

Editorial

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

Elizabeth T. Jacobs,^{1,2} Mark R. Haussler,³ and María Elena Martínez^{1,2}

¹Arizona Cancer Center, ²Mel and Enid Zuckerman, Arizona College of Public Health, and ³Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, Arizona

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 impor-

tance 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 anti-proliferative 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 D₃ [1,25-(OH)₂D₃] 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)₂D₃, 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 acid 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.

Requests for reprints: Elizabeth T. Jacobs, Arizona Cancer Center, University of Arizona, P.O. Box 245024, Tucson, AZ 85724-5024. Phone: 520-626-0341; Fax: 520-626-9275.

E-mail: jacobse@u.arizona.edu

Copyright © 2005 American Association for Cancer Research.

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

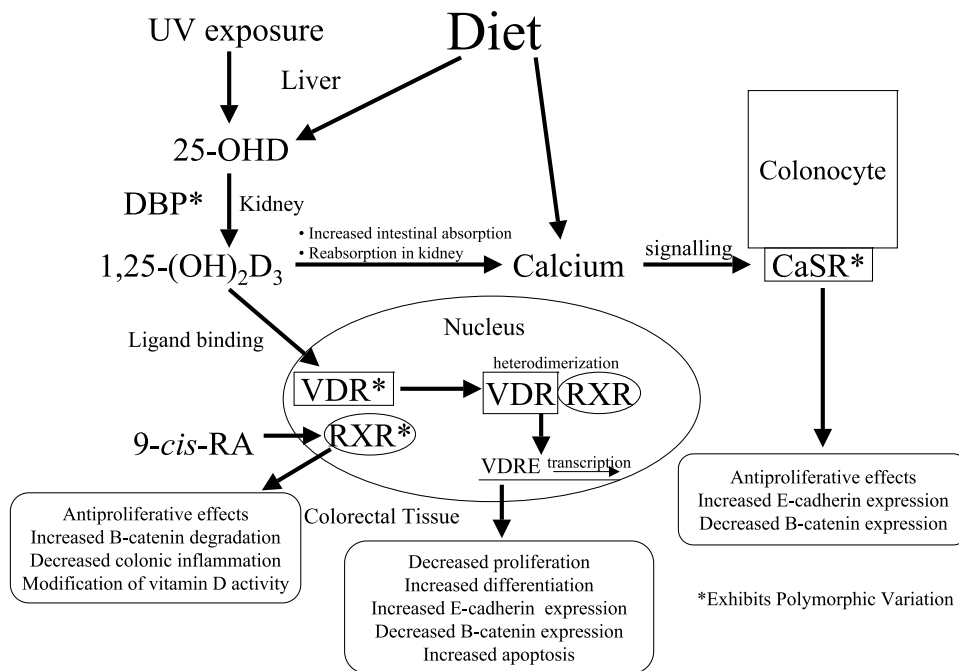


Figure 1. The intricate relationship between the effects of vitamin D, calcium, and 9-cis-retinoic acid on tissues of the colorectum. Abbreviations: 25-OHD, 25-dihydroxyvitamin D; DBP, vitamin D binding protein; 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 9-cis RA, 9-cis retinoic acid; VDR, vitamin D receptor; RXR, retinoid X receptor; VDRE, vitamin D response element; CaSR, calcium sensing receptor.

1,25-(OH)₂D₃ (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)₂D₃ (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 RXR α expressed in normal and neoplastic colorectal tissue (30) and colorectal cancer cell lines (17). In addition, recent investigations have indicated an important role for RXR α 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, RXR α 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 RXR α 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 RXR β (34), a subtype of RXR expressed in several human tissues. As has been observed with VDR polymorphisms, variation in the gene for RXR α 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.

References

1. Grau MV, Baron JA, Sandler RS, Haile RW, Beach ML, Church TR. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. *J Natl Cancer Inst* 2003;95:1765-71.
2. Peters U, McGlynn KA, Chatterjee N, et al. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2001;10:1267-74.
3. Haussler MR, Haussler CA, Jurutka PW, et al. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *J Endocrinol* 1997;154 Suppl:S57-73.
4. Platz EA, Hankinson SE, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiol Biomarkers Prev* 2000;9:1059-65.
5. Martinez ME, Marshall JR, Sampliner R, Wilkinson J, Alberts DS. Calcium, vitamin D, and risk of adenoma recurrence (United States). *Cancer Causes Control* 2002;13:213-20.
6. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med* 1999;340:101-7.
7. Wu K, Willett WC, Fuchs CS, Colditz GA, Giovannucci EL. Calcium intake and risk of colon cancer in women and men. *J Natl Cancer Inst* 2002;94:437-46.
8. Jacobs ET, Martinez ME, Alberts DS. Research and public health implications of the intricate relationship between calcium and vitamin

- D in the prevention of colorectal neoplasia. *J Natl Cancer Inst* 2003;95:1736–7.
9. Newmark HL, Wargovich MJ, Bruce WR. Colon cancer and dietary fat, phosphate, and calcium: a hypothesis. *J Natl Cancer Inst* 1984;72:1323–5.
 10. Nagengast FM, Grubben MJ, van Munster IP. Role of bile acids in colorectal carcinogenesis. *Eur J Cancer* 1995;31A:1067–70.
 11. Lapre JA, De Vries HT, Van der Meer R. Cytotoxicity of fecal water is dependent on the type of dietary fat and is reduced by supplemental calcium phosphate in rats. *J Nutr* 1993;123:578–85.
 12. Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. *N Engl J Med* 1985;313:1381–4.
 13. Pence BC. Role of calcium in colon cancer prevention: experimental and clinical studies. *Mutation Res* 1993;290:87–95.
 14. Hambly RJ, Saunders M, Rijken PJ, Rowland IR. Influence of dietary components associated with high or low risk of colon cancer on apoptosis in the rat colon. *Food Chem Toxicol* 2002;40:801–8.
 15. Chakrabarty S, Radjendirane V, Appelman H, Varani J. Extracellular calcium and calcium sensing receptor function in human colon carcinomas: promotion of E-cadherin expression and suppression of β -catenin/TCF activation. *Cancer Res* 2003;63:67–71.
 16. Wong NA, Pignatelli M. β -Catenin—a linchpin in colorectal carcinogenesis? *Am J Pathol* 2002;160:389–401.
 17. Kane KF, Langman MJ, Williams GR. Antiproliferative responses to two human colon cancer cell lines to vitamin D₃ are differently modified by 9-*cis*-retinoic acid. *Cancer Res* 1996;56:623–32.
 18. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D₃ analog induces a G₁-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. *Endocrinology* 2000;141:3931–9.
 19. Wang QM, Jones JB, Studzinski GP. Cyclin-dependent kinase inhibitor p27 as a mediator of the G₁-S phase block induced by 1,25-dihydroxyvitamin D₃ in HL60 cells. *Cancer Res* 1996;56:264–7.
 20. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1 α ,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol Endocrinol* 2001;15:1370–80.
 21. Palmer HG, Gonzalez-Sancho JM, Espada J, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β -catenin signaling. *J Cell Biol* 2001;154:369–87.
 22. Elstner E, Linker-Israeli M, Umiel T, et al. Combination of a potent 20-epi-vitamin D₃ analogue (KH 1060) with 9-*cis*-retinoic acid irreversibly inhibits clonal growth, decreases bcl-2 expression, and induces apoptosis in HL-60 leukemic cells. *Cancer Res* 1996;56:3570–6.
 23. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. *Science* 2001;294:1866–70.
 24. Haussler MR, Whitfield GK, Haussler CA. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res* 1998;13:325–49.
 25. Fitzgerald P, Teng M, Chandraratna RA, Heyman RA, Allegretto EA. Retinoic acid receptor α expression correlates with retinoid-induced growth inhibition of human breast cancer cells regardless of estrogen receptor status. *Cancer Res* 1997;57:2642–50.
 26. Laaksovirta S, Rajala P, Nurmi M, Tammela TL, Laato M. The cytostatic effect of 9-*cis*-retinoic acid, tretinoin, and isotretinoin on three different human bladder cancer cell lines *in vitro*. *Urol Res* 1999;27:17–22.
 27. Brown EM, Pollak M, Hebert SC. The extracellular calcium-sensing receptor: its role in health and disease. *Annu Rev Med* 1998;49:15–29.
 28. Speer G, Cseh K, Mucci K, et al. Calcium-sensing receptor A986S polymorphism in human rectal cancer. *Int J Colorectal Dis* 2002;17:20–4.
 29. White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. *Trends Endocrinol Metabol* 2000;11:320–7.
 30. Kane KF, Langman MJ, Williams GR. 1,25-Dihydroxyvitamin D₃ and retinoid X receptor expression in human colorectal neoplasms. *Gut* 1995;36:255–8.
 31. Desreumaux P, Dubuquoy L, Nutten S, et al. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPAR γ) heterodimer. A basis for new therapeutic strategies. *J Exp Med* 2001;193:827–38.
 32. Xiao JH, Ghosh N, Hinchman C, et al. Adenomatous polyposis coli (APC)-independent regulation of β -catenin degradation via a retinoid X receptor-mediated pathway. *J Biol Chem* 2003;278:29954–62.
 33. Arai H, Miyamoto K, Taketani Y, et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *J Bone Miner Res* 1997;12:915–21.
 34. Rajsbaum R, Fici D, Fraser PA, Flores-Villanueva PO, Awdeh ZL. Polymorphism of the human retinoid X receptor β and linkage disequilibrium with HLA-DPB1. *Tissue Antigens* 2001;58:24–9.
 35. Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol Endocrinol* 2000;14:401–20.
 36. Ingles SA, Wang J, Coetzee GA, Lee ER, Frankl HD, Haile RW. Vitamin D receptor polymorphisms and risk of colorectal adenomas (United States). *Cancer Causes Control* 2001;12:607–14.
 37. Xu Y, Shibata A, McNeal JE, Stamey TA, Feldman D, Peehl DM. Vitamin D receptor start codon polymorphism (FokI) and prostate cancer progression. *Cancer Epidemiol Biomarkers Prev* 2003;12:23–7.
 38. Ikuyama T, Hamasaki T, Inatomi H, Katoh T, Muratani T, Matsumoto T. Association of vitamin D receptor gene polymorphism with renal cell carcinoma in Japanese. *Endocr J* 2002;49:433–8.
 39. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. *Br J Cancer* 2001;85:171–5.
 40. Kliewer SA, Umeson K, Mangelsdorf DJ, Evans RM. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D₃ signalling. *Nature* 1992;355:446–9.
 41. Sarraf P, Mueller E, Smith WM, et al. Loss-of-function mutations in PPAR- γ associated with human colon cancer. *Mol Cell* 1999;3:799–804.

BLOOD CANCER DISCOVERY

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

Elizabeth T. Jacobs, Mark R. Haussler and María Elena Martínez

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

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/14/9/2061>

Cited articles This article cites 41 articles, 15 of which you can access for free at:
<http://cebp.aacrjournals.org/content/14/9/2061.full#ref-list-1>

Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/14/9/2061.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/14/9/2061>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.