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REVIEW

Vitamin D endocrine system and osteoblasts

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The interaction between vitamin D and osteoblasts is complex. In the current review we will give an overview of the current knowledge of the vitamin D endocrine system in osteoblasts. The presence of the vitamin D receptor in osteoblasts enables direct effects of 1α,25dihydroxyvitamin D3 (1α,25D3) on osteoblasts, but the magnitude of the effects is subject to the presence of many other factors. Vitamin D affects osteoblast proliferation, as well as differentiation and mineralization, but these effects vary with the timing of treatment, dosage and origin of the osteoblasts. Vitamin D effects on differentiation and mineralization are mostly stimulatory in human and rat osteoblasts, and inhibitory in murine osteoblasts. Several genes and mechanisms are studied to explain the effects of 1α,25D3 on osteoblast differentiation and bone formation. Besides the classical VDR, osteoblasts also express a membrane-localized receptor, and *in vitro* studies have shown that osteoblasts are capable of the synthesis of 1α,25D3.

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Introduction

In bone, 1α,25dihydroxyvitamin D_3 (1α,25D3) is important for mineralization, either indirectly via control of calcium absorption in the intestine and reabsorption in the kidney or via direct action on osteoblasts.[1–3] Several *in vivo* murine studies have indicated the direct effects of 1α,25D3 on bone; however, the effects described are different between studies. Transgenic mice overexpressing the vitamin D receptor (*VDR*) under control of an osteoblast-specific promoter showed increased trabecular bone volume and increased bone strength, indicating an anabolic effect of 1α,25D3.[4] However, similar characteristics such as increased trabecular thickness and increased osteoid volume and osteoblast number have been reported in the global *VDR* knockout mice,[5] pointing to a negative effect of 1α,25D3 on trabecular development. This latter observation is supported by a recent study showing increased bone mass in osteoblast-specific *VDR* knockout mice.[6] However, in this transgenic mouse model the bone formation parameters were unaltered and the effect on bone mass was through reduced bone resorption. Thus, both osteoblast-specific overexpression and knockout of *VDR* lead to increased bone mass, revealing opposite conclusions on the role of vitamin D in osteoblasts and bone metabolism. Although an explanation for this discrepancy is yet unclear, these data point to a direct effect of 1α,25D3 on bone involving osteoblasts.

In the current review, we will discuss the vitamin D endocrine system in osteoblasts, including its receptor, as well as vitamin D metabolism and effects on osteoblast activity.

VDR

The classical VDR is a member of the nuclear receptor family. Upon binding 1α,25D3, the VDR heterodimerizes with the retionoic X receptor and binds as a dimer to the vitamin D response element in the DNA to regulate gene expression.[7] The VDR is present is osteoblasts and its expression can be regulated by 1α,25D3 itself (homologous upregulation) and by other factors such as parathyroid hormone (PTH), glucocorticoids, transforming growth factor-β and epidermal growth factor.[8–13] A recent study identified multiple enhancer sites in the *VDR* promoter.[14] Cyclic adenosine monophosphate response element-binding protein binding to the *VDR* promoter was reported as the potential explanation for the heterologous upregulation of VDR expression by PTH. Also, the CCAAT enhancer-binding protein binding to *VDR* promoter has been linked to PTH VDR upregulation.[15] 1α,25D3 upregulated C/EBPβ binding to the *VDR* promoter, which may have a role in the homologous regulation of VDR.[14] C/EBPβ interacts with VDR in the regulation of CYP24 expression in osteoblasts.[15,16] RUNX2 is a key transcription factor in osteoblast differentiation. Interaction of VDR with RUNX2 in the regulation of osteocalcin and osteopontin expression by osteoblasts has been shown.[17,18] It is now well established that 1α,25D3 and VDR regulate gene transcription in osteoblasts and in all other target cells via interaction with a multitude of other transcription factors and DNA and histone-modifying proteins.[7]

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24-Hydroxylase (CYP24)

CYP24 is the most sensitive gene for 1α,25D3 regulation and is expressed in all target cells—that is, cells expressing VDR. CYP24 encodes the enzyme 24-hydroxylase. Hydroxylation of 1α,25D3 at the C-24 position is the first and rate-limiting step in the degradation of 1α,25D3.[19–21] The VDR level in osteoblasts is tightly coupled to the induction of CYP24 expression and 24-hydroxylase activity and thereby to the degradation of 1α,25D3. Thus, homologous upregulation of VDR concomitantly induces the inactivation of 1α,25D3 and thereby limits its effect.[8,22] Other 1α,25D3 responses do not follow the change in VDR level. For example, although transforming growth factor-β-induced upregulation of VDR is followed by an increase in 24-hydroxylase activity, 1α,25D3 stimulation of osteocalcin expression is inhibited.[23] The impact of VDR regulation may depend on the type of regulator and/or target cell.[12,24] Besides regulation of VDR level, VDR activity can also be regulated by phosphorylation. 1α,25D3 itself as well as activation of protein kinase C involving casein kinase II can phosphorylate VDR and thereby affect 1α,25D3 transcriptional activity.[25–27]

Hydroxylation of 1α,25D3 or 25(OH)D3 at the C-24 position does not directly lead to an inactive vitamin D molecule. Classic studies by Henry and Norman demonstrated the significance of 24,25D3 for normal chicken egg hatchability and calcium and phosphorus homeostasis.[28,29] Several human and animal (chicken, mouse, rat) studies showed effects of 24,25D either alone or in combination with other hormones on bone metabolism. It has already been shown in 1980 that 24,25D3 directly stimulates calcification of bone synergistically with PTH and that 24,25D3 decreased the number and size of resorption sites in bone.[30,31] 24,25D3 restored the reduction in bone mineral apposition rate in vitamin D-deficient rats and enhanced the bone mineral apposition rate restoration by PTH in parathyroidectomized rats.[32] One study showed, on the basis of histomorphometric data, no effect of 24,25D3 in ovariectomized rats.[33] Another study with ovariectomized rats showed that 24,25D3 in contrast to 1α,25D3 increased the breaking force with minimal effect on mineral content. The authors suggested that 1α,25D3 and 24,25D3 act differently on the matrix and mineral phase of the bone.[34] 24,25D3, together with 1α,25D3, improved bone mechanical strength in chickens.[35] 24,25D3 treatment improves fracture healing,[36] and interestingly 24,25D3 serum levels are correlated to fracture healing.[37] A role for 24,25D3 in fracture repair is supported by studies in the *CYP24* knockout mouse.[38] However, in a human study no positive association of 24,25D3 with femoral fracture was observed.[39] A study on pre-dialysis renal insufficiency patients treated with either 1α(OH)D3 alone or in combination with 24,25D3 supported a direct, that is, PTH-independent, functional role of 24,25D3 in bone. 24,25D3 together with 1α(OH)D3 but not 1α(OH)D3 alone preserved the osteoblast perimeter, and improved mineralization activity was observed.[40] These data suggest a direct role in bone cells—in particular, in osteoblasts. Van Driel *et al.* have shown in *in vitro* studies that indeed 24,25D3 has direct effects on human osteoblasts similar to that of 1α,25D3.[41] It should be noted that, as 24-hydroxylation is the first step of a degradation cascade, whether biological active levels of 24,25D3 or 1,24,25D3 can be reached depends fully on the velocity of the subsequent steps in the degradation pathway.

The presence of VDR in osteoblasts[42,43] thus enables direct effects of 1α,25D3 on osteoblasts, but the magnitude of the effects is subject to the presence of other factors. It is therefore important to consider the effects on osteoblasts in the context of interaction with other hormones (for example, PTH, cortisol),[9,12,44,45] growth factors such as transforming growth factor-β, insulin-like growth factor-I, bone morphogenetic factor, interferon, hepatocyte growth factor, epidermal growth factor,[3,46–51] and other signaling molecules such as the peroxisome proliferator-activated receptor ligand rosiglitazone and Wnt signaling.[52] Alternatively, 1α,25D3 or non-hypercalcemic 1α,25D3 analogs may change the activity of other hormones, factors and signaling cascades. 1α,25D3 enhanced, for example, the 17β-estradiol effect in female but not in male human osteoblasts, as assessed by increased creatine kinase response.[53]

Proliferation

The proliferative effects of 1α,25D3 on osteoblasts of various origin, such as mouse, rat and human, have been studied. 1α,25D3 can inhibit the proliferation of osteoblasts[54–61] and also stimulate osteoblast proliferation.[56,62,63] 1α,25D3 has been reported to decrease the number of viable MC3T3 osteoblasts[64] as well as to inhibit[65] and induce osteoblast apoptosis.[66] In contrast, in human SV-HFO osteoblasts, no clear 1α,25D3 effect on proliferation was observed.[67] Overall, the data point to an impact of 1α,25D3 on osteoblast proliferation, cell viability, apoptosis and processes related to the cell cycle. However, the 1α,25D3 effect appears to vary with the timing of treatment, dosage and origin of the osteoblast.[68–70]

Differentiation and Mineralization

1α,25D3 is a regulator of bone metabolism and functions by stimulating the production of bone matrix proteins (for example, collagen, osteopontin, osteocalcin, matrix Gla protein) and activity of alkaline phosphatase activity involved in mineralization. 1,25D3 interacts with the osteoblast differentiation regulatory Wnt signaling cascade. 1α,25D3-activated VDR binds in osteoblasts of various origin to the promoter of the gene-encoding LRP5, the Frizzled co-receptor-initiating canonical Wnt signaling, and increases LRP5 mRNA levels.[71] 1α,25D3 enhanced β-catenin signaling in human TE-85 osteoblasts.[45]

So far, 1α,25D3 has been shown to stimulate bone formation and mineralization in all studies using human osteoblasts and to stimulate osteogenic differentiation from human mesenchymal stem/stromal cells (MSCs).[41,67,72–76] 1,25D3 enhanced mineralization through its effects on human osteoblasts before the onset of mineralization.[2] Thus, 1,25D3 is not directly involved in the process of mineral deposition but more likely in a process preparing the environment/extracellular matrix for mineralization. Gene expression profiling studies demonstrated that the 1,25D3 effect is not likely primarily due to changes in expression of the environment/extracellular matrix proteins and thereby not due to composition of the environment/extracellular matrix.[2] Some studies on the expression and production of procollagen type I by human osteoblasts have demonstrated stimulation,[77,78] whereas others have shown no effect.[78–80] However, 1α,25D3 significantly induced the production of

alkaline phosphatase-positive matrix vesicles[81] providing a means to enhance mineralization.[2] Studies on ovariectomized rats supported a positive effect of 1α,25D3 and of an 1α,25D3 analog on bone formation and mineralization.[82,83] However, studies on the expression of collagen type I in isolated rat osteoblasts showed either an inhibition or no effect.[84,85]

In contrast, 1α,25D3 inhibits differentiation and mineralization in cultures of murine osteoblasts such as MC3T3 cells,[3,50,64,86] and murine osteoblasts lacking the VDR have increased osteogenic potential.[87] Collagen type I has been shown to be stimulated, inhibited or not affected by 1α,25D3[88,89] Inhibition of mineralization is supported by the observation that 1α,25D3 increases, in a VDR-dependent manner, the expression of ectonucleotide pyrophosphatase phosphodiesterase (ENPP1) and progressive ankylosis in murine osteoblasts, leading to an increase in the mineralization inhibitor pyrophosphate.[90] This and other studies also demonstrated a 1α,25D3 increase in osteopontin, which has been shown to inhibit mineralization.[91] However, there have been specific transgenic murine models, for example, with osteoblast-specific *VDR* overexpression, that show increased bone formation and mineralization.[4,92,93] Further, a study has demonstrated a positive effect of the 1α,25D3 analog on bone nodule formation and mineralization in murine calvarial osteoblast cultures of wild-type but not *VDR*-null mice[83] and a study demonstrated increased mineralization in a study with MC3T3 cells.[89]

Overall, the current observations show variation in the effects of vitamin D on differentiation and mineralization, with the effects overall being stimulatory in human and rat osteoblasts and inhibitory in murine osteoblasts.[50,67] In line with this, in human osteoblasts 1α,25D3 has been shown to increase *RUNX2* expression,[61,73,94] whereas in murine osteoblasts 1α,25D3 suppresses the *RUNX2* promoter and inhibits *RUNX2* expression.[73,95] The role of osteocalcin needs to be emphasized upon in this discrepancy between mouse and human osteoblasts. Human and mouse osteocalcin genes are differently regulated by 1α,25D3.[96] In contrast to human and rat osteoblasts in which $1,25(OH)_2D_3$ stimulates *BGLAP* expression, $1,25(OH)_2D_3$ inhibits *BGLAP* expression in murine osteoblasts,[97,98] further supporting differences between murine and human/rat osteoblasts with respect to vitamin D responsiveness and mineralization (**Table 1**).

A full explanation for this apparent discrepancy between human and murine osteoblasts is absent. Extracellular milieu (see above section VDR) as well as the intracellular milieu of the cell is important for the eventual effect of the 1α,25D3 as exemplified by the fact that insulin-like growth factor-binding protein-6 can bind to the VDR and inhibit 1α,25D3 induction of alkaline phosphatase activity.[99] Also, extracellular conditions like phosphate concentration[100] or growth factors and cytokines may determine the eventual effect of 1α,25D3.[46,91,101]

Table 1 Summary of 1α,25(OH)D3 on differentiation-related responses in human, mouse and rat osteoblasts

	Human	*Mouse*	*Rat*
Alkaline phosphatase	↑	↓/↑/=	↑/↓
Collagen type I	↑/=	↓/↑/=	↓/=
Osteocalcin	↑	↓	↑
Mineralization	↑	↓/↑	↑

These characteristics may contribute to the differences in 1α,25D3 effects observed in human and murine osteoblasts. However, within studies on murine osteoblasts also differences were observed, with inhibition of mineralization in cultures of calvarial osteoblasts and no effect in osteoblasts derived from long bones.[102] Respective parts of the mammalian skeleton differ from each other in origin, mode of osteogenesis and function. The axial and appendicular skeletons originate from the mesoderm, whereas the skull bones originate from the cranial neural crest. Regarding formation, the calvaria are formed by intramembranous bone formation and the long bones by endochondral formation. Interestingly, the most widely used murine osteoblast cell line, MC3T3, is of calvarial origin.[103] For the MC3T3 cells, the number of passages influences the grade of mineralization[104] which may also have an impact on 1α,25D3 action. However, passaging of a human osteoblast cell line did not affect VDR level and the 1α,25D3 response.[105] Although different from cell passaging, age of the donor of MSCs has been shown to be negatively correlated with the 1α,25D3 stimulation of alkaline phosphatase activity and osteocalcin expression in osteogenic human MSCs.[76] Unfortunately, stimulation of mineralization was not analyzed with respect to age in this study. An additional factor that influences the 1α,25D3 effect is the differentiation stage of osteoblasts and basal level of expression of the gene/protein of interest.[2,89,106]

In vivo studies with rats demonstrated a direct anabolic effect of 1α,25D3 on bone. Both chronic treatment with 1α,25D3[107] and short-term treatment[108] increased bone formation. The number of osteoblast precursors and osteoblasts was increased by both treatment regimens, which may explain the increased bone formation. However, in hypocalcemic *VDR* knockout mice lacking 1α,25D3 signaling, an increase in osteoblast number and osteoid volume was observed.[5] Alternatively, evidence for a direct 1α,25D3 bone anabolic effect was provided by the results of a study with osteopenic ovariectomized rats.[109] These apparent contradictive data suggest species differences in the effect of 1α,25D3 on bone. Besides species differences, a number of additional parameters like diet (that is, composition and concentrations of minerals), age, sex, timing of treatment, duration of treatment,[2,94,106,110–113] dosages, etc. should be taken into account when comparing *in vivo* studies. However, it is not always possible to include these modifying factors in the comparison and interpretation as they are often missing or not reported in sufficient detail. These data together with the discussed differences in *in vitro* mineralization and osteocalcin expression in osteoblasts warrant careful interpretation of the data while translating to the human situation.[90,114,115]

Gene Expression and Gene Expression Profiling Studies

Besides the already mentioned effects of 1α,25D3 on *RUNX2*, several other genes and potential mechanisms have been studied in order to explain the effect of 1α,25D3 on osteoblast differentiation and bone formation. 1α,25D3-induced *c-MYC* expression has been implicated as an accelerator of human osteoblast differentiation by enhancing BMP-2-induced bone formation.[116] Expression of insulin-like growth factor-binding proteins-2, -3 and -4 in human MSCs is induced by 1α,25D3 and may have a role in the stimulation of osteogenic differentiation,[117] although this functional role for the binding proteins

needs to be demonstrated. Forkhead Box O (*FoxO*) transcription factors were differentially regulated by 1α,25D3 in MC3T3 osteoblasts; *FoxO3a* was upregulated, *FoxO1* was downregulated and *FoxO4* level was not changed. Knockdown of the *FoxOs* did not change the 1α,25D3-induced expression of *CYP24* and cell cycle regulators, nor the inhibition of proliferation by 1α,25D3.[118] Unfortunately, the effect on 1α,25D3 inhibition of mineralization by these murine MC3T3 osteoblasts was not reported. The changes in *FoxO* expression were linked to an increase in reactive oxygen species accumulation. This may be linked to cellular metabolism and bone formation, as this is a high-energy-demanding process.[119–121] Glucose, insulin and 1α,25D3 regulation of proliferation, alkaline phosphatase activity and production of (uncarboxylated) osteocalcin has been studied in isolated rat osteoblasts but no coupling to mineralization was made.[122]

In view of the relationship between bone formation and angiogenesis, the observation of 1α,25D3-increased vascular endothelial growth factor (VEGF) expression in human and rat osteoblasts is of interest,[123–125] and the vascular endothelial growth factor has been shown to have a role in the 1α,25D3 anabolic effect.[126]

A recent observation provided evidence for microRNA (miRNA)-637 and miRNA-1228 in the 1α,25D3 stimulation of human osteoblast differentiation for the first time.[127] It is becoming evident that miRNAs have an important role in osteoblast differentiation and bone formation,[128] and in the near future more data on their role in 1α,25D3 action in osteoblasts will come forward.

Activin A inhibits osteoblast differentiation and mineralization.[129] 1α,25D3 stimulated the expression of activin A in human osteoblasts.[130] Thus, 1α,25D3, as stimulator of osteoblast differentiation and mineralization, also stimulates the production of an inhibitor. The hypothesis that this serves a role in preventing over-mineralization is supported by the observations that the activin A blocker follistatin enhances 1α,25D3-stimulated mineralization.[130] The induction of carboxylated osteocalcin by 1α,25D3 may fit this hypothesis and provide further support. 1α,25D3-induced accumulation of osteocalcin in the extracellular matrix of human osteoblast cultures is inhibited by warfarin (antagonist of vitamin K), whereas vitamin K_2 (cofactor of γ-carboxylase) enhanced the 1,25 D3 effect.[131] Vitamin K_2 metabolism is stimulated by 1α,25D3,[132] and 1α,25D3-stimulated mineralization was significantly augmented by warfarin.[130] Although not fully delineated, these data on activin A, follistatin, warfarin and vitamin K put forward a 1α,25D3-induced regulatory mechanism to control and guarantee optimal mineralization.[130] Differences in these regulatory loops may also be part of the differences in 1α,25D3 effects between human and murine studies.

Several gene expression profiling studies have been performed to examine the effect of 1α,25D3 on RNA expression in osteoblasts. The reported inhibition of proliferation of murine MC3T3 osteoblasts was linked to downregulation of DNA replication genes in the same cells.[54] Gene expression profiling on multiple days during the differentiation phase before mineralization did not reveal a specific set of DNA replication genes being regulated in human osteoblasts.[2] Gene ontology analyses did identify genes that were linked to the cell cycle phase, RNA splicing translation and cell death as being most significantly overrepresented.[2] Interestingly, this study demonstrated a strong difference between the genes regulated in the period preceding the mineralization and those regulated during mineralization. Only 0.6% (3 genes) of the genes regulated in the mineralizing period were also regulated before mineralization.[2] A gene expression profiling study investigating the effect of 24-h of 1α,25D3 treatment on primary human osteoblasts identified by Ingenuity IPA (http://www.ingenuity.com) various biological functions and/or diseases related to bone metabolism. Cellular Processes or Molecular Function IPA analyses identified functions related to skeletal development.[133] In relation to skeletal development, 1α,25D3-induced expression in murine and human osteoblasts of the odd-skipped related genes *Osr1* and *Osr2*, known from expression in the developing limb, is of interest.[134]

Gene expression profiling after 24 h of treatment with 1,25D3 identified functions and processes related to the immune system.[133] In line with this is the observation of a gene profiling study showing interferon-related genes being overrepresented after 1α,25D3 treatment of human osteoblasts. The interferon signaling-related genes were downregulated by 1α,25D3.[48] Interferon-β inhibits mineralization through an effect in the very early phase of osteoblast differentiation, which is overruled by 1α,25D3.[48,135]

Besides regulating bone formation, 1α,25D3 may also regulate bone resorption through expression of the regulators of osteoclast formation receptor activator of nuclear factor-κ B ligand and osteoprotegerin.[45,67,68,80,112,136–138] The relationship between 1α,25D3 stimulation of bone formation and induction of regulators of bone resorption with respect to osteoblast differentiation is yet unclear. In other words, does 1α,25D3 regulate these processes at different stages of osteoblast differentiation?

Membrane Receptor/non-VDR-mediated Effects

Besides the classical VDR, the presence of a membrane-localized receptor for 1α,25D3 has been described to be present in osteoblasts.[139] This receptor, described as 1,25D3 membrane-associated rapid response-binding protein (1,25D3MARSS) or protein-disulfide isomerase-associated 3 (Pdia3) and also known as ERp60, ERp57 or Grp58, may have a role in rapid responses to 1α,25D3.[140] Rapid effects of 1,25D3 on intracellular ionized calcium in isolated murine osteoblasts have been reported already in 1987.[141] A role of rapid calcium signaling is supported by a study using 1,25D3 and a vitamin D analog previously shown to induce rapid calcium influx without binding to the VDR. Gene expression profiling showed that these compounds induce in 3 h the same set of genes.[142] Pdia3 mediates the rapid effects of 1,25D3 on prostaglandin E2 production and protein kinase C activation.[143] Previously, we had shown that protein kinase C is involved in homologous VDR upregulation and osteocalcin production in rat osteoblasts.[137] Data obtained with wild-type and *VDR* knockout osteoblasts suggested that 1,25D3 affects mechanical loading-induced nitric oxide production in a VDR-independent manner.[144] It is unclear whether Pdia3 is involved in this effect. A recent study showed interaction between 1,25D3 and BMP2 in the regulation of osteoblast marker gene expression and mineralization in MC3T3 osteoblasts in which both VDR and Pdia3 are involved.[3] However, earlier data showed that 1α,25D3-induced differentiation of human osteoblasts and mineralization was blocked by the VDR antagonist ZK159222, whereas the

membrane receptor antagonist 1β,25D3 did not change the 1α,25D3 action.[41] thereby questioning a role for a vitamin D membrane receptor in the action of 1α,25D3 on osteoblast differentiation and mineralization.

1α-Hydroxylase (CYP27B1)

Studies published in 1980 and 1981 already reported that cells isolated from chicken calvaria[145] and a human osteosarcoma cell line as well as bone cells isolated from an ileac crest biopsy[146] can produce 1,25D3. Although 1α-hydroxylase (CYP27B1) expression has been shown in a range of normal and diseased states, bone was never considered a 1,25D3-synthesizing tissue. Functional consequences of the observed 1α-hydroxylase activity for osteoblast biology were unclear. In 2006, we demonstrated that CYP27B1 is expressed and that 25(OH)D3 is hydroxylated at the C-1 position in human and mouse osteoblasts.[67] Most importantly, this study also demonstrated functional consequences of 1α-hydroxylation in human osteoblast differentiation. Incubation with 25(OH)D3 induced the expression of *CYP24* and osteocalcin and stimulated alkaline phosphatase activity and mineralization, which was blocked by the 1α-hydroxylase inhibitor ketoconazole. This was supported by a study using small interfereing RNA to silence *CYP27B1* in human osteoblasts.[55] Further support for the functionality of 1α-hydroxylase came from a study showing the requirement of *CYP27B1* for proliferation and the osteogenic differentiation of human MSCs.[147] This effect was age dependent with reduced *CYP27B1* expression in MSCs of older subjects and resistance to 25(OH)D3-induced osteoblast formation.[148] Inhibition of histone deacytylase blocked the 1α-hydroxylase-dependent 25(OH)D3 stimulation of alkaline phosphatase activity in human MSCs.[149]

So far, these data on 1,25D3 synthesis by osteoblasts are derived from *in vitro* studies. The *in vivo* significance of CYP27B1 expression in osteoblasts and osteoclasts has yet to be proven, for example, by osteoblast-specific *CYP27B1* knockout mice. However, the observed human-murine differences should be taken into account, but this may be overcome in the future because of the progress in generating transgenic rats. Although *in vivo* proof is lacking, the principle of local synthesis of 1,25D3 in bone provides a basis for explaining the correlations of bone as well as of other parameters with 25(OH)D3 and not with 1,25D3 as discussed by Anderson and colleagues.[150,151] A preliminary report showed a decrease in *CYP27B1* expression in MSCs of chronic kidney disease patients, but 25(OH)D3 was still able to induce alkaline phosphatase activity similar to that in control MSCs.[152] Additional studies are needed to confirm and extend this observation but it may suggest a role for autocrine/paracrine 1,25D3 formation and adequate 25(OH)D3 levels for proper bone metabolism in chronic kidney disease.

Regulation of CYP27B1

Two regulators of renal 1α-hydroxylase, PTH and ambient calcium, did not change CYP27B1 in differentiated human osteoblasts.[67] In contrast, PTH stimulated CYP27B1 expression in human MSCs.[148] A similar difference was observed for the effect of 25(OH)D3. CYP27B1 expression was stimulated by 25(OH)D3 in human MSCs,[153] whereas it was not affected in mature osteoblasts.[67] These data point to an apparent difference in CYP27B1 regulation between MSCs and mature osteoblasts. 1,25D3 inhibits CYP27B1 expression in human MSCs, resembling the effect in the kidney.[153] Insulin-like growth factor-I stimulates CYP2B1 in human MSCs.[153] Interferon-β reduces while interleukin-1 increases CYP27B1 expression in mature human osteoblasts.[48,67] The effect of interleukin-1 points to the involvement of nuclear factor-κB in the stimulation of CYP27B1 expression in human osteoblasts. The observed inhibition of nuclear factor-κB by interferon-β in synoviocytes[154] and regulation of CYP27B1 in human dendritic cells support this.[155] However, a positive role for nuclear factor-κB has been challenged by CYP27B1 promoter studies, but these studies were performed in human embryonic kidney cells (HEK-293 cells).[156] This limited set of data on regulation of CYP27B1 demonstrate that its regulation in osteoblasts is far more complex than in the kidney, involves local regulators like cytokines and growth factors, and may differ depending on differentiation stage.

Human osteoblasts also express the vitamin D binding protein receptors cubulin and megalin that are involved in cellular uptake of 25(OH)D3.[55,67] Also vitamin D3-25-hydroxylases CYP2R1 and CYP3A4 mRNA are expressed in human osteoblasts;[67] however, 25-hydroxylase functionality in osteoblasts needs to be proven. In conclusion, the expression of these enzymes and vitamin D binding protein receptors together with CYP24 and VDR expression demonstrates that the complete vitamin D endocrine system is present in osteoblasts enabling auto- and paracrine effects in bone and bone marrow.

Conflict of Interest

The authors declare no conflict of interest.

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