The purpose of this review is to discuss the mechanisms by which vitamin D analogs alter cellular growth, with an emphasis on 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]-mediated regulation of growth factors and growth factor receptors. We believe this represents an important pathway by which vitamin D analogs change cellular growth in a variety of cells. It is well known that the primary function of 1,25(OH)2D3 is the regulation of calcium and phosphorus metabolism (6). The observation that 1,25(OH)2D3 reduces the rate of cellular growth and induces the differentiation of myeloblasts (M1 cells) and promyelocytes (HL-60 cells) into macrophages (1), and that 1,25(OH)2D3 inhibits the proliferation of cancer cells (4), initially showed that the sterol is capable of functioning in areas regulating cell growth in a variety of cells. It is well known that the primary function of 1,25(OH)2D3 is the regulation of calcium and phosphorus metabolism (6). The observation that 1,25(OH)2D3 reduces the rate of cellular growth and induces the differentiation of myeloblasts (M1 cells) and promyelocytes (HL-60 cells) into macrophages (1), and that 1,25(OH)2D3 inhibits the proliferation of cancer cells (4), initially showed that the sterol is capable of functioning in areas regulating cell functions distinct from transcellular calcium transport. The effects of 1,25(OH)2D3 on the growth and differentiation of several different cell types have now been reported and suggest that 1,25(OH)2D3, or noncalcemic vitamin D analogs, may be of therapeutic value in the treatment of disorders characterized by abnormal cell growth.

The effects of vitamin D3 and its metabolites on cells maintained in culture, or the effects observed after the topical application of vitamin D analogs to the skin at relatively high concentrations, stand in contrast to the effects of vitamin D deficiency seen in the whole organism. In vitamin D deficiency, growth retardation is a striking feature (7). These observations can be reconciled if one realizes that growth retardation in vitamin D deficiency is a consequence of calcium and phosphorus deficiencies. In vitamin D-dependent rickets type II, a disorder characterized by resistance to 1,25(OH)2D3 because of mutations in the vitamin D receptor (VDR), calcium infusions increase the growth rate in affected children (2). Recently, VDR null mutant mice were shown to be similar to their wild-type or heterozygous littermates in size, growth rate, and behavior at birth (10) because during gestation, sufficient transfer of calcium and phosphorus occurs across the placenta. Symptoms typical of rickets were seen in the null mutant mice after weaning, when non-vitamin D-dependent calcium transport mechanisms in the intestine are known to disappear (10). Feeding null mutant mice a diet containing lactose and high concentrations of calcium overcomes the growth retardation seen in these animals. It should also be noted that the effects of 1,25(OH)2D3 on cell proliferation are variable. Although 1,25(OH)2D3 generally has an antiproliferative effect on cells maintained in culture, 1,25(OH)2D3 in some instances has been shown to accelerate growth in vivo or in vitro. Cell culture conditions, as well as direct responses mediated by 1,25(OH)2D3 through receptor or nonreceptor mechanisms, may account for the apparent divergent biological activity. For example, the presence or absence of serum in cell culture, or the concentrations of the sterol or calcium in the medium, have an effect on the response of a given cell type to 1,25(OH)2D3 (8).

1,25(OH)2D3 regulates the expression of growth factors such as β-type transforming growth factors (TGF-β), insulin-like growth factor (IGF), nerve growth factor (NGF), glial cell line-derived neurotrophic factor, interleukins, hepatocyte growth factor and vascular endothelial growth factor (14). Table 1 summarizes some of these effects; it is not comprehensive, and the reader is referred to other reviews for details (14). These growth factors are expressed in many vitamin D-sensitive organs. Although information concerning the regulation of growth factors by 1,25(OH)2D3 was previously available, there was until recently little direct proof of...
of their role in 1,25(OH)2D3-mediated regulation of cell growth.

1,25(OH)2D3, growth factors, and the skin

Several investigators have shown that 1,25(OH)2D3 regulates the rate of proliferation of keratinocytes and skin fibroblasts in vitro and in vivo (8, 14). 1,25(OH)2D3 and noncalcemic vitamin D analogs are used in the treatment of hyperproliferative skin disorders such as psoriasis (8, 14). The mechanism by which 1,25(OH)2D3 decreases the growth of skin fibroblasts and keratinocytes, however, has not been clearly elucidated. We examined the effects of 1,25(OH)2D3 on human keratinocyte proliferation and the role of growth factors in 1,25(OH)2D3-mediated control of keratinocyte growth (8). When human keratinocytes are grown in a serum-free or defined culture medium, concentrations of 10-8 M or greater of 1,25(OH)2D3 inhibit keratinocyte growth. Growth inhibition is not stringent but is exerted mainly in the G1 phase of the cell cycle. The inhibition of cellular growth is associated with the differentiation and development of a more mature cellular phenotype. Interestingly, in defined medium where 1,25(OH)2D3 is depleted and at lower or physiological concentrations (≤10-9 M), cellular proliferation is enhanced. We have found that inhibition of cellular growth of human keratinocytes maintained in culture is caused by the release of TGF-β2 from cells (8). A dose-dependent increase in TGF-β2 release and a dose-dependent decrease in cellular proliferation were observed when the cells were treated with 1,25(OH)2D3; TGF-β1 and TGF-β3 concentrations did not increase. The addition of a pan-specific antibody against TGF-β partially blocked the growth inhibitory activities of the hormone, thus suggesting that at least some of the effects of 1,25(OH)2D3 on growth were mediated via the release of TGF-β2.

1,25(OH)2D3, growth factors, and bone

TGF-β, along with systemic hormones and cytokines, regulates bone remodeling, bone fracture repair, bone induction, and development (12). The amount of TGF-β in the bone of vitamin D-deficient rats is selectively reduced because of a deficiency of vitamin D and not calcium. Recently, we have shown that 1,25(OH)2D3 causes a decrease in the growth of human osteoblasts, which is accompanied by an increase in the expression of TGF-β2 but not TGF-β1 (15). Addition of an antibody directed against TGF-β partially blocks the growth inhibitory effects of 1,25(OH)2D3 suggesting that the upregulation of TGF-β2 plays a role in the growth retardation seen after the treatment of osteoblasts with 1,25(OH)2D3. 1,25(OH)2D3-induced TGF-β2 expression is regulated at the level of gene transcription and increased TGF-β2 messenger RNA (mRNA) stability (15). Interestingly, an increase in the amount of TGF-β type I and II receptor mRNA was also observed in osteoblasts treated with 1,25(OH)2D3 (14). Upregulation of TGF-β type I and II receptors could significantly contribute to 1,25(OH)2D3 inhibition of osteoblast growth. In bone, the release of growth factors such as TGF-β likely influences the activity of cells adjacent to

| TABLE 1. Summary of effect of 1,25(OH)2D3 on growth factors in selected cell lines |
|-----------------------------|---------------------------------|-----------------|-----------------|
| Cell Type                  | Effect                          | 1,25(OH)2D3, M  | Reference       |
| Human keratinocytes        | Increased TGF-β2 expression     | 10^{-6}–10^{-10}| 8               |
| Human osteoblasts          | Increased TGF-β2 and TGF-β1     | 10^{-6}–10^{-10}| 15              |
| Human bone cells           | Decreased IGF I release         | 10^{-8}         | 12              |
| Mouse calvaria             | Increased IGF II expression     | 10^{-7}–10^{-10}| 11              |
| Mouse clonal osteoblastic cells | Increased IGF type receptor and IGFBP-2,-3, and -4 expression | 10^{-6}–10^{-11}| 12              |
| Rat prostatic epithelial cells | Increased TGF-β2 and -3 expression | 10^{-7}         | 5               |
| Human prostate             | Increased IGFBP-6 expression    | 10^{-6}–10^{-9} | 5               |
| Mouse neuroblastoma cells  | Increased NGF expression and AP-1 binding activity | 10^{-8}         | 13              |
| Glial cells                | Increased NGF expression        | 10^{-6}–10^{-10}| 13              |
| Mouse fibroblasts          | Increased NGF expression        | 10^{-7}         | 13              |
| Rat osteosarcoma cells     | Increased NGF expression        | 10^{-6}–10^{-11}| 13              |

1,25(OH)2D3, 1,25-dihydroxyvitamin D3; TGF-β, β-type transforming growth factor; IGF, insulin-like growth factor; IGFBP, IGF binding protein; NGF, nerve growth factor; AP-1, activator protein-1.

“...at least some of the effects of 1,25(OH)2D3 on growth were mediated via the release of TGF-β2.”
osteoblasts such as osteoclasts and thus may play an important role in bone remodeling (12).

IGF-I and -II and insulin-like growth factor binding proteins (IGFBP) are regulated in bone by 1,25(OH)₂D₃ (11, 12). IGF-I and -II increase the synthesis of collagen in osteoblasts as well as increasing cell replication in bone (12). 1,25(OH)₂D₃ decreases IGF-I release from human bone cells maintained in culture; in mouse calvaria maintained in culture, 1,25(OH)₂D₃ increases IGF-II synthesis (11, 12). In addition to effects on the release of IGF, 1,25(OH)₂D₃ increases the number of type 1 IGF receptors in mouse clonal osteoblastic cells as well as upregulating the expression of IGFBP-2, -3, and -4 (11). The published findings do not allow a firm conclusion to be made as to how 1,25(OH)₂D₃ influences bone cell growth via the IGF system.

1,25(OH)₂D₃, growth factors, and the prostate

As previously mentioned, 1,25(OH)₂D₃ may be of therapeutic value in regulating disorders characterized by abnormal cell growth. In particular, 1,25(OH)₂D₃ has been shown to regulate prostate cell proliferation, and recent studies have implicated vitamin D deficiency as a potential risk factor in the etiology of prostate cancer (5). Although the precise mechanism is unclear, two recent studies suggest that TGF-β and IGFBP play a role in 1,25(OH)₂D₃-regulated prostate cell growth. 1,25(OH)₂D₃ has been shown to increase TGF-β2 and TGF-β3 expression in NRP-152 cells, an epithelial cell line derived from rat prostate (5). Addition of a neutralizing TGF-β antibody was able to block the production of other 1,25(OH)₂D₃-regulated metabolites, suggesting that the upregulation of TGF-β plays a direct role in the effects of 1,25(OH)₂D₃ on this cell line. In addition, 1,25(OH)₂D₃ has been shown to increase the expression of IGFBP-6 in human prostate cells in a dose-dependent manner. It is possible that the mechanism for 1,25(OH)₂D₃-regulated prostate cell growth may ultimately involve both TGF-β and IGFBP.

1,25(OH)₂D₃, growth factors, and the nervous system

1,25(OH)₂D₃ inhibits cell growth and induces NGF expression in cultured neuroblastoma cells, in glial cells, in rat brain in vivo, in cultured fibroblasts, and in osteoblasts (13). NGF is secreted in a target-derived, paracrine manner, and subsequent binding of NGF to cell surface receptors on sensory, sympathetic, and central nervous system neurons results in a pleiotropic response that has been shown to be important for neuronal development and survival (3). The effects of 1,25(OH)₂D₃ on mouse neuroblastoma cells are dependent on the expression of NGF, because antibodies neutralizing NGF activity partially blocked the antiproliferative effects of 1,25(OH)₂D₃. Recent results from our laboratory using osteosarcoma ROS 17/2.8 cells and a series of NGF promoter/human growth hormone reporter plasmids have shown that the activator protein-1 (AP-1) site located within the first intron of the NGF gene plays a critical role in 1,25(OH)₂D₃-induced NGF expression in this cell line (13). 1,25(OH)₂D₃ increases AP-1 binding activity in these cells, suggesting that 1,25(OH)₂D₃ regulates NGF expression indirectly by regulating members of the Fos and Jun protooncogene family, which constitute the AP-1 transcription factor (13). 1,25(OH)₂D₃ treatment also increases AP-1 binding activity in myelogenous leukemia cells by increasing the expression of JunD, a member of the Jun family (9).

Conclusions

Since the initial observation that 1,25(OH)₂D₃ influences breast epithelial cell growth and differentiation (1), numerous other cell types in vitro and in vivo have been shown to respond to 1,25(OH)₂D₃ treatment in a similar fashion. 1,25(OH)₂D₃ has been shown to affect the expression of growth factors, immediate-early response genes, and protein kinases. As we have shown above, an important mechanism by which 1,25(OH)₂D₃ influences cell growth is the altered expression of various growth factors. The mechanism by which 1,25(OH)₂D₃ alters cell growth via an alteration of growth factors needs further examination.

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References

The synthesis reaction may be approached with the "downhill" transport of protons to the ATP synthesis reaction. In bacteria, the F0F1 ATP synthase couples the energy of the proton motive force or pH gradient to the synthesis of ATP. Whether in mitochondria, chloroplasts, or eubacteria, the F0F1 ATP synthase couples the "downhill" transport of protons (the proton motive force or pH gradient) to the synthesis of ATP. The process of coupling energy stored in the electrochemical gradient of protons (the proton motive force or pH gradient) to the synthesis of ATP is a fundamental aspect of cellular physiology.

Molecular Features of Energy Coupling

The structural and mechanistic aspects of energy coupling in all active transport proteins remain an elusive goal. The long-standing nature of this problem has been caused in part by a dearth of structural information describing the large, integral membrane proteins, some of which are multiple-subunit complexes. Recently, the structural knowledge of the F0F1 ATP synthase has dramatically increased. Although only a partial structure of the soluble F1 domain was obtained using ATP derived from glycolysis to drive a structural knowledge of the F0F1 ATP synthase has dramatically increased. Although only a partial structure of the soluble F1 domain was obtained from bovine mitochondria (1), the X-ray crystallographic structure at 2.8-Å resolution has led to several insights into the catalytic and coupling mechanisms. Furthermore, the structure has provided new insights into the catalytic and coupling mechanisms.

Catalytic turnover, site-site cooperativity, and H+ transport inorganic phosphate (Pi). The process of coupling energy from ADP and H+ translocation is coupled to ATP synthesis in the F0F1 ATP synthase via a rotary mechanism. Catalytic turnover, site-site cooperativity, and H+ transport inorganic phosphate (Pi). The process of coupling energy from ADP and H+ translocation is coupled to ATP synthesis in the F0F1 ATP synthase via a rotary mechanism.

Robert K. Nakamoto

In the F0F1 ATP Synthase

Molecular Features of Energy Coupling