Editorial

Vitamin D Activity and Colorectal Neoplasia: A Pathway Approach to Epidemiologic Studies

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The relationship of vitamin D with colorectal and other cancers is a rapidly expanding field of study. The pathway through which vitamin D exerts transcriptional effects is complex and involves the action of other nutrients. Recent epidemiologic work underscores the importance of considering the joint effects of calcium and vitamin D in studies of colorectal neoplasia (1, 2). A key feature of vitamin D is its role in maintaining calcium homeostasis, which further highlights the interrelationship of these nutrients. However, the action of a vitamin A derivative, 9-*cis* retinoic acid and its receptor, the retinoid X receptor (RXR), has seldom been investigated in epidemiologic studies of vitamin D, despite the importance of RXR to the transcriptional activity of the vitamin D receptor (VDR; ref. 3). The intricate biological relationship among these nutrients makes it difficult to assess their individual roles in the development of colorectal neoplasia. In this commentary, we present a pathway that outlines the independent and interactive effects of nutrients that impact vitamin D activity in the colorectum. Although the pathway that we present is relatively simplified, our purpose is to highlight the various gene-nutrient relationships that should be considered when conducting studies of vitamin D and colorectal cancer. In this context, we specifically emphasize the actions of 9-*cis* retinoic acid and RXR, two neglected factors in this pathway. Based on these actions, we recommend that future epidemiologic investigations consider the interrelationships of these nutrients and associated genetic polymorphisms, in addition to studying the effects of each individually.

As a brief introduction to the epidemiologic data for the action of vitamin D and related nutrients, there is epidemiologic support for a protective effect of vitamin D on risk of colorectal neoplasia (2, 4); however, the data are equivocal (5). For calcium, there are a wealth of prospective and clinical data indicating that this nutrient is associated with a lower risk of colorectal neoplasia (5-7). In a clinical trial conducted by Baron et al. (6), subjects assigned to daily supplementation with calcium carbonate had a significantly reduced risk of adenoma recurrence compared with the placebo group. Further analyses of this clinical trial revealed that higher serum 25-dihydroxy-vitamin D levels were inversely associated with adenoma recurrence among participants in the calcium-supplemented group and not for those in the placebo group (1); this finding lends further support to the importance of the intricate biological relationship of these two nutrients to colorectal neoplasia (8). Although the role of calcium and vitamin D in colorectal carcinogenesis has been intensively investigated, the activity of 9-*cis* retinoic acid and RXR have not; this is in spite of the fact that RXR has been established as a component of vitamin D transcriptional activity. Nonetheless, putative protective mechanisms of action for 9-*cis* retinoic acid, calcium and vitamin D, and colorectal neoplasia have been proposed, as summarized in Fig. 1 and described below.

Various chemopreventive properties have been suggested for calcium and include the binding of bile acids (9, 10), decreased fecal water cytotoxicity (11), inhibition of cellular proliferation (12, 13), induction of apoptosis (14), promotion of the tumor suppressor gene, *E-cadherin* (15), and inhibition of *β-catenin* (15), a proto-oncogene that has been implicated in several stages of colorectal carcinogenesis (16). For vitamin D and its analogues, the proposed mechanisms include antiproliferative effects (17), increased expression of the cyclin-dependent kinase inhibitor proteins, $p27^{Kip1}$ (18, 19) and $p21^{waf1}$ (18, 20), promotion of colorectal carcinoma cell differentiation by induction of *E-cadherin* (21), and inhibition of *β-catenin* (16, 21). Furthermore, analogues of 1,25-dihydroxy-vitamin D_3 [1,25-$(OH)_2D_3$] act as inducers of apoptosis, and have shown the ability to inhibit the expression of bcl-2, a suppressor of apoptosis, in HL-60 cells (22).

Investigations of the effects of 9-*cis* retinoic acid in the colon have been relatively sparse. Until recently, the role of RXR as an important regulatory component has been largely overshadowed by the potent effects of its heterodimer partners, including the peroxisome proliferator–activated receptor-γ (23) and VDR (24). Nonetheless, 9-*cis* retinoic acid has been shown to inhibit growth of breast and bladder cancer cell lines (25, 26). In colon cancer cell lines, 9-*cis* retinoic acid has been shown to modify the antiproliferative response of 1,25-$(OH)_2D_3$, exhibiting an increased antiproliferative response in Caco-2 cells but blocking it in HT-29 cells (17). The reasons for these differential responses are unclear, but the data support a role for 9-*cis* retinoic as an important modulator of vitamin D activity. In addition to the pathways that describe the downstream biological activities induced by vitamin D, calcium, and 9-*cis* retinoic acid, the activity of related receptors and binding proteins for these nutrients must also be considered (Fig. 1).

The calcium-sensing receptor is necessary for the detection and maintenance of extracellular calcium levels by influencing parathyroid hormone secretion and calcium reabsorption (27). Calcium-sensing receptors may regulate the amount of calcium available to intestinal cells, and variation in the amount or activity of calcium-sensing receptors may have implications for colorectal carcinogenesis. Indeed, polymorphisms of this gene have been associated with risk of advanced stage rectal cancer (28).

The vitamin D-binding protein is important for the transport of the two major forms of circulating vitamin D, 25-(OH)D and

Cancer Epidemiol Biomarkers Prev 2005;14(9):2061–3

Received 7/12/05; accepted 7/26/05.

Grant support: Supported in part by the Specialized Program of Research Excellence in Gastrointestinal Cancer (CA95060), and the Colon Cancer Prevention Program Project Grant (National Cancer Institute/NIH PO1 CA41108). Dr. Jacobs is supported by a Career Development Award (1K07CA106269-01A1) from the National Cancer Institute. Dr. Haussler is supported by NIH Grants DK-33351 and DK-063930.

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doi:10.1158/1055-9965.EPI-05-0472

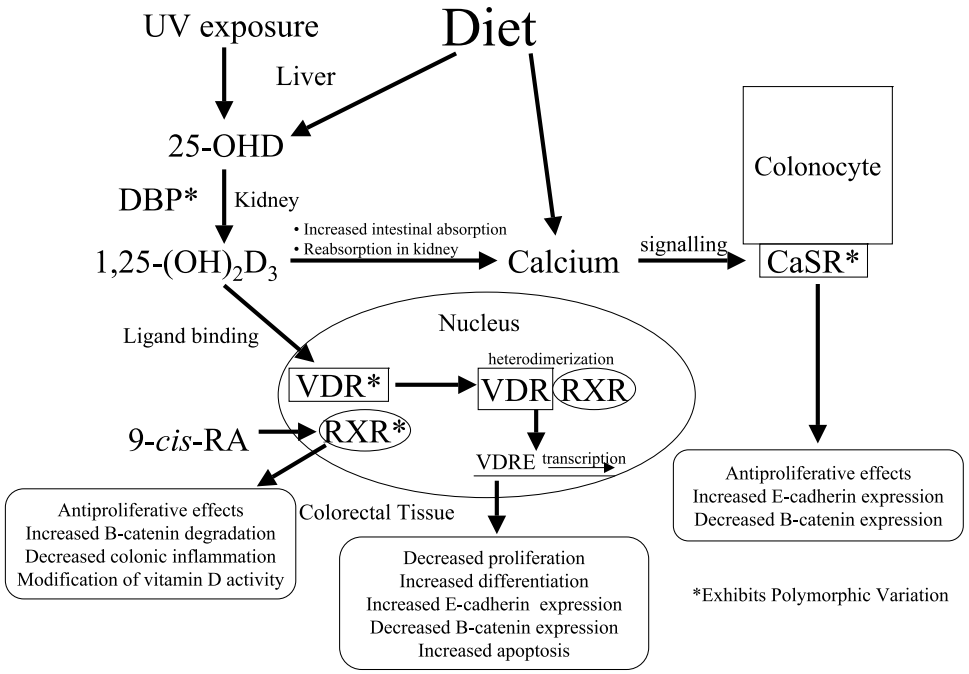

Figure 1. The intricate relationship between the effects of vitamin D, calcium, and 9-*cis*-retinoic acid on tissues of the colorectum. Abbreviations: 25-OHD, 25-dihydroxyvitamin D; DBP, vitamin D binding protein; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 9-*cis* RA, 9-*cis* retinoic acid; VDR, vitamin D receptor; RXR, retinoid X receptor; VDRE, vitamin D response element; CaSR, calcium sensing receptor.

1,25-$(OH)_2D_3$ (29). Although this protein has been shown to be highly polymorphic, there have been few studies of its genetic variation and cancer. Following vitamin D transport to target tissues via vitamin D-binding protein, the VDR is critical to the action of 1,25-$(OH)_2D_3$ (3). After binding its ligand, VDR must heterodimerize with the RXR to exert transcriptional effects on target genes (24).

There are three isoforms of RXR (α, β, and γ), with abundant RXRα expressed in normal and neoplastic colorectal tissue (30) and colorectal cancer cell lines (17). In addition, recent investigations have indicated an important role for RXRα in colorectal pathogenesis, both independently and in conjunction with its heterodimer partners. RXR has been shown to have a role in decreasing colonic inflammation in mouse models (31). In addition, RXRα may exert chemopreventive or chemotherapeutic effects in the colon via the regulation of *β-catenin* (32). Dysregulation of *β-catenin* is a common outcome of mutations observed in colorectal cancer, resulting in higher concentrations of *β-catenin* and ultimately increased activation of oncogenes (32). RXR agonists have been shown to enhance interaction between RXRα and *β-catenin*, resulting in more efficient *β-catenin* degradation and subsequent antiproliferative effects (32).

Alterations in the concentration or function of the receptors, binding proteins, or ligands presented in Fig. 1 could have major implications on actions within the cell. Polymorphic variation has been observed in several nuclear receptors (33, 34), including *VDR*, and has been shown to affect transcriptional activity (35). In addition, *VDR* polymorphisms have been associated with colorectal adenoma formation (36), as well as with risk of several types of cancer (37-39), although results have been equivocal (1, 2). Recently, polymorphic variation has been described in the gene for *RXRβ* (34), a subtype of RXR expressed in several human tissues. As has been observed with *VDR* polymorphisms, variation in the gene for *RXRα* may elicit functional changes that are important for colorectal cancer risk. Therefore, investigations to identify the functional effects of genetic variation in *RXR* and its role in the vitamin D pathway will be critical to further the understanding of the mechanism of action of vitamin D.

In summary, the involvement of several nutrients and genes in altering vitamin D activity strongly indicates the need for a pathway approach to the study of its biological effects, including greater emphasis on the activity of 9-*cis* retinoic acid and RXR. Study of the biological effects of RXR is an especially rich area for research, given its role as a heterodimer partner for several steroid nuclear receptors (40), including peroxisome proliferator–activated receptor-γ (23), a molecule that has itself been shown to have implications for colorectal carcinogenesis (41). We believe that examining vitamin D and/or *VDR* alone in epidemiologic studies, without consideration of additional key factors involved in the proposed pathway, may result in misleading or incomplete mechanisms of action. We have presented a simplified pathway to facilitate further discussion of well-known interactions among the nutrients and genes discussed. However, the vitamin D pathway is clearly more complex. Because this is a rapidly evolving field of cancer research, future studies will undoubtedly continue to uncover additional gene-gene and gene-nutrient interactions that will need to be considered along with factors proposed in the present work.

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Cancer Epidemiol Biomarkers Prev 2005;14:2061-2063.