Review

Modulation of Vitamin D Synthesis and Catabolism in Colorectal Mucosa: A New Target for Cancer Prevention

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Abstract. *Sporadic colorectal cancer is a disease of advancing age and the percentage of the population which reaches an advanced age is strongly increasing. Multiple factors are responsible for the etiology of this cancer since the colorectal mucosa is directly influenced by nutrients reaching the colonic lumen and impacting on mucosal cells. The vitamin D system appears to be central to several preventative molecular pathways. Insufficiency of the serum precursor 25-hydroxyvitamin D$_3$ has been linked by epidemiology to enhanced colon tumor incidence, most likely because it is a major determinant of 1,25-dihydroxyvitamin D$_3$ synthesis in colonic mucosal cells. Bound to its receptor, vitamin D regulates colonic proliferation, differentiation and apoptosis in an autocrine/ paracrine manner. During early malignancy, vitamin D synthesis is enhanced to counteract hyperproliferation, whereas in high-grade tumors catabolism by far surpasses synthesis. The colonic vitamin D system is regulated by several known natural factors. One of the most important ones is nutritional calcium that, if supply is low, will result in enhanced catabolism of colonic 1,25-dihydroxyvitamin D$_3$. Estrogenic compounds can increase expression and activity of the synthesizing 25-hydroxyvitamin D-1α-hydroxylase. Due to enhanced synthesis of the active metabolite, this can lead to protection against colorectal tumors in women. During tumor progression, expression of 25-hydroxyvitamin D-1α-hydroxylase as well as of the catabolizing 25-hydroxyvitamin D-24-hydroxylase appears to be under epigenetic control as demonstrated by studies with phytoestrogens and folate. It is commonly accepted that sporadic colorectal cancer pathogenesis is multifactorial and these are*

just a few examples of the regulatory capacity of natural (nutrient) substances for improving the colonic vitamin D system. However, protection by vitamin D might have central importance, with nutrients increasing the efficiency of the vitamin D system in a targeted manner. This could result in prevention of hyperproliferation or retardation of progression to clinically manifest primary colonic tumors.

Accumulating evidence that a compromised vitamin D status is associated with a significant risk not only of bone diseases but also of several types of cancer has raised considerable attention (1). Vitamin D insufficiency may annually account for several thousand premature deaths from the most frequent malignancies in Western industrialized countries, such as colon (2), mammary (3) and prostate cancer (4). This review will deal primarily with mechanistic aspects of vitamin D insufficiency in pathogenesis and prevention of colon cancer.

Up to 80% of the body's need for vitamin D can be met by UV-B-induced synthesis of vitamin D$_3$ in the skin. Therefore, vitamin D insufficiency is frequently observed in individuals with limited sun exposure, such as in the chronically ill, in immobilized or housebound elderly people, who also have a reduced capacity of vitamin D$_3$ synthesis in the skin. Yet a compromised vitamin D status is also a common phenomenon in the free-living normal population of younger age, particularly during winter months at latitudes above 34° north or south, or when living/working conditions, especially in industrialized countries, reduce the possibility for sufficient sun exposure. Other factors that are prohibitive for sunlight-induced vitamin D$_3$ production in the epidermis include extensive use of sunscreens, dark pigmentation of the skin or traditional clothing that covers most of the body.

To achieve vitamin D adequacy, the amount of endogenously produced vitamin D$_3$ has to be complemented by supply from food sources (*e.g.* milk, milk products and fatty fish), food additives or supplements, which may also contain vitamin D$_2$. However, in many countries vitamin D intake from nutrient supply is too low to sustain an optimal vitamin D status in the general population.

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Regardless of whether it is produced in the skin or absorbed from the gut, vitamin D_3 is transported either to adipose tissue for storage, or to the liver for conversion to 25-hydroxyvitamin D_3 ($25(OH)D_3$). This process is catalyzed mainly by the mitochondrial cytochrome P450-based enzyme CYP27A1, though microsomal cytochrome P450 enzymes may also be involved (5). Circulating 25(OH)D (the term 25(OH)D is used to denote the sum of $25(OH)D_3$ and $25(OH)D_2$, the latter from dictary sources) binds with high affinity to the vitamin D-binding protein (DBP), and is thereby protected from rapid degradation. Because of its long halflife, plasma 25(OH)D is a reliable indicator of an individual's vitamin D status.

While circulating 25(OH)D levels of 20-30 nmol/l will indeed prevent rickets, levels closer to 75-80 nmol/l are needed to support other physiological functions of vitamin D (6), especially those involved in limiting growth of cells and increasing differentiation (see also Figure 1). These non-classical effects of vitamin D will be subsequently described in detail.

Figure 1. *Comparison of renal and colonic $1,25(OH)_2D_3$ synthesis and catabolism: limitation by $25(OH)D_3$ insufficiency and regulation in colonocytes by calcium, estrogens and folate.*

1,25-Dihydroxyvitamin D_3 Synthesis in the Kidney and at Extrarenal Sites

Because 25(OH)D is biologically rather inactive, it has to be converted by C1α-hydroxylation to the hormonally active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). This occurs primarily in proximal tubule cells of the kidney catalyzed by another cytochrome P450 enzyme, CYP27B1. While plasma concentrations of $25(OH)D_3$ are in the nanomolar range, $1,25(OH)_2D_3$ circulates at 1,000fold lower levels, *i.e.* between 75-200 pmol/l. Renal CYP27B1 activity is up-regulated by serum Ca^{2+} and parathyroid hormone (PTH), but down-regulated by end-product inhibition from $1,25(OH)_2D_3$. In a negative feed-back loop, $1,25(OH)_2D_3$ induces its own degradation, namely by up-regulation of another cytochrome P450 enzyme: the *CYP24A1*-encoded 25(OH)D-24-hydroxylase, which inactivates $25(OH)D_3$ as well as $1,25(OH)_2D_3$, the latter with 10-fold higher efficiency.

Although there was increasing epidemiological evidence for a role of $25(OH)D_3$ in tumor prevention, an actual mechanistic link between low serum concentrations of $25(OH)D_3$ and high cancer incidence was not apparent. Not before synthesis and degradation of $1,25(OH)_2D_3$ was demonstrated in a variety of cancer cells, such as those derived from colon, breast and prostate gland (see (7), was it accepted that this steroid hormone has a function not only in systemic mineral ion homeostasis, but also possibly as an important local regulator of a variety of cell functions. Demonstration of $1,25(OH)_2D_3$ synthesis at various extrarenal sites and of its strong dependence on availability of $25(OH)D_3$ explained why cancer incidence is associated primarily with low serum levels of $25(OH)D_3$ and not so much with $1,25(OH)_2D_3$. This assumption was supported by
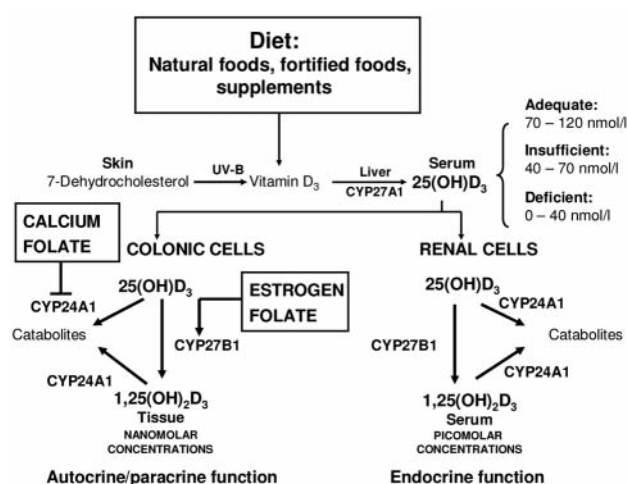
a recent human pilot study (8) which demonstrated for the first time that rectal crypt cell proliferation correlated inversely with serum $25(OH)D_3$ levels. Further experimental proof for the importance of intracellularly produced $1,25(OH)_2D_3$ for regulation of cell functions was provided by a study from Rowling *et al.* (9) who have shown that VDR-mediated actions depended more on megalin-mediated endocytosis of $25(OH)D_3$ than on ambient $1,25(OH)_2D_3$. Also Lechner *et al.* (10) induced the characteristic antimitogenic effect of $1,25(OH)_2D_3$ when human colon carcinoma cells were treated with $25(OH)D_3$, although only if they were CYP27B1 positive.

It should be noted that, although mineral homeostasis is regulated by circulating $1,25(OH)_2D_3$ in picomolar concentrations, growth inhibition of neoplastic cells requires the presence of $1,25(OH)_2D_3$ in tumor tissues at 1,000-fold higher, *i.e.* nanomolar concentrations. These can conceivably be achieved by high local CYP27B1 activity under optimal substrate conditions. Proof of our assumption would require accurate determination of $1,25(OH)_2D_3$ tissue concentrations. Recently, our laboratory has achieved this major endeavor. Parallel to induction of *CYP27B1* mRNA, we measured enhanced levels of $1,25(OH)_2D_3$ in colonic mucosa of mice as described in more detail later (see section Colonic Synthesis and Degradation of $1,25(OH)_2D_3$ Is Regulated by Growth Factors and Hormones; see also the paper by Nittke *et al.* in this volume of Anticancer Research).

CYP24A1, the catabolic vitamin D hydroxylase, is also present in nonrenal cells. It has been suggested that CYP24A1 is undetectable in vitamin D target cells unless induced by $1,25(OH)_2D_3$ through a VDR-mediated mechanism. Recently, we demonstrated (11) that this is apparently not the case in

human colon cancer cells, since during tumor progression, expression of *CYP24A1* mRNA increases whereas that of *CYP27B1* declines (see section on Epigenetic Regulation).

Vitamin D Signalling for Cancer Prevention

Besides its classic role in calcium and phosphate homeostasis, $1,25(OH)_2D_3$ has nonclassic roles which include prodifferentiating and antiproliferative activities as well as a proapoptotic and antiangiogenic effects (for review see (12)). $1,25(OH)_2D_3$ exerts transcriptional activation and repression of target genes by binding to the VDR. Studies in knockout mice deficient in the VDR as well as in key members of the vitamin D metabolic pathway demonstrated transcriptional effects in many target genes (12). While knockout clearly resulted in bone-related diseases, alopecia, infertility, growth retardation *etc.*, none of the *in vivo* models deficient in parts of the vitamin D system developed tumors spontaneously, but incidence was more rapidly enhanced when provided with a carcinogen, or when VDR knockout was coupled to tumor suppressor gene deactivation (13). Only in the colon did VDR knockout in mice lead to hyperproliferation and increasing levels of a marker for DNA damage (14).

VDR target gene expression is modulated through synergistic $1,25(OH)_2D_3$-binding and dimerization with the retinoic X receptor. This induces phosphorylation and conformational change of the VDR followed by the release of co-repressors (nuclear receptor co-repressors, NCoRs; silencing mediator for retinoid and thyroid hormone receptors, SMRT; histone deacetylase, HDAC) and repositioning of the VDR activation function 2 domains. The complex then binds within the promoter region to vitamin D response elements (VDREs). This initiates the recruitment of co-activators as prerequisite for full transcriptional activation (see (15)).

In kidney cells, the induction of the *CYP24A1* gene by $1,25(OH)_2D_3$ is by far the strongest molecular event. In nonrenal cells, besides regulation of vitamin D hydroxylases and other genes described above, vitamin D exerts direct and indirect effects. Importantly, signaling from $1,25(OH)_2D_3$/VDR is transduced into differentiation of human colon carcinoma cells *via* induction of E-cadherin and inactivation of the β-catenin/T-cell transcription factor-4 (TCF-4) complex (16), a regulatory level at which calcium and vitamin D interact on the Wnt pathway. Some mediators of cell cycle disruption such as certain cyclins and cyclin-dependent kinase inhibitors (p21, p27) are directly transcriptionally regulated by $1,25(OH)_2D_3$/VDR. However, many cell cycle-regulating genes that are affected by $1,25(OH)_2D_3$ do not contain VDREs and these are modulated due to cross-talk with other pathways affected by $1,25(OH)_2D_3$: the down-regulation of the epidermal growth factor receptor (EGFR) by $1,25(OH)_2D_3$ is a good example of this.

Colonic Synthesis and Degradation of $1,25(OH)_2D_3$ Is Regulated by Growth Factors and Hormones

Growth factors. There is obviously widespread potential for extrarenal synthesis of $1,25(OH)_2D_3$, since positivity for CYP27B1 was demonstrated by Zehnder *et al*. in many different types of cells (17). We observed that with colon cell hyperproliferation during early malignancy, expression of the synthesizing hydroxylase CYP27B1 is increased as well as that of the VDR. Only during outright human malignancy in high-grade colon tumors is expression again repressed, in contrast to that of CYP24A1 (18, 19). These experimental data caused us to hypothesize that extrarenally synthesized $1,25(OH)_2D_3$ could be a physiological mechanism that normally exists to prevent cancerous growth as long as cells still maintain a certain level of differentiation. We suggested therefore that the $1,25(OH)_2D_3$/VDR system can be activated in colon epithelial cells in response to mitogenic stimulation, *e.g.* by EGF or transforming growth factor α (TGF-α) (20, 21). A strong autocrine/paracrine antimitogenic action of $1,25(OH)_2D_3$ would retard further tumor growth as long as cancer cells retain a certain degree of differentiation and high levels of CYP27B1 activity and of VDR expression.

However, during progression to high-grade malignancy, signaling from the $1,25(OH)_2D_3$/VDR system would be too weak to effectively counteract proliferative effects from, for example, EGFR activation (21), especially since cells from poorly differentiated (grade, G3) colonic neoplasms generally have only low VDR and CYP27B1 expression, whereas there is high positivity for *EGFR* mRNA shown by *in situ* hybridization (21). We confirmed these hypotheses by demonstrating that in differentiated colon cell lines, EGF stimulated expression of VDR and CYP27B1, whereas in a primary culture derived from an only moderately differentiated tumor, expression of VDR and of CYP27B1 was actually reduced by EGF treatment (22).

Regulation by sex hormones. Although men and women suffer from similar rates of colorectal cancer deaths in their lifetime, the age-adjusted risk for colorectal cancer is less for women than for men (23). This strongly indicates a protective role of female sex hormones, particularly of estrogens, against colorectal cancer (see (24, 25)). A meta-analysis of studies showed a 34% reduction in the incidence of this tumor in postmenopausal women receiving hormone replacement therapy (26). A detailed mechanism of action for estrogens in lowering colon cancer risk is not known yet.

While the colon cannot be considered an estrogen-dependent tissue, it must be defined as an estrogen-responsive organ. Expression of estrogen receptor (ER) subtypes α and β have been detected in cancer cell lines. Whereas human colonic mucosa expresses primarily the ER-

β type regardless of gender (27), ER-α is mainly expressed in the breast and the urogenital tract (28). Both receptors bind estrogen, but they activate promoters in different modes. Studies of breast and prostate carcinogenesis suggested opposite roles for ER-α and ER-β in proliferation and differentiation (29), where hormone binding to ER-β exerts a protective action. With respect to colon cancer, the concept of a protective role of ER-β gained support recently: decreasing levels of this receptor were reported during colonic tumorigenesis compared with expression in the adjacent normal mucosa from the same patient (30).

Estrogens may indirectly oppose progression of malignancies by changing VDR expression or vitamin D metabolism in colonic epithelial cells. Liel *et al.* (31) reported that estrogen increased VDR activity in epithelial cells of the rat gastrointestinal tract. In the colon adenocarcinoma-derived cell line Caco-2, which is ER-β positive but negative for ER-α, we demonstrated an increase of *CYP27B1* mRNA expression and also of enzymatic activity after treatment with 17β-estradiol (32). Based on these findings a clinical pilot trial was designed in which postmenopausal women with a past history of rectal adenoma were given 17β-estradiol daily for one month to reach premenopausal serum levels. Rectal biopsies were obtained at the beginning and end of the trial. A predominant result was the elevation of *VDR* mRNA (33). We also observed significant induction of *CYP27B1* mRNA in parallel with a decrease in cyclooxygenase-2 (*COX-2*) mRNA expression in those patients who had particularly high levels of the inflammatory marker at the beginning of the trial (unpublished).

To study further the modification of vitamin D hydroxylase activity by 17β-estradiol, we used a mouse model to measure actual $1,25(OH)_2D_3$ synthesis and accumulation in colonic mucosa. In female compared with male mice, *CYP27B1* mRNA doubled and $1,25(OH)_2D_3$ concentration in the mucosa was increased by more than 50%. This occurred in the proximal colon only and suggests that there may be site-specific action of 17β-estradiol (Nittke *et al.*, see this volume of Anticancer Research). In this respect it is significant, that the estrogen receptor *ESR1* is more methylated (inactivated) (34) in the distal than in the proximal colon of elderly patients undergoing colonoscopy. This would imply that in women, tumors of the proximal colon become clinically manifest at a much later age than in men (see Brozek *et al.* in this volume of Anticancer Research).

Epigenetic Regulation of CYP27B1 and of CYP24A1 Expression

While modification of gene sequences of *CYP27B1* and *CYP24A1* by splicing variation or polymorphisms has been actively investigated by various groups, not much information has been gained by these studies on altered enzymatic activities of hydroxylases. In epigenetic

regulation, it is not a gene sequence that is modified but gene expression, and this could have direct impact on activity as well. For instance, DNA methylation of cytosine residues of CpG islands in the promoter region of genes is associated with transcriptional silencing of gene expression in mammalian cells, while decreased methylation of CpG islands enhances gene activity. The CpG island methylator phenotype (CIMP) is a distinct phenotype in sporadic colorectal cancer. A CIMP-high status is significantly associated with tumors of the proximal colon. Relative survival can also be associated with methylation status (35). In the normal colon, methylation is age and also apparently site related. When evaluating the promoter region of the estrogen receptor (*ESR1*), it was found to be more highly methylated (inactivated) in the human distal than in the proximal colon (34). This suggests that in women, the distal colon is less protected by vitamin D against tumor incidence (see above).

Other genes modified by epigenetic events could be those coding for the vitamin D system. Kim *et al.* (36) demonstrated that the negative response element in the *CYP27B1* promoter is regulated by the ligand-activated vitamin D receptor through recruitment of histone deacetylase, a critical step for chromatin structure remodelling in suppression of the *CYP27B1* gene. In addition, this transrepression by VDR requires DNA methylation in the *CYP27B1* gene promoter. However, this study was carried out on kidney cells and not tumor-derived cells. In a mouse model of chemically induced colon cancer, protection against tumor incidence by estrogen was associated with reduced CpG island methylation of the *VDR* promoter and enhanced VDR expression (37). When we tested colon cancer cell lines derived from moderately differentiated G2 tumors (COGA-1 cells) and from undifferentiated G3 tumors (COGA-13 cells) for expression of vitamin D hydroxylases and compared results with the differentiated colon cancer cell line Caco-2, it became evident that Caco-2 cells had high levels of *CYP27B1* mRNA, while COGA-1 and COGA-13 had low expression or none. In contrast, constitutive *CYP24A1* expression was extremely high in COGA-13, and not apparent in COGA-1 and Caco-2 cells. Addition of the methyltransferase inhibitor 5-aza-2'-deoxycytidine induced *CYP24A1* mRNA expression significantly in Caco-2 and also in COGA-1 cells. In COGA-13 cells however, the methyltransferase inhibitor did not further raise the already high basal *CYP24A1* expression. Interestingly, CYP27B1 appeared to be under epigenetic control as well, since COGA-1 and COGA-13 cells showed a distinct elevation of *CYP27B1* mRNA after treatment with 5-aza-2'-deoxycytidine (unpublished observation).

Differences in expression of vitamin D hydroxylases in the course of tumor progression as observed in colon cancer patients (19, 38) could be caused by epigenetic regulation of

gene activity *via* methylation/demethylation processes, as well as by histone acetylation/deacetylation. In low-grade cancerous lesions, CYP27B1 expression is exceedingly high and accumulation of newly synthesized $1,25(OH)_2D_3$ in the colonic mucosa could be responsible for up-regulation of transcriptional activity of *CYP24A1* (39) as well as for inhibition of tumor cell growth. This enhanced expression of CYP27B1 could be due, at least in part, to epigenetic regulation. In highly malignant tumors, expression of the catabolic vitamin D hydroxylase by far exceeds that of CYP27B1. Our hypothesis therefore is that during cancer progression, CYP27B1 could be inactivated by epigenetic mechanisms, whereas that of CYP24A1 could be activated. Analysis of a selected (small) number of tumor biopsies suggested that in poorly differentiated cancerous lesions, regions of the *CYP24A1* promoter were demethylated and those of *CYP27B1* were methylated (unpublished observation).

Tumor Prevention by Nutrition

While several meta-analyses and prospective studies (see (40)) indicate that vitamin D can be considered a tumor progression-preventive steroid hormone, it is commonly accepted that sporadic colorectal cancer pathogenesis is multifactorial. However, protection by vitamin D might have central importance, with other factors increasing the efficiency of the vitamin D system in a targeted manner. This could result in prevention of hyperproliferation or slowing of progression to clinically manifest primary tumors. The colon as a major digestive organ is influenced by many nutritional substances that may modify also expression of the vitamin D system. Among such substances are calcium, soy and folate.

Calcium. A high percentage of an apparently healthy population is both insufficient for vitamin D and for calcium. Nutritional calcium intake is inversely associated with colorectal cancer incidence. Risk reductions are in the range of 15-40% for the highest *vs*. the lowest intake categories. There is increasing evidence that calcium and vitamin D status act largely together in control of colon epithelial cell proliferation. In the Polyp Prevention Study Group trial, supplementation of calcium with vitamin D during follow-up was inversely associated with adenoma recurrence, and even more so with multiple recurrences (41). An interaction between nutritional calcium and vitamin D in protection against colorectal cancer may be due to the ability of luminal calcium to suppress degradation of $1,25(OH)_2D_3$ synthesized in colonocytes: CYP24A1 expression was doubled in mice fed low dietary calcium and expression of the cyclin-dependent kinase inhibitor p21 was reduced by 50% . This could lead to reduced accumulation of the antimitotic prodifferentiating $1,25(OH)_2D_3$ resulting in hyperproliferation of colonic crypts (42). In addition, bile acids, when not bound and eliminated

by nutritional calcium, could damage mucosal cells and cause aberrant crypt foci. Free bile acids in the gut lumen, for instance lithocholic acid, can bind to the VDR and can thus induce expression of *CYP24A1* (43). This in turn could reduce available levels of $1,25(OH)_2D_3$ even further.

Phytoestrogens. Reduced incidence, especially of hormone-related cancer such as mammary and prostate tumors, but of colon tumors as well, has been linked to the consumption of a typical Asian diet containing soy. Soy and red clover are prominent sources of phytoestrogens that bind preferentially to estrogen receptor (ER)-β. Presence of this receptor is associated with a normal prostate and mammary gland, whereas it is diminished during tumor progression. ER-β is prominently expressed in normal colonic mucosa. When mice are fed a diet containing soy or the isoflavone genistein, expression of the synthesizing vitamin D hydroxylase CYP27B1 is enhanced and that of the catabolic hydroxylase CYP24A1 is reduced (44, 45). This optimization of vitamin D hydroxylase expression is especially prominent when animals were fed a low calcium diet (46). Interestingly expression of COX-2, an inflammatory bowel marker, was elevated by low dietary calcium, but was suppressed by genistein (47)

Folate. As a water-soluble vitamin of the B family, folate is essential for synthesis, repair and methylation of DNA. As a methyl donor, folate could play an important role in epigenetic regulation of gene expression. Methylation/demethylation processes (*i.e.* epigenetic regulation) in promoter sequences of vitamin D hydroxylases may lead to reduced respectively enhanced expression of these enzymes (44). While folic acid was supplemented to foods in the USA in the late 1990s to curb incidence of neural tube defects, and blood folate concentrations increased in the survey period shortly thereafter, there has been a decline since. While the causes for this are unknown, this might have an impact on cancer occurrence (48).

Sporadic carcinomas evolve over a lifetime and could therefore be at least equally affected by low folic acid intake as is neural tube development. Older age and inadequate folate intake lead to altered methylation patterns (49). Evidence is increasing that a low folate status predisposes to development of several common malignancies including colorectal cancer (50). Giovannucci *et al*. (51) and others demonstrated that prolonged intake of folate above currently recommended levels significantly reduced the risk of colorectal cancer.

To investigate the relevance of folate for regulation of the vitamin D system, we used C57/BL6 mice on the semisynthetic AIN76A diet, which contained, among others, 5% fat, 0.025 μg/g vitamin D_3, 5 mg/g calcium and 2 μg/g folic acid (52, 53). When this basal diet was modified to contain high fat, low

calcium, low vitamin D_3 and low folic acid, mice exhibited signs of hyperplasia and hyperproliferation in the colon mucosa (53), which were accompanied by a more than 2.5-fold elevated *CYP24A1* mRNA expression (54). When calcium and vitamin D_3 in the diet was optimized while fat was still high and folic acid low, *CYP24A1* mRNA expression fell by 50%, but was still higher than in the colonic mucosa of mice fed the basal (control) diet. Finally, when the diet contained high fat, low calcium and low vitamin D, but folic acid content was optimized, only then was any increment in colonic CYP24A1 due to dietary manipulations completely abolished (54). This suggests that adequate folic acid consumption could override the detrimental action of vitamin D and calcium insufficiency. Our data on epigenetic regulation of vitamin D hydroxylases *in vitro* in human colon cells also indicate that the up-regulation of catabolic 24-hydroxylase expression could be a main determinant of a lack of colonic $1,25(OH)_2D_3$ during human colon tumor progression (see section Epigenetic Regulation of CYP27B1 and of CYP24A1 Expression).

Conclusion

Evidence is increasing that extrarenal $1,25(OH)_2D_3$ synthesis can be stimulated to prevent tumor progression especially in the colon. Efficient synthesis of the active vitamin D metabolite depends primarily on ample serum levels of the precursor $25(OH)D_3$. However, equally important is the controlled expression and activity of the synthesizing (CYP27B1) and catabolic (CYP24A1) hydroxylases. Adequate calcium intake may protect the colonic mucosa from aggressive bile acids and may also prevent vitamin D catabolism. Interaction of vitamin D and calcium also occurs along the Wnt pathway which, in a broad sense, controls proliferation and differentiation of colonocytes. Women are better protected against colorectal cancer, apparently due to their sex hormones which, on the one hand, elevate VDR expression *via* ER-β, the sole estrogen receptor expressed in the colon, while on the other hand, estradiol increases colonic $1,25(OH)_2D_3$ concentration by stimulating CYP27B1 expression. Phytoestrogens preferentially activate ER-β and thus stimulate CYP27B1 expression. Low rates of colorectal cancer incidence in both genders in soy-consuming populations could be due to appropriate modulation of the anti-inflammatory and anticancer potential of vitamin D by phytoestrogens. During colorectal cancer progression, the initially enhanced vitamin D synthesis as a defense against hyperproliferation is curbed and catabolism becomes prominent. Evidence is accumulating that expression of both CYP27B1 and CYP24A1 is under epigenetic control. Both folate and phytoestrogen, by virtue of their capacity to modulate epigenetic regulation, could raise CYP27B1 expression and reduce the enormous increase in catabolic CYP24A1 during tumor progression.

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