Expression of Vitamin D Receptor and 25-Hydroxyvitamin D3-1α-Hydroxylase in Normal and Malignant Human Colon

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Abstract
Considerable evidence exists to support the use of vitamin D to prevent and/or treat colorectal cancer. However, the routine use of bioactive vitamin D, 1,25-dihydroxyvitamin D3, is limited by the side effect of toxic hypercalcemia. Recent studies, however, suggest that colonic epithelial cells express 25-hydroxyvitamin D3-1α-hydroxylase, an enzyme that converts nonactive pro-vitamin D, 25-hydroxycholecalciferol [25(OH)D3], to its bioactive form. Yet, nothing is known as to the cellular expression of 1α-hydroxylase and the vitamin D receptor (VDR) in the earliest histopathologic structures associated with malignant transformation such as aberrant crypt foci (ACF) and polyps [addressing the possibility of using nonactive 25(OH)D3 for chemoprevention], nor is anything known as to the expression of these proteins in colorectal cancer as a function of tumor cell differentiation or metastasis [relevant to using 25(OH)D3 for chemotherapy]. In this study, we show that 1α-hydroxylase is present at equal high levels in normal colonic epithelium as in ACFs, polyps, and colorectal cancer irrespective of tumor cell differentiation. In contrast, VDR levels were low in normal colonic epithelial cells; were increased in ACFs, polyps, and well-differentiated tumor cells; and then declined as a function of tumor cell de-differentiation. Both 1α-hydroxylase and VDR levels were negligible in tumor cells metastasizing to regional lymph nodes. Overall, these data support using 25(OH)D3 for colorectal cancer chemoprevention but suggest that pro-vitamin D is less likely to be useful for colorectal cancer chemotherapy. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2370–6)

Introduction
Vitamin D’s ability to prevent colorectal cancer has been suspected for over a quarter of a century. One of the earliest studies supporting this link came from the observation that there was an inverse relationship between mean solar radiation and age-adjusted colorectal cancer death rates (1). Since that time, a number of epidemiologic studies have suggested a link between vitamin D and/or calcium levels and the incidence of human colorectal cancer formation (reviewed in ref. 2). The validity of these epidemiologic observations was significantly enhanced by the results of the CaliPolyp Prevention Study, a double-blind placebo-controlled study that showed a decreased recurrence rate of colorectal adenomas in patients receiving calcium carbonate (3, 4). Patients receiving the greatest benefit from calcium supplementation were those with decreased serum levels of 25-hydroxycholecalciferol [25(OH)D3; ref. 3], highlighting the importance of vitamin D in inhibiting colorectal cancer formation.

Although vitamin D status in humans is typically assessed by measuring serum 25(OH)D3 levels, this nonactive pro-vitamin requires hydroxylation at carbon 1 by 25-hydroxyvitamin D3-1α-hydroxylase to generate bioactive 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]. Whereas the primary function of 1,25(OH)2D3 is to regulate calcium absorption and maintain mineral homeostasis, upon binding to its cognate vitamin D receptor (VDR), 1,25(OH)2D3 also increases the proliferation and enhances the differentiation of colorectal cancer cells (5) as well as alters the transcription of a large number of genes involved in inhibiting carcinogenesis (6, 7).

Unfortunately, 1,25(OH)2D3 has a narrow therapeutic index, with its propensity for causing toxic hypercalcemia precluding its routine use in otherwise healthy patients. Recently, however, a number of investigators have suggested that 1α-hydroxylase is present in normal (8, 9) and malignant (10, 11) epithelial cells lining the adult human colon, suggesting that nonactive 25(OH)D3 might represent an efficacious treatment modality for treating patients with known colorectal polyps or cancer (i.e., chemotherapy), or preventing these lesions from forming in the first place (i.e., chemoprevention). Yet, the studies done to date have not systematically evaluated VDR and 1α-hydroxylase expression in colon cancer as a function of tumor cell differentiation or metastasis, critical to assessing 25(OH)D3’s potential as a chemotherapeutic agent, nor have they evaluated the expression profile of these proteins in early neoplastic lesions such as aberrant crypt foci (ACF), critical to assessing 25(OH)D3’s potential as a chemopreventive agent.

To assess 25(OH)D3’s potential for colorectal cancer chemoprevention and/or chemotherapy, we herein report on cellular VDR and 1α-hydroxylase protein expression in human ACFs, polyps, and colorectal cancer’s of defined differentiation along with associated lymph node metastases. To do this, we used quantitative immunohistochemistry to precisely determine VDR and 1α-hydroxylase expression in formalin-fixed surgically resected tissues at the cellular level. We herein show that 1α-hydroxylase levels are consistently high in all nonmetastatic tissues, irrespective of histology. In contrast, we show that VDR levels are increased in ACFs and well-differentiated tumors but progressively diminish with colorectal cancer de-differentiation. Lastly, we show that both 1α-hydroxylase and VDR expression is low to negligible in metastatic tissues, regardless of the histopathologic stage assigned to either the primary tumor or tumor contained...
within the metastasis. Overall, these findings suggest that 25(OH)D3 has potential for use in colorectal cancer chemoprevention but may be less efficacious for colorectal cancer chemotherapy.

Materials and Methods

Materials. Anti-VDR antibody was from Abcam (Cambridge, MA). Sheep Anti-murine 25-hydroxyvitamin D3-1α-hydroxylase antibody was from The Binding Site (San Diego, CA). Goat anti-sheep horseradish peroxidase (HRP)–labeled antibody, goat anti-mouse IgG-HRP, and rabbit anti-goat IgG-HRP was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). TBST wash buffer, target retrieval solution, protein block serum, antibody diluent, EnVision+ HRP, (DAB)-rabbit system, 3,3′-diaminobenzidine (DAB) chromogen, and automated hematoxylin were all from DAKO (Carpinteria, CA). Auto/ iodine, Redusol, and Permound and polyvinylidene difluoride membranes were from Fisher Scientific (Pittsburgh, PA). Mammalian protease inhibitor cocktail was purchased from Sigma (St. Louis, MO). Enhanced Chemiluminescence plus Western Blotting Detection System was from Amersham (Piscataway, NJ); 30% acrylamide/Bis solution and Precision Plus protein standards were from Bio-Rad Laboratories, Inc. (Richmond, CA); and bicinchoninic acid protein assay kit was from Pierce (Rockford, IL). Calctiriol (1α, 25-dihydroxyvitamin D3) was purchased from BIOMOL International, LP (Plymouth Meeting, PA).

Tumor Specimens and Histologic Grading. Colon cancers were randomly selected from the University of Illinois at Chicago Gastrointestinal Tumor Bank. The University of Illinois at Chicago and Veterans Administration Institutional Review Boards approved use of these tissues for this study under the stipulation that no linked clinical data could be used as a part of their evaluation and analysis.

Differentiation was assessed as previously described (12-14). Briefly, well-differentiated tumors were defined by the presence of well-formed glands containing malignant columnar cells displaying small regular nuclei. The complete absence of gland formation, or the presence of bizarre shaped glands, identified poorly differentiated tumors. Moderately differentiated tumors possessed well-formed glands, but the cells were less columnar or frankly cuboidal, with reduced cell polarity and more dysplastic nuclei than those observed in well-differentiated tumors.

Western Analysis. Confluent cells were rinsed in PBS and lysed in radioimmunoprecipitation assay buffer containing a 1:20 dilution of mammalian protease inhibitor cocktail. Protein concentrations were determined using the bicinchoninic acid protein assay kit as described by manufacturer. In all instances, 20 μg of protein were loaded and electrophoresed across a 10% polyacrylamide gel under denaturing and reducing conditions. The resolved proteins were electrophoretically transferred to polyvinylidene difluoride membranes. Membranes were incubated with primary antibody for 2 hours at the following concentrations: VDR, 1:1,750; α(OH), 1:150; and actin, 1:100 followed by two sequential 10-minute washes with TBST. VDR and actin immunoreactive bands were visualized using a HRP-conjugated goat anti-rabbit IgG, whereas α(OH) was visualized using a HRP-conjugated rabbit anti-goat IgG and the Enhanced Chemiluminescence Plus detection system.

Quantitative Immunohistochemistry. Tissues were sectioned (4 μm thick) using a Sakura Accu-Cut SRM 200 Rotary Microtome (Torrance, CA) and processed for antigen retrieval in the following manner. Sections were rehydrated in graded alcohol and a running water bath, placed in auto/iodine for 1 minute, rinsed in Tris-buffered saline Tween 20 (TBST) buffer, rinsed twice for 2 minutes in Redusol, and once again in TBST buffer. The slides were then placed in Target Retrieval Solution at 100°C for 20 minutes, allowed to cool to room temperature, and rinsed once again in TBST.

Immunohistochemistry was done on a DAKO Autostainer Universal Staining System using a two-step indirect immunoperoxidase technique. Briefly, tissues were incubated in a 3% H2O2 solution to quench endogenous peroxidase activity, rinsed with TBST, blocked with protein block serum for 30 minutes, and rinsed in TBST. VDR primary antibody was applied (1:1,750, as determined by antibody titration) for 1 hour at room temperature before rinsing with TBST. Next, labeled polymer rabbit HRP was added for 30 minutes, rinsed thoroughly with TBST followed by incubation with DAB chromogen for 8 minutes, and then counterstained for 2 minutes with hematoxylin.

1α-Hydroxylase primary antibody was applied (1:150, as determined by antibody titration) for 45 minutes at room temperature before rinsing with TBST. Next, rabbit anti-sheep HRP-labeled antibody was applied (1:100) for 45 minutes. Slides were rinsed with TBST followed by DAB chromogen incubation for 5 minutes and counterstained for 2 minutes with hematoxylin. All tissues were then dehydrated in graded alcohol and xylene and cover-slipped using Permound. For all specimens, control tissues were processed identically and at the same time, except that they were not exposed to primary antibody. Thus, all differences between the experimental tissue and the control tissue are ultimately due to DAB identification of the relevant protein.

Chromogen abundance was quantified by quantitative immunohistochemistry as previously described (15, 16). Briefly, images were acquired using a Diagnostic Instruments SPOT RT Digital Scanning Camera (Sterling Heights, MI) attached to a Nikon E600 microscope (Stamford, CT), and image files saved in tagged-image file format. The amount of chromogen per pixel was determined by subtracting the mathematical energy (E_M) of the control slide (i.e., not exposed to primary antibody) from that in the homologous region of the experimental slide (i.e., exposed to primary antibody). Chromogen quantity (E_M) is expressed as energy units per pixels (eu/pix).

Standards and Statistics. In all instances, immunohistochemical quality control was achieved as follows. Because all runs were done using a computer-controlled DAKO Autostainer, conditions were identical at all times. Additionally, a section from a single tumor was included in all runs to control for experimental variability. The amount of chromogen as determined by quantitative immunohistochemistry revealed run-to-run variability of <5%. Data obtained by quantitative immunohistochemistry was evaluated by using the online statistical calculator provided by the College of Saint Benedict/ St. John’s University (http://www.physics.csbsju.edu/) using the statistical test as identified in the text, with P < 0.05 considered significant.

Results

Few studies exist examining 1α-hydroxylase or VDR protein expression in the colon, whereas no studies have systematically evaluated their expression as a function of tumor cell differentiation. To evaluate the expression of these proteins, we used commercially available polyclonal antibodies: the VDR antibody recognizes amino acids 395 to 413 of the human protein, whereas the 1α-hydroxylase antibody recognizes the murine protein (specific epitope not defined by the manufacturer). To confirm the specificity of these antibodies, we first evaluated them by Western blot analysis against whole protein lysates obtained from NCM460 cells, a nonmalignant human colon epithelial cell line, and Caco-2 and HT-29 cells, human
colon cancer cell lines. The antibody for 1α-hydroxylase identified a single band at 56 kDa, whereas that for VDR identified a band at 52 kDa (Fig. 1A). We further confirmed the specificity of the 1α-hydroxylase antibody, because the specific epitope was not defined by the manufacturer. Because it has been previously shown that 1,25(OH)2D3 suppresses 1α-hydroxylase expression in Caco-2 cells (8), we assessed the response of these cells to this secosteroid. In all instances, 50,000 cells were plated in defined medium in 12-well plates and cultured under standard conditions for 24 hours. Cells were then cultured for another 48 hours in serum-free medium alone, serum-free medium containing 0.1% ethanol (v/v), or serum-free medium supplemented with 10 nmol/L calcitrol in 0.1% ethanol (v/v). Caco-2 cells alone or exposed to ethanol vehicle showed strong evidence of 1α-hydroxylase expression by Western analysis, but this band was specifically and significantly down-regulated after exposure to 10 nmol/L 1,25(OH)2D3 for 48 hours (Fig. 1B) thus confirming the specificity of this commercially available antibody.

We next used these antibodies to evaluate 1α-hydroxylase or VDR expression in resected human colon cancers. To do this, we randomly selected 10 colorectal cancer from the University of Illinois at Chicago Gastrointestinal Tumor Bank. Tumor blocks selected for evaluation were those that contained the tumor margin thereby allowing us to evaluate 1α-hydroxylase or VDR protein expression in normal and malignant colonic epithelial cells from the same patient (Figs. 2 and 3). Because colorectal cancer is heterogeneously differentiated (12, 17, 18), we also could assess the expression of these proteins as a function of tumor cell differentiation, again with all evaluation done in the same patient. Overall, the 10 colorectal cancers studied contained 53 separate and distinct regions of well-differentiated tumor, 66 that were moderately differentiated, and 32 that were poorly differentiated.

Overall, 1α-hydroxylase expression seemed consistently strong in normal and malignant colon epithelial cells, irrespective of tumor cell differentiation (Fig. 2A-D). In contrast, minimal VDR expression was observed in normal colonic epithelial cells, with the expression localized predominantly in the nucleus (Fig. 3A). With malignant transformation, total cellular VDR expression increased markedly in well-differentiated tumors (Fig. 3B) but then decreased with tumor cell de-differentiation (Fig. 3C-D).

To more accurately quantify 1α-hydroxylase and VDR expression in these tissues, we used our novel technique of...
quantitative immunohistochemistry (15, 16), which we have shown quantifies chromogen in a manner that accurately reflects the actual amount of protein present (16). Consistent with our empirical observations as reviewed above, the amount of chromogen due to 1α-hydroxylase was of similar high levels in normal colonic epithelial cells as in well-differentiated and moderately differentiated colorectal cancer (Fig. 4). In contrast, 1α-hydroxylase levels were significantly decreased in poorly differentiated tumor cells and in metastases to regional lymph nodes (Fig. 4; \( P < 0.05 \), ANOVA). We also studied three tubular, three villous, and three tubulovillous adenomas, neoplastic lesions that can progress to colorectal cancer. These adenomas, irrespective of histopathology, all expressed similar levels of 1α-hydroxylase as observed in normal colonocytes and well-differentiated and moderately differentiated colorectal cancer (Fig. 4). In contrast, VDR expression was 5-fold higher in polyps and 12-fold higher in well-differentiated colorectal cancer than in normal colonic epithelial cells (Fig. 4; \( P < 0.05 \), ANOVA). However, this elevation decreased in de-differentiated colorectal cancer such that poorly differentiated tumor cells expressed VDR levels similar to what we observed in polyps, an amount that was significantly less than observed in well-differentiated tumor cells (\( P < 0.05 \), ANOVA).

The earliest histopathologic lesion associated with colorectal cancer malignant transformation is the ACF (19, 20). These lesions are not typically seen during routine colonoscopy but can be visualized with high-magnification endoscopes when suitable contrast stains, such as methylene blue, are used (ref. 21; Fig. 5A). Because screening colonoscopy at the University of Illinois at Chicago is routinely done using magnification chromocolonoscopy, our Gastrointestinal Tumor Bank has a large collection of human ACFs for study. We therefore randomly selected five ACF for immunohistochemical evaluation. Whereas 1α-hydroxylase expression levels were similar to what we observed in normal colonic epithelia and in polyps (Figs. 4 and 5B), the amount of VDR detected was the same as observed in polyps (Figs. 4 and 5C). Overall, then, these data suggest that 1α-hydroxylase and VDR expression levels in colorectal cancer precursor lesions such as ACFs and polyps may well allow for pro-vitamin D drugs such as 25(OH)D3 to be used for colorectal cancer chemoprevention.

Importantly, there has been no significant improvement in the survival of patients with solid tumors that metastasize, including those affecting the colon, since the 1950s (22). Hence, we next studied all resected lymph nodes from additional five patients with known lymph node metastases. Overall, 24 separate lymph nodes (range, 2-9 per patient) were evaluated, of which 12 contained metastatic colorectal cancer. Of these, five contained metastatic tumor deposits that histologically were well differentiated (Fig. 6). We quantified the amount of VDR chromogen in well-differentiated tumor cells contained within the primary cancer and in the five well-differentiated lymph node metastases. High levels of VDR-specific chromogen were detected in well-differentiated tumor cells located within the primary cancer (402 ± 28 eu/pix, mean ± SE), whereas little to no VDR-specific chromogen was detected in well-differentiated tumor deposits metastatic to regional lymph nodes (34 ± 5 eu/pix; \( P < 0.05 \), unpaired \( t \) test; Fig. 7).

Because VDR acts by translocating to the nucleus after binding ligand, we used our technique of quantitative immunohistochemistry to assess the relative amounts of VDR expressed in nuclear and nonnuclear (i.e., cytoplasmic) regions (Fig. 8). Intriguingly, the ratio of nuclear to cytoplasmic VDR exceeded 4 in normal colonic epithelial cells, indicating that most of this protein was in the nucleus (Fig. 8, inset; Fig. 3A). However, this ratio dropped to <1.0 for ACFs, the earliest lesion associated with colorectal cancer malignant transformation.

Figure 3. Expression of VDR in human colon cancer and adjacent nonmalignant tissue. VDR is expressed at low levels in normal colonic epithelial cells, with the majority of expression in the nucleus (A). In contrast, VDR expression is increased and predominantly cytoplasmic in well-differentiated tumor cells (B). However, with increasing de-differentiation, significantly less VDR expression is appreciated in moderately differentiated tumor cells (C) and in poorly differentiated tumor cells (D). Magnification, ×40 and ×1,000 (inset).
This ratio remained between 0.9 and 0.4 for all histopathologic types evaluated except for poorly differentiated colorectal cancer and tumors metastatic to regional lymph nodes (ratio, <0.2; Fig. 8, inset; Fig. 3D). Thus, these data suggests that an ever-declining percentage of VDR translocates, or is able to translocate, to the nucleus of colonic epithelial cells that are not histologically normal. In concert with our observation that 1a-hydroxylase and VDR are essentially not present in metastatic cells, these data suggest that pro-vitamin D analogues such as 25(OH)D3 may not be efficacious for colorectal cancer chemotherapy.

Discussion

Vitamin D deficiency is well established as a risk factor for colorectal cancer (reviewed in ref. 2). Upon binding to the VDR, 1,25(OH)2D3 decreases the proliferation of a variety of human colon cancer cell lines (23, 24) as well as of cells within resected human rectal mucosa (25). Moreover, VDR-null mice exhibit decreased markers of cellular proliferation and increased levels of markers reflecting DNA oxidative stress (26), suggesting that the vitamin D-receptor complex acts by attenuating oxidative DNA damage and preventing malignant transformation from occurring. Additionally, once malignant transformation has occurred, 1,25(OH)2D3 induces apoptosis (27, 28) as well as pro-differentiating effects (27) on various colorectal cancer cell lines. Thus, vitamin D may have colorectal cancer chemotherapeutic in addition to chemopreventive effects.

Despite the evidence for increasing hypovitaminosis D in the general population (29-31), widespread use of 1,25(OH)2D3 is not likely to occur given its narrow therapeutic range and side effect profile, even in individuals with known colorectal cancer. However, a role for nontoxic 25(OH)D3 in colorectal cancer chemoprevention and chemotherapy might be reasonable if 1a-hydroxylase is present, along with the VDR, in relevant locations so as to allow for local generation of bioactive vitamin D. Hence, the purpose of the current study was to systematically assess 1a-hydroxylase and VDR cellular expression in normal and neoplastic colonic epithelium.

Figure 4. Amount of 1a-hydroxylase (○) and VDR (●) present in normal colonic epithelium, ACF, polyps, colon cancers of defined differentiation, and tumors metastasizing to regional lymph nodes. In all instances, immunohistochemistry was done and chromogen amount quantified as described in Materials and Methods. Points, means; bars, 95% confidence intervals. The amount of 1a-hydroxylase in lymph nodes is significantly different from the amount present in all other tissues (ANOVA, P < 0.01). The amount of VDR present in ACFs and polyps are similar and significantly different from that expressed by normal colonic epithelial cells, as well as in well-differentiated and moderately differentiated cancers and metastases to lymph nodes (ANOVA, P < 0.01). Abbreviations: NL, normal colonic epithelium; T, tubulovillous, V, villous, TV, tubulovillous; W, well differentiated; M, moderately differentiated; P, poorly differentiated; LN, lymph node.

Figure 5. Expression of 1a-hydroxylase and VDR in a human ACF. A. ACF detected during magnification chromocolonoscopy (arrowhead). B. 1a-Hydroxylase expression in ACF (boxed region) with surrounding normal epithelium. Magnification, ×100. C. VDR expression in ACF (boxed region) with surrounding normal epithelium. Magnification, ×100. Insets, greater cellular detail provided. Magnification, ×400.
Clinically, the most important implication of tumor differentiation relates to the fact that it predicts the development of metastases for most solid tumors (36, 37). In contrast, colon cancers are unusual and differ from most other solid tumors, including even rectal cancers (17), insofar they tend to be heterogeneously differentiated (12, 18). Thus, assessing an entire colon cancer’s “differentiation,” as is usually done by clinical pathologists when assessing tumor “grade,” does not provide information useful for predicting a patient’s outcome or survival. However, when the differentiation of specific cell populations within a colon cancer is considered, this variable does yield prognostic information (38, 39). For example, individual tumors containing well-differentiated cells at the leading edge (39), or at the point of budding (38), do better even in the face of local lymph node invasion than tumors not associated with local metastases but which are comprised of less well differentiated cells. Thus, differentiation and the factors regulating tumor cell appearance are as important in colon cancer as for other solid tumors, providing that this analysis be done at the level of the individual tumor cell. In this study, we make use of our powerful technique of quantitative immunohistochemistry (15, 16) to show for the first time the degree to which both 1α-hydroxylase and VDR are expressed at the cellular level in colonic epithelia as a function of histology. Whereas 1α-hydroxylase is expressed in uniformly high levels in normal and neoplastic colonic epithelial cells, VDR expression is low in normal colonic epithelia, is increased in better differentiated tumor cells, and decreases in poorly differentiated tumor cells, within any particular colorectal cancer. Most importantly, we show that the expression of both 1α-hydroxylase and VDR is decreased in lymph node metastases, irrespective of the degree to which the tumor cells are differentiated in the metastatic lesion.

Our technique for quantitative immunohistochemistry also permitted us to determine the amount of VDR chromogen present in nuclear and cytoplasmic immunohistochemical regions of each cell. In so doing, we could calculate the relative amount present in the nucleus compared with the cytoplasm, a marker of VDR nuclear translocation and thus of “activity.” Nuclear-to-cytoplasmic VDR ratios drop dramatically in even ACFs and decline further thereafter in neoplastic lesions as a function of histology. Thus, these findings indicate that, despite uniformly high levels of 1α-hydroxylase, pro-vitamin D [i.e., 25(OH)D3] may be less likely to be of value for colorectal cancer...
chemotherapy. Although the significance of this finding is not clear, it is possible, given the observation that VDR polymorphisms can result in nonfunctional protein (40, 41) and that tumor de-differentiation might be associated with increasing mutation of the VDR gene. If so, this would be similar to what has been observed for the gene for the colon cancer morphogen, gastrin-releasing peptide receptor (13).

This study is also the first to evaluate 1α-hydroxylase and VDR expression in the earliest lesion associated with malignant transformation in the colon, ACF. ACFs were first defined in the colons of rodents exposed to carcinogens and identified soon thereafter in human colons (20). ACFs are associated with adenomatous polyp (42) and colorectal cancer formation in humans (20) and have become an accepted biomarker for assessing the efficacy of chemopreventive drugs in various nonhuman colorectal cancer models (43). Whereas polyps are relatively fixed as lesions, ACF seem fluid and can readily change in number over time (44). Thus, our finding that ACFs, as well as premalignant polyps, express high levels of 1α-hydroxylase and VDR, suggest that pro-vitamin D may be of value as a chemopreventive agent.

In summary, we herein show that whereas (a) 1α-hydroxylase is present in uniformly high levels in normal and neoplastic colorectal epithelia, except for tumor cells metastatic to other tissues, ACF seem fluid and can readily change in number over time (44). Thus, our finding that ACFs, as well as premalignant polyps, express high levels of 1α-hydroxylase and VDR, suggest that pro-vitamin D may be of value as a chemopreventive agent.

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