulin-like growth factor 1 (IGF-1) as a common mediator for many osteoanabolic agents. Not only do both $25(\text{OH})\text{D}$ and $1\alpha,25(\text{OH})_2\text{D}$ stimulate IGF-1 gene expression and osteoblast differentiation in hMSCs, other pro-osteoblastogenic agents, such as parathyroid hormone (PTH),²⁶ estradiol (E2),³¹ and dehydroepiandrosterone (DHEA)³¹ stimulate IGF-1 and osteoblast differentiation in hMSCs.⁹ IGF-1 itself stimulates *in vitro* biosynthesis of $1\alpha,25(\text{OH})_2\text{D}$ and osteoblast differentiation in hMSCs.¹⁵ The activation of IGF-1 signaling was shown to be necessary for both PTH²⁶ and DHEA³¹ to stimulate osteoblast differentiation in hMSCs.

Figure 4. Correlation between bone mineral density (BMD) and estimated glomerular filtration rate (eGFR). In a cohort²⁸ of 35 study subjects, whole-body BMD was positively correlated with eGFR.

Figure 5.

Comparison of constitutive gene expression in hMSCs from a hemodialysis subject and a control subject. There was lower expression of CYP27B1 (1 α -hydroxylase) and CYP27A1 (25-hydroxylase), greater expression of CYP24A1 (24-hydroxylase), and similar expression of CYP2R1 (25-hydroxylase) and VDR (vitamin D receptor) in hMSCs from a hemodialysis subject, compared with age/gender-matched control subjects.

Figure 6.

Summary of vitamin D metabolism and the regulation of CYP27B1 in hMSCs. (A) Dietary or skin-derived vitamin D undergoes a first hydroxylation in the liver to 25(OH)D and a second hydroxylation in the kidney to the active hormone 1α,25(OH)₂D, which stimulates osteoblastogenesis. Thus, 1α,25(OH)₂D produced locally by hMSCs may act in an autocrine/paracrine manner to stimulate osteoblastogenesis. CKD reduces 1α,25(OH)₂D production in kidney and human MSCs. (B) Numerous factors regulate CYP27B1/1α-hydroxylase in hMSCs, including stimulation by PTH, 25(OH)D, IGF-1, and estradiol (E2) and downregulation by high doses of 1α,25(OH)₂D, CKD, and FGF23 and aging.

Table 1

Comparison of CYP27B1 regulation in renal cells and human MSCs

Regulator	Renal		MSCs	
	Regulation	Ref.	Regulation	Ref.
1 α ,25(OH) $_2$ D	↓	32, 49	↓ ↔	15, 50, 51
25(OH)D	↔ ↓	52	Regulation in dose-dependent manner. ↑ ↔ ↓	15, 51, 53
Calcium	↓	46	↓	47
Phosphate	↓	42, 48	Unknown	
PTH	↑	32, 40	↑	26
IGF-1	↑	43	↑	15, 26
Age	↓	44	↓	26
CKD	↓	54	↓	30, 41
FGF23	↓	42	↓	41
Estrogen	↑	55, 56	↑	53

Note: ↑, upregulation or positive correlation; ↓, downregulation or inverse correlation; ↔, no effect or correlation.