EDITORIALS

Toward Therapeutic Intervention of Cancer by Vitamin D Compounds

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One area of cancer chemoprevention that has been intensively studied in recent years is biologic modifiers of cancer cells that are designed to retard proliferation (1-3), to induce differentiation of these cells to a quiescent, nondividing state (4-7), and/or to promote cell death (5-8). In this issue of the Journal, Mehta et al. (9) report the effect of a novel vitamin D compound in a murine mammary gland chemoprevention model. The secosteroid hormone known as 1α,25-dihydroxyvitamin D₃ [1α,25(OH)₂D₃] has been described as a key regulator of serum calcium. In the last two decades, however, it has also been found to have diverse biologic effects in normal and malignant tissues. These responses include the in vitro inhibition of proliferation and induction of differentiation of various cancer cells, such as those from the human hematopoietic system, breast, ovaries, colon, brain, and prostate (10-17). Initiation of these genomic responses is through a specific steroid hormone nuclear vitamin D₃ receptor (VDR) acting as a ligand-inducible transcription factor that binds the vitamin D₃ response element contained within the promoter/enhancer region of target genes (18).

Despite the intense research that has focused on 1α,25(OH)₂D₃ since it was first characterized in 1971 (19), the exact mode of action by which it inhibits cancer cells remains largely unknown. In normal tissues not directly involved in calcium regulation, for example, the well-studied system of keratinocytes, exposure of these cells to 1α,25(OH)₂D₃ increases the synthesis of transforming growth factor (TGF)-β1 and TGF-β2 (20), decreases expression of epidermal growth factor receptors (21), and leads to dephosphorylation of the retinoblastoma protein (22). In normal prostate cells, it exerts a differentiating effect in combination with testosterone (23). At pharmacologically active doses, 1α,25(OH)₂D₃ can suppress the immune system (24-27) and can enhance monocyte–macrophage differentiation (28). Other specific, genomic effects observed in cancer cells exposed to 1α,25(OH)₂D₃ include cell cycle arrest in G₁. Many factors can lead to cell cycle arrest, but the cyclin-dependent kinase inhibitors known as p21(WAF1) and p27Kip1 are pivotal to this process; the p21(WAF1) gene contains a vitamin D₃ response element within its promoter region (29) and expression of the gene increased in response to 1α,25(OH)₂D₃. Also, expression of p27Kip1 is markedly induced in certain cancer cell types (e.g., myeloid leukemia and prostate cancer) after their exposure to 1α,25(OH)₂D₃ (17,30-32).

A major focus of chemoprevention research in the field of vitamin D and cancer has been to synthesize analogues of 1α,25(OH)₂D₃ that have prominent antiproliferative effects against cancer cells without resulting in hypercalcemia when they are administered in vivo at pharmacologically active doses. This research has resulted in several analogues that have dramatic antiproliferative behavior, most noticeably analogues with double and triple bonds in the C/D ring and side chain (33,34), addition of three to six hexafluoride groups to the end of the side chain (17,28,35), or placement of the side chain in the 20-epi configuration (36-38). Initial clinical trials are under way; for instance, an ongoing phase I study in the U.K. is examining the effects of these analogues on breast cancer. Studies in vitro have shown that vitamin D₃ analogues can inhibit the clonal proliferation of breast cancer cells at the 10⁻¹¹-10⁻⁹ M range, with an associated increase in expression of bax and concurrent decrease in bcl-2 expression (37). Furthermore, potent hexafluoride analogues can reduce the breast cancer incidence and burden in N-nitroso-N-methylurea-treated rats (39). One area in which the therapeutic potential of vitamin D₃ has been realized is in the treatment of psoriasis, where the topical application of potent analogues, including calcipotriene (Dovonex), controls the disease and does not significantly interfere with serum calcium levels (40).

The reason for the increased antiproliferative potency of the analogues is becoming clearer, as illustrated in Fig. 1. Vitamin D₃ analogues usually bind less well to the D-binding protein in the blood and are, therefore, more readily available to enter the cells (41). Analogues may also extend the half-life of the VDR (42), or they may induce novel VDR conformations (43), which may either allow more efficient interactions with vitamin D₃ response elements and/or expand the array of vitamin D₃ response elements that can be activated. In addition, metabolic products of analogues may result in potent intermediates in vivo. For example, compared with the parental analogues, the 24-oxo metabolites have the same in vitro anticancer activities but have fewer effects on calcium levels in sera (44,45).

In vivo, VDR forms heterodimers with the retinoid X recep-

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tor (18), and the combination of 9-cis-retinoic acid and 1α,25(OH)2D3 can synergistically increase expression of a reporter gene construct containing a vitamin D3 response element within its promoter (46). Cooperation between these two receptor pathways has been the basis for combination therapy; we have previously demonstrated (47,48) synergistic inhibition of proliferation of human myeloid leukemia cells and MCF-7 breast cancer cells by a potent vitamin D3 analogue in combination with 9-cis-retinoic acid. Thus, combinations of retinoids and vitamin D ligands may be an attractive prospect for control of deregulated cell growth. Also, various steroid hormone receptor enhancer proteins have been identified (49,50); by recruiting them in vivo, we may be able to accentuate further the positive therapeutic genomic effects of 1α,25(OH)2D3.

The study by Mehta et al. reported in this issue of the Journal presents an entirely novel class of vitamin D compounds (vitamin D5). Utilizing a mammary gland lesion model to assess chemoprevention, the authors demonstrated preventive effects in vitro but no significant effect on serum calcium levels in vivo. Thus, the therapeutic index (ratio of its antiproliferative to its calcemic effects) for this compound is sufficiently high to warrant further investigations using other cancer cell types and model systems. The study by Mehta et al. and ongoing fundamental research into the effects of vitamin D compounds on cancer cells are elucidating the molecular effects and highlighting the therapeutic potential of these highly interesting compounds. Several analogues have already been identified that have significant inhibitory effects but that do not induce hypercalcemia; Mehta et al. add another compound to this list. Many of these compounds are potential candidates for clinical investigations.

References


Cervical cancer is the second leading cause of cancer mortality in women worldwide, with approximately half a million new cases occurring annually (1). More than 90% of cervical cancers and their precursors, so-called cervical intraepithelial neoplasia, contain human papillomavirus (HPV) DNA sequences, and it is now well established that HPV has a major causal role in the development of cervical neoplasia (2). It has also been recognized that HPV infection of the cervix and lower genital tract is one of the most common sexually transmitted diseases (3). Prevalence rates of HPV infection (based on nucleic acid amplification techniques) can be only crudely estimated because they are influenced by several factors. These rates vary substantially according to the population studied and are highly age dependent. In the United States, HPV prevalence ranges from 25%-30% in asymptomatic women aged 15-25 years to approximately 5% in women aged 33-55 years, an age group in which most cervical cancers are detected (2,4). In contrast, there are approximately 15,000 incident cases of cervical cancers in the United States annually (5). These observations have led investigators to postulate that other factors, in addition to HPV, play a role in cervical carcinogenesis.

Several lines of evidence suggest that cell-mediated immune responses are important in controlling both HPV infections and HPV-associated neoplasms [for review, see (6)]. First, the prevalence of HPV-related diseases (infections and neoplasms) is increased in transplant recipients and in patients infected with human immunodeficiency virus (HIV); both types of patients are known to have impaired cell-mediated immunity (7,8). Second, studies on animals have demonstrated that immunized animals are protected from papillomavirus infection and from the development of neoplasia. Immunization also facilitates the regression of tumors is thought to be mediated by Th1 cytokine responses and impaired by Th2 cytokine responses. The IL-2- and IFN γ-producing Th1 response is likely to be the major component that contributes to the development of cell-mediated immunity against HPV infections and HPV-associated neoplasms.

Notes

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Analysis of Cytokine Profiles in Patients With Human Papillomavirus-Associated Neoplasms

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Cell-mediated immunity is regulated by cytokines that are secreted by T helper cells. In general, T-helper cells can be classified as Th1 and/or Th2 cells on the basis of the different types of cytokines they secrete. Th1 cells secrete interleukin (IL) 2 (IL-2) and interferon gamma (IFN γ). Th2 cells produce IL-4, IL-5, IL-10, and IL-13. The Th1 lymphocytes are the most important effector cells in inflammatory reactions associated with vigorous delayed-type hypersensitivity but low antibody production, as occurs in contact dermatitis and in viral or intracellular bacterial infections [for review, see (12,13)]. The functional phenotype of most Th2 cells may account for both the persistent production of certain antibody isotypes, particularly immunoglobulin G1 and immunoglobulin E, and the eosinophilia observed in human helminthic infections and allergic disorders. Although the Th1 and Th2 phenotypes were first described in mice, clones of Th1 and Th2 cells have also been isolated from humans. For example, most CD4+ T cells that infiltrate the thyroid gland in patients with autoimmune thyroid diseases are of the Th1 type, since they can be induced to produce IFN γ but not IL-4. In contrast, the great majority of allergen-specific T-cell clones derived from patients with allergic disorders express the Th2 phenotype, as evidenced by their production of IL-4 and IL-5 and their limited, if any, production of IFN γ (13). Lymphocyte-mediated protection from viral infections as well as control of tumors is thought to be mediated by Th1 cytokine responses and impaired by Th2 cytokine responses. The IL-2- and IFN γ-producing Th1 response is likely to be the major component that contributes to the development of cell-mediated immunity against HPV infections and HPV-associated neoplasms.

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