Vitamin D and Colon Carcinogenesis1–3

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ABSTRACT Colorectal cancer is the third most commonly occurring cancer in the United States and accounts for ~11% of cancer deaths. Many epidemiological studies have shown an association between dietary factors, including calcium and vitamin D, and the incidence of colon cancer. Recently the Calcium Polyp Prevention Study demonstrated that calcium supplementation can reduce the recurrence of colon polyps, but the effect depends on serum vitamin D levels. We used the Apcmin mouse model of intestinal cancer to investigate the effects of vitamin D treatment and calcium intake independently on polyp formation. We found that 1,25-dihydroxycholecalciferol was potent in inhibiting tumor load; however, the dose used to achieve this antiproliferative effect led to deleterious effects on serum calcium homeostasis. These effects were minimized by use of a synthetic analogue with reduced toxicity. Additionally, we tested the effect of a modified-calcium diet in Apcmin mice but did not find a protective effect, perhaps because of a reduction in circulating levels of 25-hydroxycholecalciferol with increasing levels of dietary calcium. A number of other studies that use rodent models with vitamin D supplementation or deficiency illustrate the efficacy of vitamin D in colon cancer prevention. The mechanisms of direct action of vitamin D on colonic epithelium include regulation of growth factor and cytokine synthesis and signaling, as well as modulation of the cell cycle, apoptosis, and differentiation. Because of the apparent synergistic effect of vitamin D and calcium, cosupplementation of both nutrients in cancer prevention programs may be advised. J. Nutr. 134: 3463S–3471S, 2004.

KEY WORDS: vitamin D • calcium • colorectal • carcinogenesis • vitamin D analogs • rodent models

Diet and colon cancer risk

Cancer of the colon and the rectum is the third most commonly occurring cancer in both males and females in the United States, accounting for ~11% of cancer deaths. According to American Cancer Society estimates, some 57,100 people in the United States died of colorectal cancer and at least twice that number of new cases were diagnosed in 2003 (1). Dietary factors are considered to be important influences of risk of colorectal cancer. It has been estimated that up to 90% of U.S. deaths from cancer of the colon could be prevented by feasible dietary intervention (2). A good illustration of this fact is that economically developed countries in Europe, North America, and Australasia have especially high rates of cancers of the colon and the rectum, whereas economically developing countries in Africa, Latin America, and Asia have lower rates (2). As developing countries become urbanized, the rates of colorectal cancer increase, which appear to parallel a change in dietary patterns toward a more urban diet. This urban-industrial or Western diet is characterized by lower consumption of cereals, tubers, and similar starchy foods, and higher consumption of fat, meat, and meat products; alcohol; and processed foods containing substantial amounts of fat and sugar. Western diets are relatively deficient in calcium and vitamin D (2). Migrant studies have also been used to provide circumstantial evidence that environmental factors, including diet, have a strong effect on cancer incidence. In these studies, changes in the patterns of cancer as people of the same genetic background move from rural, lesser developed areas to urban developed areas are evaluated. In one example, Chinese men who migrate from economically developing Shanghai to urban Hong Kong, Los Angeles, and Hawaii experience a 2-fold
increase in colorectal cancer incidence. At the same time, rates of prostate cancer increased by 10–15 times between Shanghai and the United States, whereas stomach cancer rates dropped dramatically (3).

A number of excellent reviews have compiled the epidemiological evidence correlating dietary factors with colorectal cancer (2,4–7). The most consistent observation associating particular foods with risk for colon cancer is that high vegetable consumption moderately decreases risk and high red meat intake increases risk of colon cancer (8). Some studies of colorectal adenomas show a protective association with dietary fiber, but, overall, the epidemiology of fiber and colorectal cancer is inconsistent. The etiology of cancer of the rectum appears to be similar to the rest of the colon, and the few studies that report dietary risk factors for rectal cancer only show that they do not differ substantially from those for colon cancer (5).

**Physiology of vitamin D and calcium homeostasis**

1α,25-Dihydroxycholecalciferol [1α,25(OH)2D3] is a sec-osteroid hormone with a crucial role in the maintenance of calcium homeostasis. The metabolic pathway leading to the most bioactive form of vitamin D requires several biosynthetic steps. Cholecalciferol (vitamin D-3) is either made from cholesterol precursor in the skin (7-dehydrocholesterol) or consumed in the diet. Dietary sources include fatty fish such as salmon, mackerel, and sardines (as well as cod liver oil); supplemented foods such as milk and orange juice; some breads and cereals; and vitamin supplements (9). Whether vitamin D-3 derives from endogenous or exogenous sources, vitamin D-25-hydroxylase in the liver hydroxylates it at carbon 25 to form 25-hydroxycholecalciferol [25(OH)D3]. Circulating 25(OH)D3 is further hydroxylated at carbon 1 by 25-hydroxyvitamin D3-1α-hydroxylase in the kidney to generate the most potent form, 1,25-dihydroxyvitamin D3 [1α,25(OH)2D3 or calcitriol]. Vitamin D nutritional status is measured by serum 25(OH)D3 concentration, which is ~1000 times the concentration of 1α,25(OH)2D3 and has a half-life of ~2 wk. Renal production of 1α,25(OH)2D3 is tightly regulated by parathyroid hormone, serum calcium and phosphorus, and 1α,25(OH)2D3 itself, and therefore serum 1α,25(OH)2D3 is a poor indicator of vitamin D status (9). The major target tissues of 1α,25(OH)2D3 are intestine and bone, and the classical role of the hormone is to regulate calcium absorption in the intestine, to maintain mineral homeostasis in the kidney, and to regulate bone remodeling.

Tissues other than those involved with mineral metabolism have specific vitamin D nuclear receptors, suggesting alternative roles for vitamin D. Normal and cancer cells that express vitamin D receptors respond to 1α,25(OH)2D3 by decreasing proliferation and enhancing maturation or differentiation (9). In addition, many cell types, including notably colon cancer cells (10,11), can synthesize 1α,25(OH)2D3, suggesting autocrine/paracrine actions in manipulating cell growth.

**Vitamin D and colorectal cancer in populations**

One of the earliest observations suggesting that vitamin D may play a role in cancer prevention came from the observa-
Laboratory. These data showed that among subjects with baseline 25(OH)D3 levels at or below the median [75.6 nmol/L (29.1 µg/L)], calcium supplementation was not associated with adenoma recurrence. However, calcium supplementation was associated with reduced risk (risk ratio = 0.71) of adenoma recurrence among individuals with baseline 25(OH)D3 levels above the median, indicating vitamin D status is an important factor in determining the effectiveness of calcium supplementation to inhibit human colorectal carcinogenesis (19). The Calcium Polyp Prevention Study group is now initiating a new supplementation study where patients with a history of adenomas will receive either 1000 IU/d vitamin D or calcium supplements, both, or placebo (26).

Vitamin D treatment in rodent models

Rodent models provide an opportunity to produce experimental evidence linking individual factors and their mechanisms of action (Table 1). These models allow investigations of specific gene-nutrient interactions that are critical to the chemopreventive actions at specific points in the carcinogenic process. The neoplastic phenotype in epithelial cells results from a complex multistep process in which cells accumulate alterations in multiple genes involved in cell growth and differentiation. Mutational activation of oncogenes, which leads to enhanced cell growth, and the inactivation of certain tumor suppressor genes, reversing their suppression of cell proliferation, are significant in the development of colorectal cancer (27). Events that occur early in colorectal carcinogenesis include mutation or loss of the APC gene (adenomatous polyposis coli; a tumor suppressor gene), mutation of K-ras (a proto-oncogene), and generalized disorganization of DNA methylation; later events include loss of TP53 (a tumor suppressor gene) (28). Mutations in the APC gene are particularly important and are rate limiting for sporadic and familial colorectal cancers (29,30). At least 4–5 genes are altered to form a malignant tumor, and it appears that the temporal order rather than the accumulation of these genetic changes is most important in determining the neoplastic phenotype and the likelihood of tumor progression (29). The primary rodent models of intestinal tumorigenesis used in vitamin D studies are produced either through carcinogen treatment, by germinal manipulation of genes involved in the process of carcinogenesis such as those listed above, particularly of APC, immunodeficient mice implanted with human tumor xenografts, as well as wild-type mice fed a Western-style diet. Common carcinogens used to induce intestinal neoplasia in rats and mice include azoxymethane (AOM) and 1,2-dimethylhydrazine (DMH). The carcinogenesis process of F344 rats that have been treated with these carcinogens resembles that of humans (31). Studies using these models have been conducted to determine the effect of vitamin D alone or in combination with calcium on the development of intestinal cancer. Rats on a high-fat (20% corn oil) diet had increased tumor incidence after DMH treatment, and this increase was ameliorated by either supplemental calcium or vitamin D (32,33). Conversely, vitamin D deficiency abolished the protective effects of calcium on colon cancer in DMH-treated rats (34,35). When rats were placed on a stress diet (20% fat reduced Ca2+, high phosphorus:Ca2+ ratio, and low vitamin D3 content) before treatment with DMH, supplemental vitamin D3 abrogated the increased hyperproliferation induced by the stress diet and decreased tumor multiplicity and adenocarcinoma incidence (36). Administration of 1α,25(OH)2D3 before treatment with DMH obliterated the peak in ornithine decarboxylase activity (a sign of increased mucosal cell proliferation) seen with DMH administration and reduced by 50% the number of colon adenocarcinomas (37). However, in this and a similar study (38), 1α,25(OH)2D3 did not prevent tumor formation when administered after DMH. One mechanism of the chemopreventive action of 1α,25(OH)2D3 treatment elucidated in AOM-treated rats was an inhibition in angiogenesis defined as a decrease in immunohistochemical staining for vascular endothelial growth factor and microvessel counts (39).

Some of the better-defined rodent models of intestinal cancer tumorigenesis are mice containing mutations in the Apc gene derived from ethylnitrosourea treatment (Apcmin) (40) or targeted mutations in the Apc gene (41,42). Use of these models in experiments is advantageous in that they do not require administration of exogenous carcinogens (and therefore are safer to the researcher), mice develop many tumors (requiring fewer mice per experiment), the tumors develop early in the their lives (so studies can be shorter), and mice develop predominately adenomas or aberrant crypts rather than carcinomas (providing a model of cancer initia-
tion). The gene mutated also accounts for a defined inherited colon cancer syndrome in humans, familial adenomatous polyposis (29). The Min mutation creates a stop codon at codon 850, resulting in a truncated APC protein, which loses its tumor suppressor function. The homozygous Min/Min mice die as embryos, but on the C57BL/6J (or B6) background, Min/+ mice develop multiple intestinal neoplasias (hence, Min designation) throughout the intestinal tract within a few weeks after birth. Min mice rarely survive beyond 150 d; death is apparently caused by secondary effects, such as anemia and intestinal blockage (43,44). On the B6 background, the Min mutation is dominant and fully penetrant in the intestinal phenotype.

We tested the effects of vitamin D treatments on polyposis formation in the Apc\textsuperscript{Min} model. In this study, 3 groups of female Apc\textsuperscript{Min} mice aged 4–5 wk were randomly assigned to 3 groups: a 1α,25(OH)\textsubscript{2}D\textsubscript{3}–treated group (n = 11) was injected with 0.01 mg 1α,25(OH)\textsubscript{2}D\textsubscript{3}; an analogue-treated group (n = 10) was treated with 5 mg 1α,25-(OH)\textsubscript{2}–16-ene-19-nor-24-oxo D\textsubscript{3} (a gift from Hoffman-LaRoche), each intraperitoneally 3 times per wk; and a control group (n = 12) was sham injected with PBS. A sulindac-treated (160 mg/L in drinking water) group (n = 10) served as positive control, because this nonsteroidal anti-inflammatory drug was shown to reduce polyposis formation in the Apc\textsuperscript{Min} mouse (45). All mice were fed a standard AIN-93G purified diet containing vitamin D at 1000 IU/kg and calcium (as calcium carbonate) at 5 g/kg. After 10 wk of treatment, 2 observers unaware of the treatment scored polyp number and size over 4-cm segments of the small intestine and all of the large intestine. Tumor number was not affected by 1α,25(OH)\textsubscript{2}D\textsubscript{3} or analogue treatment. However, a significant decrease in total tumor area was ob-

### TABLE 1

Summary of Vitamin D effects in rodent models of intestinal cancer

<table>
<thead>
<tr>
<th>Rodent Model</th>
<th>Treatment(s)</th>
<th>Effects of Vitamin D</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats + DMH + high-fat diet</td>
<td>Dietary vitamin D-3 (and calcium) before DMH</td>
<td>Vitamin D alone inhibits fat-induced promotion</td>
<td>32</td>
</tr>
<tr>
<td>Rats + DMH + high-fat diet</td>
<td>Dietary vitamin D-3 (and calcium) after DMH</td>
<td>Synergy of calcium + vitamin D to inhibit fat-induced promotion (not significant)</td>
<td>33</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Injection 1α,25(OH)\textsubscript{2}D\textsubscript{3} before, with, or after DMH</td>
<td>Reduction in tumor number (initiation) only when vitamin D given before DMH</td>
<td>37</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Dietary 1α,25(OH)\textsubscript{2}D\textsubscript{3} analog before, with, or after DMH</td>
<td>Diminished formation of aberrant crypt foci before and simultaneous with DMH (with 16-wk treatment) and after DMH (with 16-wk treatment)</td>
<td>57</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Injection 1α,25(OH)\textsubscript{2}D\textsubscript{3} analog after DMH</td>
<td>Diminished formation of aberrant crypt foci</td>
<td>60</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Dietary 1α,25(OH)\textsubscript{2}D\textsubscript{3} analog with or after DMH</td>
<td>Decreased incidence tumors during postinitiation phase</td>
<td>58</td>
</tr>
<tr>
<td>Rats + AOM</td>
<td>Dietary 1α,25(OH)\textsubscript{2}D\textsubscript{3} analog before, with, or after DMH</td>
<td>Decreased incidence tumors during initiation phase</td>
<td>61</td>
</tr>
<tr>
<td>Rats + AOM</td>
<td>Injection 1α,25(OH)\textsubscript{2}D\textsubscript{3} (and analog) with and after AOM</td>
<td>Decreased proliferation and angiogenesis</td>
<td>39</td>
</tr>
<tr>
<td>Rats + MNU</td>
<td>Intragastric 1α,25(OH)\textsubscript{2}D\textsubscript{3} analog</td>
<td>Diminished number of colonic tumors</td>
<td>59</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Dietary vitamin D-3 and analogs before DMH</td>
<td>Diminished metastases in analog-treated rats</td>
<td>56</td>
</tr>
<tr>
<td>HT-29 or SW-620 tumor xenografts in nude mice</td>
<td>Injection 1α,25(OH)\textsubscript{2}D\textsubscript{2} and analog</td>
<td>Analog decreased HT-29 cell proliferation in vivo (not SW-620)</td>
<td>63</td>
</tr>
<tr>
<td>LoVo tumor xenografts in nude mice</td>
<td>Injection of 1α,25(OH)\textsubscript{2}D\textsubscript{2} analog</td>
<td>Analog-induced dose-dependent decrease in LoVo cell proliferation in vivo</td>
<td>64</td>
</tr>
<tr>
<td>Apc\textsuperscript{Min} Mice</td>
<td>Injection 1α,25(OH)\textsubscript{2}D\textsubscript{2} (and analog)</td>
<td>Decreased tumor load</td>
<td>46</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Dietary vitamin D-3 deficiency with high/normal calcium before DMH</td>
<td>Vitamin D deficiency abolished protective effects of calcium supplementation on tumor formation</td>
<td>34</td>
</tr>
<tr>
<td>Rats + DMH</td>
<td>Dietary vitamin D-3 deficiency with high/normal calcium before DMH</td>
<td>Vitamin D deficiency abolished protective effects of calcium supplementation on K-ras mutation frequency</td>
<td>35</td>
</tr>
<tr>
<td>Rats ± DMH</td>
<td>“Stress” diet (high fat, low calcium, high P:Ca, low vitamin D) = dietary vitamin D supplement before ± DMH</td>
<td>Supplemen tal vitamin D-3 abrogated increased hyperproliferation and adenocarcinoma formation induced by stress diet + DMH</td>
<td>36</td>
</tr>
<tr>
<td>Mice/rats + Western diet (short-term)</td>
<td>Increased fat, phosphate and low vitamin D and calcium</td>
<td>Colonic hyperproliferation</td>
<td>51</td>
</tr>
<tr>
<td>Mice + Western diet (short term)</td>
<td>Increased fat, phosphate and low vitamin D and calcium</td>
<td>Hyperproliferation reduced by calcium supplementation</td>
<td>52,53</td>
</tr>
<tr>
<td>Mice + Western diet (long term)</td>
<td>Increased fat, phosphate and low vitamin D and calcium</td>
<td>Hyperproliferation and hyperplasia</td>
<td>54</td>
</tr>
<tr>
<td>Mice + “new” Western diet (long term)</td>
<td>Increased fat, phosphate and low vitamin D, calcium, folic acid, methionine, choline, and vitamin B\textsubscript{12}</td>
<td>Adenoma and carcinoma development</td>
<td>55</td>
</tr>
<tr>
<td>Apc\textsuperscript{1638N} mice + Western diet</td>
<td>Increased fat, phosphate + low vitamin D and calcium</td>
<td>Increased number polyps</td>
<td>49</td>
</tr>
</tbody>
</table>
served over the entire gastrointestinal tract in the analog (36% decrease; \( P < 0.05 \)) and the 1α,25(OH)\(_2\)D\(_3\) groups (46% decrease; \( P < 0.001 \)) vs. the control group. Further analysis of these data by segment revealed the difference in tumor area with analog treatment to be greatest in the medial small intestine. 1α,25(OH)\(_2\)D\(_3\) treatment was most effective in the distal small intestine. No significant differences in tumor area were seen in the large intestine with analogue or 1α,25(OH)\(_2\)D\(_3\) treatment. Sulindac treatment resulted in a significant decrease in total and in each small intestinal segment in polyp number (49%; \( P < 0.001 \)) and in polyp area (70%; \( P < 0.001 \)). RT-PCR of total RNA derived from each intestinal segment showed that the vitamin D receptor was expressed throughout the small intestine and colon. Serum calcium levels in the analog group were not elevated at wk 4 of treatment and were only moderately elevated (22%) by wk 8 (\( P < 0.001 \)). In contrast, serum calcium in the 1α,25(OH)\(_2\)D\(_3\) group was significantly elevated (\( P < 0.001 \)) at both wk 4 (23%) and wk 8 (45%). Food intake and growth were comparable for the control, analog, and sulindac groups; however, body weight (26%; \( P < 0.001 \)) and food intake (27%; \( P < 0.001 \)) were significantly decreased at wk 10 in the 1α,25(OH)\(_2\)D\(_3\) group relative to the control group, indicating possible adverse effects of 1α,25(OH)\(_2\)D\(_3\) at this dose not seen with the analog (46).

Although levels of calcium are tightly regulated and normally show little variation, the amount of calcium present in the intestinal lumen can vary substantially as a result of diet. The mechanism by which calcium intake may regulate epithelial cell differentiation and proliferation is unknown. The hypotheses proposed for how calcium may have a beneficial effect toward preventing colon carcinogenesis center mainly around 2 possibilities: 1) calcium exerts its effect indirectly through mechanisms related to binding toxic or irritating luminal agents (e.g., FFA from dietary fat and endogenous bile acids) in the colon and 2) calcium directly regulates epithelial cell turnover (47).

Therefore, we also tested the effect of feeding a modified calcium diet in this model. Female Apc\(^{min}\) mice aged 4–5 wk were randomly assigned to 3 groups and were fed a pelleted purified diet derived from AIN-93G but containing either 2.5 g calcium; 25(OH)\(_2\)D\(_3\); or 10 (high calcium; \( n = 27 \)) g calcium per kg diet. Calcium was supplied as calcium carbonate in the standard mineral premix, and vitamin D was included at 1000 IU/kg. After 12 wk of treatment, intestinal polyp number and size were scored as before. We found no effect on polyp number or tumor load in any segment of the intestine or over the entire intestine. Body weight and food intake were comparable in all treated groups. However, the high-calcium diet resulted in a 2.4-fold increase (\( P < 0.05 \)) in fecal bile acids after the 12-wk treatment, showing that the treatment was effective in precipitating bile acids from the intestinal lumen. Because no additional vitamin D was provided in the presence of increased calcium supplementation, the lack of effect of altered calcium may have resulted from changes in vitamin D metabolism. To test this further, we evaluated by radioimmunoassay the levels of 25(OH)D\(_3\) in samples taken at the beginning and termination of the experiment. We found that there was a 33% decrease in 25(OH)D\(_3\) (\( P < 0.05 \)) in the high-calcium group at the end compared with the beginning of the experiment, suggesting that feeding a high-calcium diet causes vitamin D depletion that in turn results in increased risk for carcinogenesis (48).

Feeding Western (high in fat and phosphate, low in calcium and vitamin D) diets to mice with targeted mutations in the Apc gene (1638N mice) results in an increased number of polyps relative to control (standard AIN-76A) diets (49). Further targeted inactivation of both alleles of the gene encoding p21/Waf1, a key inhibitor of cyclin-dependent kinase activity that regulates the cell cycle, in 1638N mice resulted in an increased frequency and size of intestinal tumors (50). When these mice were maintained on a Western diet, they developed even more and larger intestinal tumors and had diminished survival. Remarkably, even wild-type mice and rats fed a Western diet for a short time (12 wk) will develop colon-cell hyperproliferation (51); this effect is ameliorated by replenishing calcium (52,53). When mice are fed a Western diet for their entire life span, they develop hyperproliferation and hyperplasia, followed by other changes in the large intestine, including whole-crypt dysplastic lesions and focal hyperplasias, indicative of tumorogenesis (54). The Western diet has been further modified to a “new” Western diet to more accurately depict the deficiencies of a Western diet. This diet also has decreased levels of nutrients that are required for biochemical reactions involving methyl group availability (i.e., folic acid, methionine, choline, and vitamin B-12). When the “new” Western diet is fed to normal mice, adenoma and carcinoma develop in the colon of these mice without carcinogen exposure (55), illustrating the procarcinogenic nature of the Western diet.

**Use of 1α,25(OH)\(_2\)D\(_3\) analogs**

Clinical trials using 1α,25(OH)\(_2\)D\(_3\) for the treatment of malignancy in humans are limited because of the hypercalcemia that develops with high-dose administration. However, hundreds of compounds have been designed and synthesized that are similar in structure to 1α,25(OH)\(_2\)D\(_3\) but that exhibit reduced hypercalcemic responses while retaining their chemopreventive properties. Virtually every part of the molecule has been modified by synthetic chemists to accentuate either the antiproliferative and proapoptotic properties and/or diminish the calcemic properties. In addition to the analogue used in our own study, several synthetic analogs of 1α,25(OH)\(_2\)D\(_3\) with diminished hypercalcemic responses were shown to be effective in preventing intestinal tumorigenesis and spontaneous metastases in carcinogen-treated rats, including 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-D\(_3\) and 1,25-dihydroxy-16,23Z,diene-26,27-hexafluoro-19-nor-D\(_3\) (56); 24R,25-dihydroxyvitamin D\(_3\) (57,58); 1α-hydroxyvitamin D\(_3\) (59); and 22-oxa-calcitriol (60). The fluorinated analogue 1α,25-dihydroxy-16-ene-23-yn-26,27-hexafluorocalciferon was effective in reducing tumor burden and abolished development of adenocarcinomas in an AOM-treated rat model when administered during initiation (before or during AOM treatment) with no toxicity (61). The chemopreventive effect was through inhibition of crypt-cell hyperproliferation and aberrant crypt foci development. Also, the analogue blocked AOM-induced alterations in cyclin D1 and E-cadherin protein in aberrant crypt foci and tumors, as well as cyclooxygenase-2 and nitric oxide synthase production (62). Another fluorinated analogue, 1α,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalciferol, diminished HT-29 cell growth in nude mice, although 1α,25(OH)\(_2\)D\(_3\) itself did not (63). A well-studied low-calcemic analogue, EB1089 (Secalcitrol), also inhibited growth of subcutaneous xenografts of the human colon cancer cell line, LoVo, in a nude mouse model (64). Importantly, EB1089 is being tested in humans; a Phase I trial in England in patients with advanced breast and colorectal cancer demonstrated the safety of the drug (65). Phase II trials in European patients with inoperable pancreatic cancer and hepatocellular carcinoma showed
good patient tolerance, and, in the liver cancer patients, a subset showed a complete response to treatment (66,67).

Cellular effects of vitamin D

1α,25(OH)2D3 has direct antiproliferative properties against many cancer cells in vitro, including colon (68–71), breast (72,73), prostate (74,75), and hematopoietic cells (76). Also, 1α,25(OH)2D3 and 25(OH)2D3 reduce crypt cell proliferation in colonic tissue removed from individuals with familial adenomatous polyposis (77). Whether the antiproliferative effects of vitamin D are totally independent of calcium level is not clear; for example, the effect of vitamin D in colon cancer cells in vitro varies by extracellular calcium concentration (78) and is reduced by addition of calcium channel blockers (68).

The action of vitamin D is mediated through a high-affinity nuclear receptor. It shares common characteristics with other members of the steroid hormone receptor superfamily in that it is a ligand-activated regulator of gene transcription. The receptor is expressed in colon tumor cells (68,79), and the density of the vitamin D receptor is increased in hyperplastic polyps and in early stages of tumorigenesis but declines in late-stage neoplasia (11,80,81). With carcinogen treatment, rats show a decreased number of 1α,25(OH)2D3 binding sites in the colon (37). The level of vitamin D receptor in wild-type, heterozygote, and vitamin D receptor null mice is inversely correlated with proliferating nuclear cell antigen and cyclin D1, markers of cellular proliferation, and is positively correlated with 8-hydroxy-2′-deoxyguanosine levels, a marker of oxidative stress in the colon descendens (82). These results implicate genomic 1α,25(OH)2D3 action in prevention of hyperproliferation and oxidative DNA damage.

The activated receptor recognizes specific vitamin D response elements in a number of vitamin D–regulated genes, including p21/Waf1 and the calcium-sensing receptor. However, vitamin D regulates a number of different protooncogenes and tumor suppressor genes related to proliferation and differentiation, including p27/Kip1, c-myec, laminin, tenasin, fibronectin, cyclin C, c-fos, c-jun, phospholipase Cγ, ornithine decarboxylase, and members of the transforming growth factor (TGF)-β family (83). Because not all of these genes have vitamin D response element consensus sequences identified in their promoter regions, their regulation is thought to be indirect, through regulation of upstream events or even activation of a putative membrane-bound receptor.

The anticancer effects restricting cellular growth are thought to involve various mechanisms, including effects on growth factor and cytokine synthesis and signaling, cell-cycle progression, apoptosis, and differentiation. An example of the regulation by 1α,25(OH)2D3 of a growth-factor signaling pathway is that of TGF-β, which inhibits epithelial cell proliferation. SMAD3, a downstream protein in the TGF-β signaling pathway, is a coactivator of the vitamin D receptor and positively regulates the vitamin D signaling pathway (84–86). Cross-talk between vitamin D and TGF-β1 have been demonstrated in the growth inhibition of human colon cancer–derived cells (87). The antimitotic actions of vitamin D and its analogs seem to be mediated by the induction of G1 cell-cycle arrest, resulting from the upregulation of expression of p21/Waf1 and p27/Kip1 (88–90). In addition to inhibiting tumor growth and progression, 1α,25(OH)2D3 has anticancer action, by inducing apoptosis in various transformed cells, including colon cancer cells (88,91). The mechanisms of the proapoptotic action in colon cells is not entirely clear, but, in human colon adenoma and carcinoma cell lines, the apoptotic action of vitamin D was associated with upregulation of the expression of the proapoptotic protein Bak (92). The differentiation-promoting effect of 1α,25(OH)2D3 was demonstrated in a human colon carcinoma cell line, SW480, which expresses vitamin D receptors but not in other similar lines that do not express the receptor (93). 1α,25(OH)2D3 induced the expression of proteins associated with the mature phenotype, such as E-cadherin and cell adhesion proteins, and repressed β-catenin signaling, which has an antitumor effect in vivo.

In colon cancer, the vitamin D receptor has been shown to act as a receptor for the secondary bile acid lithocholic acid (LCA). The receptor has a high affinity for LCA and its metabolites, thus acting as an intrestinal bile acid sensor. By binding to the vitamin D receptor, both LCA and vitamin D may activate a feed-forward catabolic pathway that increases the expression of CYP3A, a cytochrome P450 enzyme that detoxifies LCA in the liver and intestines and leads to the detoxification of LCA (94). This may provide one mechanism to explain how the protective pathway of vitamin D receptor activation may become overwhelmed by high-fat diets (which increase LCA levels) or compromised when vitamin D is deficient.

Vitamin D in cancer prevention and current recommendations

As outlined, the anticancer effect of vitamin D is observ-}

able in epidemiological studies and in experimental models of colon cancer. The use of analogs of vitamin D has potential as a treatment in patients with primary (or at risk for recurrence of) colon cancer and with promising results in a limited number of clinical trials; the oncology community can expect additional trials to demonstrate the effectiveness of these compounds. As described previously, analogs provide the advantage of antiproliferative effects against cancer cells, with diminished hypercalcemia when administered at pharmacologically active doses. However, for the general population, the intake recommendations for the nutrient vitamin D, and for calcium as well, may need to be reevaluated in the context of cancer and other chronic disease prevention.

The index disease for vitamin D is rickets; with fortification of foods and vitamin D supplements, most cases of stage-3 deficiency were eliminated in the United States (95). However, there has been a resurgence in rickets in children, prompting a recent National Institutes of Health conference to discuss the topic (96). With increasing evidence of a protective role of vitamin D in many chronic diseases, an increased prevalence of acute and subclinical deficiencies in children and adults is troublesome. Observational studies in locations such as Boston, for example, among unsolicited adult medical patients and hospital employees and visitors show a high prevalence of hypovitaminosis that varies with season in the general population (97,98). A recent study conducted at UCLA designed to evaluate the role of vitamin D in glucose metabolism found that even in sunny Southern California, the mean 25(OH)D3 levels in the Asian-American and African-American subgroups of healthy young adults recruited into the trial were below the defined lower limit of hypovitaminosis D (<52 nmol/L, 20 μg/L) and those of Mexican Americans were borderline (99). Indeed, results from the Third National Health and Nutrition Examination Survey (1988–1994) showed hypovitaminosis D [defined in this study as ≤37.5 nmol/L (15 μg/L)] in 42% of African American women of reproductive age compared with 4% in white women (100).

The difficulty in setting dietary recommendations for vita-
min D intake is because of the multitude of factors that regulate vitamin D availability. Currently the Standing Committee on Scientific Evaluation of Dietary Reference Intake has proposed only an adequate intake value rather than an estimated average requirement, because of insufficient data (101). The current adequate intake value, set at 200 IU/d (5 \( \mu \)g/d) for persons 0–50 y, 400 IU/d (10 \( \mu \)g/d) for persons 51–70 y, and 600 IU/d (15 \( \mu \)g/d) for persons >70 y. The upper limit of intake recommended is 2000 IU/d (50 \( \mu \)g/d) for all persons >1 y. These recommendations assume no vitamin D input from sun-mediated cutaneous production (102). However, UV exposure is critical to the vitamin D biosynthetic pathway, therefore latitude of residence, season, lifestyle habits that keep an individual indoors, and sunscreen use modify vitamin D production and therefore dietary requirement. The elderly have increased dietary vitamin D requirements because of a 4-fold decrease in the capacity of the skin to produce vitamin D3 in adults over 65 y relative to younger adults, as well as decreased intestinal absorption (103). Adults with obesity also have decreased circulating vitamin D levels, thought to be the result of decreased bioavailability of vitamin D from cutaneous and dietary sources (104,105).

From a public health perspective, the question remains how to apply the findings of cancer protective effects of vitamin D. Some have advised brief daily sun exposure to increase vitamin D levels (9), but the risk of increased skin cancer and the difficulty in factoring in variables such as age, latitude, skin pigmentation, and sunscreen use make this controversial and difficult to implement (106). Relatively few foods are good naturally occurring sources of vitamin D, suggesting increased supplementation is advised. Because of the apparent synergistic effects of vitamin D and calcium, it appears that supplementing both nutrients is desirable. In the context of colon cancer, a current intervention trial is addressing this issue (26). Dairy products are a primary dietary source of both vitamin D and calcium; however, there are limitations to the effectiveness of recommending increased intake of these products to prevent hypovitaminosis D. Evidence exists that the levels of vitamin D in fortified dairy foods, mainly fluid milk, vary with processing, storage, and quality control (107–109). Furthermore, the race/ethnicity and age groups at greatest risk for insufficiency consume less milk relative to other groups and lactose intolerance may also limit dairy intake (110). Fortification of orange juice with calcium and vitamin D is an alternative (111), as is the daily use of multivitamin and mineral supplements. Recently, a case was made for mandating calcium and vitamin D supplementation to cereal-grain products (including wheat flour and products, corn meal, and pastas), which is currently optional under U.S. federal guidelines, to prevent not only osteoporosis but also colon cancer (112).

Conclusions

Vitamin D has been shown to have chemopreventive effects in colon carcinogenesis. This effect is confounded with that of calcium but does not preclude direct genomic and nongenomic effects of vitamin D, as shown with animal and cell culture models. Clinical intervention and experimental data suggest that supplementation with both vitamin D and calcium may diminish colon cancer incidence and is likely to affect a number of other chronic diseases, e.g., diabetes. As more information becomes available on the metabolic actions of vitamin D and on polymorphisms in genes controlling its action, the future may provide the ability to further individualize recommendations on vitamin D intake.

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LITERATURE CITED


VITAMIN D AND COLON CARCINOGENESIS


