The Antiproliferative Effect of Vitamin D₃ Analogues

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1,25-Dihydroxyvitamin D₃ (VD), the biologically active metabolite of vitamin D, is well known to regulate calcium homeostasis, but the hormone can also regulate cellular growth: at the beginning of the 1980s, VD was shown to induce differentiation and to inhibit proliferation of several normal and malignant cell types [1, 2]. Thus, VD would be an interesting drug against hyperproliferative diseases like psoriasis and cancer [3], if side-effects, like hypercalcaemia, hypercalciuria and soft tissue calcification [4] caused by the hormone's classical action, were brought under control. This led to the development of VD analogues with low calcaemic effect and/or high potency on growth arrest, and in total more than 1,000 compounds have yet been synthesized by various academic and industrial institutions [5-8]. The majority of them have a modification in the side chain. Two compounds, calcipotriol (MC903; Leo) and tacalcitol [1,24(R)-dihydroxyvitamin D₃; Teijin], are already commercially available for the topical treatment of psoriasis and the second generation of even more potent analogues are on clinical trial.

For a molecular biologist the attraction of using VD as a model system for understanding the molecular basis of hyperproliferative diseases is its simple but still rather flexible mode of action. In contrast to most other signalling molecules, VD and its analogues pass cellular membranes easily and bind with high affinity to a nuclear receptor, VDR, which is a member of a large family of structurally related ligand-inducible transcription factors. Without any involvement of membrane and intracellular signalling pathways, the stimulation of cells with VD results in a direct up- or down-regulation of perhaps up to a few hundred genes. A specific characteristic of such primary VD responding genes is a binding site for VDR, referred to as VD response element, in their promoter region. Among these genes are well-known candidates like osteocalcin, osteopontin and

calbindins, but also peptide hormones like atrial natriuretic factor [9]. A VD response element in the promoter of a gene involved in the control of the cell cycle has not yet been identified, but for quite a long time the proto-oncogene c-myc is known to be regulated by VD [10] and recently an even more prominent protein, the inhibitor of cyclin-dependent kinases p21 (WAF1/CIP1), has been shown to be up-regulated after 2 h of stimulation with VD [11].

On the level of gene regulation, VD analogues can be characterized by their half-maximal gene activation (EC₅₀) value on different types of VD response elements and by their affinity to VDR. With most of the VD analogues that we have tested so far, we obtain a good correlation between VDR binding and gene activation from a classical VD response element, which is formed by two directly repeated hexameric core binding motifs spaced by 3 nucleotides (DR3), but we observed in the same cell line no convincing correlation with the respective half-maximal growth inhibition (IC₅₀) values [12, 13]. However, when we used a novel type of VD response

elements that consists of two motifs in an inverted palindromic orientation spaced by 9 nucleotides (IP9) [14], the respective EC₅₀ values fit much better with these IC₅₀ values [12, 15]. This suggests that VD-regulated genes that are involved in the control of proliferation may contain an IP9-type rather than a DR3-type VD response element in their promoter. Moreover, this first demonstration of a promoter selectivity of VD analogues may reflect the desired selectivity between antiproliferative and calcaemic effects.

VD analogues do not only promote growth arrest but also induce differentiation of, e.g., myeloid cells, and very recently it has been reported that they can induce apoptosis [16, 17]. Recently, retinoids have been developed that either activate differentiation or inhibit proliferation [18, 19]. This was possible due to the property of the retinoic acid recep-

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tor to block the activation of the transcription factor complex AP-1 by protein-protein interaction in solution and on DNA. Such anti-AP-1 activity was initially also described for VDR [20] but appears only to function on composite sites, i.e. on response elements that bind both VDR and AP-1. We failed in detecting anti-AP-1 activity of VD analogues [12]; however, this does not exclude the possibility that VDR interacts in a ligand-dependent manner with other important regulatory proteins.

A large amount of work still has to be done. By systematic application of the powerful method of differential RNA display [21], more primary VD response elements have to be identified, especially those which are important for proliferation, differentiation and apoptosis. In vitro the potent antiproliferative effect of VD analogues has clearly been established; however, there may still be doubts on the validity of these findings for the in vivo situation.

The work described by Sato et al. [22] in this issue of *Dermatology* on the one hand takes us back to 'simple' proliferation and differentiation marker assays (ornithine decarboxylase and type I transglutaminase, respectively)

with a first-generation VD analogue, but on the other hand this work is another proof for the in vivo effects of VD analogues. The authors showed that at a concentration of 2 µg/g both tacalcitol cream and ointment have the same pharmacokinetic profile in their ability to inhibit proliferation and to induce differentiation of the epidermis of hairless mice. Several other in vivo effects of VD analogues on breast cancer [23-25], psoriasis [26] and other diseases (reviewed in [7]) have already been reported. Moreover, with the shift from in vitro to in vivo research, the pharmacological properties of VD analogues, i.e. as first priority their serum half-lives, have seriously to be taken into account (e.g. the very low calcaemic effect of calcipotriol is mainly due to its short half-life [27]).

In summary, basic and clinical investigations drew a lot of attention to the cell-cycle-regulatory properties of VD analogues. In comparison to retinoids, the side-effects of VD analogues are rather easy to control, and it is my prediction that in the near future both classes of compounds will be equal competitors for clinical application.

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