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The endocrine vitamin D system in the gut

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ABSTRACT

The active vitamin D metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) has important regulatory actions in the gut through endocrine and probably also intracrine, autocrine and paracrine mechanisms. By activating the vitamin D receptor (VDR), which is expressed at a high level in the small intestine and colon, $1,25(OH)_2D_3$ regulates numerous genes that control gut physiology and homeostasis. $1,25(OH)_2D_3$ is a major responsible for epithelial barrier function and calcium and phosphate absorption, and the host's defense against pathogens and the inflammatory response by several types of secretory and immune cells. Moreover, recent data suggest that $1,25(OH)_2D_3$ has a regulatory effect on the gut microbiota and stromal fibroblasts. Many studies have linked vitamin D deficiency to inflammatory bowel diseases (ulcerative colitis and Crohn's disease) and to an increased risk of colorectal cancer, and the possible use of VDR agonists to prevent or treat these diseases is receiving increasing interest.

Keywords: Vitamin D; Gut Homeostasis; Inflammatory Bowel Diseases; Gut Microbiota; Colorectal Cancer.

Abbreviations: $1,25(OH)_2D_3$: 1α,25-dihydroxyvitamin D_3; CDX-2: Caudal-related homeobox transcription factor 2; CRC: Colon/colorectal cancer; IBD: Inflammatory bowel diseases; IL: Interleukin; IκB: Inhibitor of κB; LCA: Lithocholic acid; miR: MicroRNA; MLCK: Myosin light chain kinase; NFκB: Nuclear factor κB; TER: Transepithelial resistance; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor-α; VDR: Vitamin D receptor; ZO: *Zonula occludens*

1. Introduction

For a long time, the gut has been known to be a major target tissue of vitamin D. 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the hormonal vitamin D metabolite from the bloodstream (endocrine action) or locally produced from circulating $25(OH)D_3$ within intestinal cells expressing the enzyme $25(OH)D_3$-1α-hydroxylase/cytochrome P_{450} CYP27B1 (intracrine, autocrine and paracrine action), is a critical regulator of gut physiology and homeostasis (Figure 1). $1,25(OH)_2D_3$ is crucial for Ca^{2+} and phosphate absorption and for epithelial integrity/barrier function and it contributes to detoxification and protection against infection (Christakos et al., 2016). Accordingly, the high affinity vitamin D receptor (VDR), a predominantly nuclear protein that regulates gene expression, is expressed in the small and large intestine/colon by epithelial and fibroblastic cells and by B-lymphocytes, T-lymphocytes ($CD4^+$ and $CD8^+$), macrophages and dendritic cells (Veldman et al., 2000). Remarkably, VDR levels are higher in intestinal epithelial cells than in other tissues and cell types (Yamamoto et al., 1999). All intestinal cell types also express CYP27B1, in many cases together with VDR, which supports intracrine, autocrine or paracrine effects of $1,25(OH)_2D_3$ (Adams and Hewison, 2008; Kundu et al., 2014). $1,25(OH)_2D_3$ is one of the main regulators of the human genome controlling the transcription rate of hundreds of genes in a tissue-specific fashion *via* its binding and modulation of VDR activity, as a transcription factor within the cell nucleus and as an inducer of non-genomic signaling in the cytosol (Ordóñez-Morán et al., 2008). Thus, the effects of $1,25(OH)_2D_3$ in the gut are mediated by the proteins encoded by its target genes, a significant proportion of which are specific to this organ.

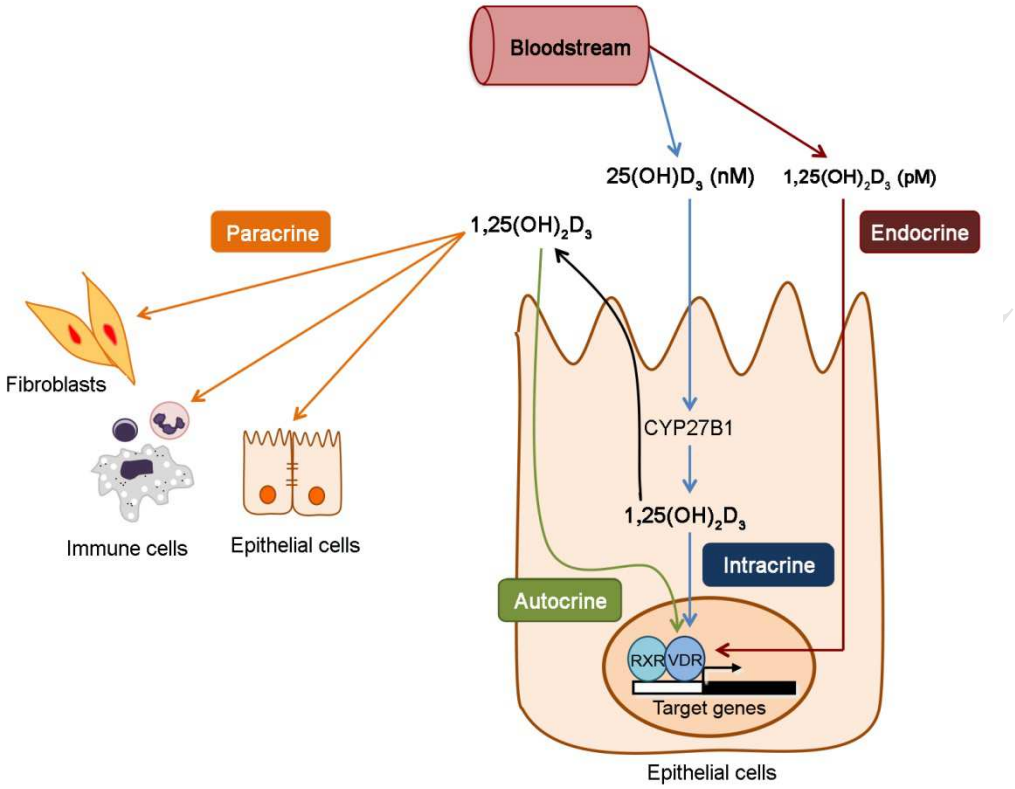

Fig. 1. Diagram of the different types of actions of $1,25(OH)_2D_3$ in the gut. Circulating $25(OH)D_3$ (nanomolar range) and $1,25(OH)_2D_3$ (picomolar range) reach intestinal cells where the latter binds VDR and regulates the transcription of target genes (endocrine action). Intestinal cells express CYP27B1 that converts $25(OH)D_3$ into $1,25(OH)_2D_3$, which can act within the synthesizing cell (intracrine action) or be secreted and act on the same cell (autocrine action) or on neighboring cells (paracrine action) as VDR is expressed in all cell types in the intestine.

The importance of $1,25(OH)_2D_3$ action in the gut is supported by the association of multiple pathologies with vitamin D deficiency, such as loss of Ca^{2+} homeostasis (rickets and osteomalacia), inflammatory bowel diseases (IBD: ulcerative colitis and Crohn's disease) and increased risk of colorectal cancer (CRC) (Figure 2). Mice lacking Vdr (*Vdr*$^{-/-}$) develop hypocalcemia and hypophosphatemia and severe bone

malformation together with other defects, but no gross abnormalities in the gut. However, these *Vdr*$^{-/-}$ mice show high levels of markers of cell proliferation and DNA oxidative damage in the colon (Kallay et al., 2002).

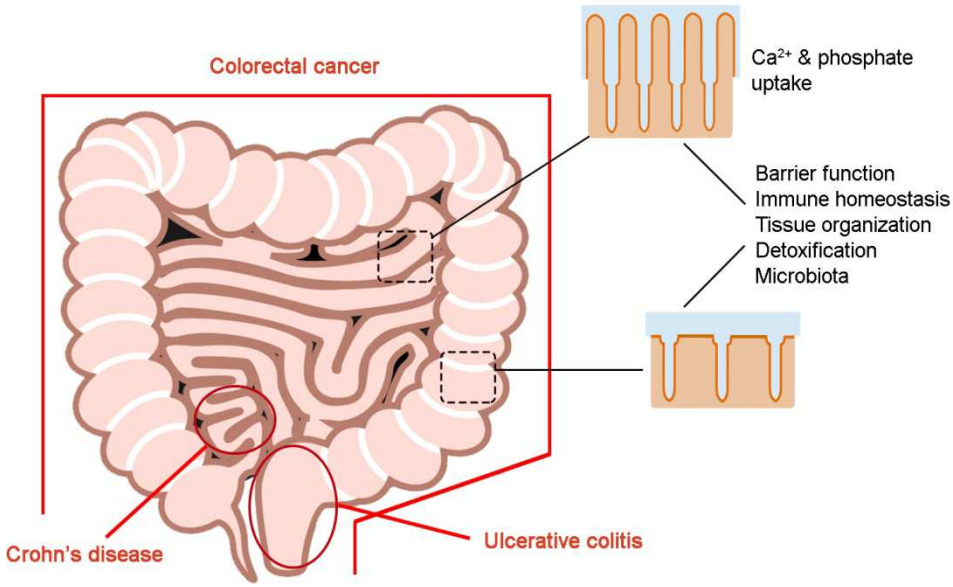

Fig. 2. Diagram depicting the main regulatory actions of 1,25$(OH)_2D_3$ in the small intestine and colon. Some diseases associated with dysfunction of the vitamin D system in the gut are depicted (in red).

2. Ca^{2+} and phosphate absorption

Control of Ca^{2+} homeostasis in the organism is the most classical and probably the principal role of 1,25$(OH)_2D_3$. In conditions of low Ca^{2+}, high level of 1,25$(OH)_2D_3$ is generated from 25$(OH)D_3$, due to induction of CYP27B1 in the kidney by the action of parathyroid hormone. Conversely, high Ca^{2+} inhibits 1,25$(OH)_2D_3$ synthesis, as does a high level of 1,25$(OH)_2D_3$ itself. As mentioned above, gut epithelial and immune cells also express CYP27B1, and thus synthesize 1,25$(OH)_2D_3$ that acts intracrinally, autocrinally and/or paracrinally on neighboring cells. The homeostatic action of 1,25$(OH)_2D_3$ on Ca^{2+} occurs in bone, kidney and, predominantly, in the intestine, where

$1,25(OH)_2D_3$ is essential for Ca^{2+} absorption in basically all segments. Whether and/or to what extent intestinal Ca^{2+} absorption depends on $1,25(OH)_2D_3$ locally produced or of endocrine origin remains unclear. It induces saturable transcellular transport, principally in the proximal small intestine (duodenum, jejunum), and nonsaturable paracellular transport. The mechanism of these two transport systems and of several key genes (*TRPV6*, calbindin D9K, *PMCA1*, alkaline phosphatase I/*ALPI*, claudin-2, claudin-12, cadherin-17 and aquaporin-8, among others) mediating them are detailed in the article by J. Fleet in this issue and in recent reviews (Christakos et al., 2016). Interestingly, a recent study by Lee and colleagues (Lee et al., 2015) has revealed a global network of genes in the mouse proximal intestine that are involved in Ca^{2+} absorption. These include previously known genes and a cohort of novel $1,25(OH)_2D_3$ target genes, some of which may be controlled by this hormone in the intestine independently from Ca^{2+} homeostasis.

$1,25(OH)_2D_3$ also contributes to regulating phosphate homeostasis, again through actions in the bone (increasing FGF23 expression) and the kidney (modulating reabsorption in the proximal tubules), together with the induction of phosphate uptake in the gut. As in the case of Ca^{2+}, a low phosphate diet increases circulating $1,25(OH)_2D_3$, and phosphate uptake combines active transport and passive diffusion. However, the effect of $1,25(OH)_2D_3$ on phosphate absorption is less important than its effect on Ca^{2+} (perhaps because the human diet is usually rich in phosphate and intestinal absorption is mostly paracellular), and much less is understood about the mechanism of action, which in rats involves the apical membrane sodium-phosphate cotransporter NaPi IIb (see also the article by Fleet and colleagues in this issue).

3. Epithelial cell differentiation: the barrier function

A fundamental characteristic of the differentiated intestinal epithelium is the establishment of a barrier to the free exchange of molecules between the internal milieu of the organism and the external medium, the intestinal lumen. This barrier also provides protection against infection by microorganisms. It is constituted by a series of adhesion structures between adjacent epithelial cells, which from apical to basal domains are: tight junctions, *adherens junctions*, desmosomes and gap junctions. Mucosal transepithelial resistance (TER) is an indicator of mucosal permeability and barrier integrity. Disruption of the barrier function takes place during inflammation and bacterial infection causing colitis and facilitating the entry of microorganisms into the intestinal tissue. As discussed in the following sections, altered barrier function is linked to abnormalities in the immune response and thus to IBDs.

$1,25(OH)_2D_3$ upregulates tight junctions proteins (occludin, *zonula occludens* (ZO)-1 and -2, and claudin-2, -7 and -12) and *adherens junctions* proteins (E-cadherin) (Pálmer et al., 2001; Fujita et al., 2008; Ordóñez-Morán et al., 2008; Zhao et al., 2012), and thus contributes to maintaining the differentiated adhesive phenotype of intestinal epithelial cells. Concordantly, $1,25(OH)_2D_3$ increases TER in cultured cells (Pálmer et al., 2001; Kong et al., 2008; Zhao et al., 2012), while a lack of *Vdr* markedly reduces TER in experimental colitis mouse models (Froicu et al., 2003; Kong et al., 2008; Liu et al., 2013).

Tumor necrosis factor (TNF)-α controls intestinal epithelial barrier through the regulation of tight junctions. It increases the permeability of human colon carcinoma cell monolayers at least in part by inducing the long isoform of myosin light chain kinase (MLCK) and myosin II regulatory light chain phosphorylation. $1,25(OH)_2D_3$

protects against TNF-α-induced injury of the intestinal barrier by suppressing the MLCK pathway in cultured HCT116, Caco-2 and SW480 cells (Du et al., 2015). This effect is mediated by the inhibition of nuclear factor *k*B (NFκB) binding to the promoter of the *MLCK* gene and is reproduced by the $1,25(OH)_2D_3$ analogue paricalcitol in a mouse model of colitis (Du et al., 2015). Likewise, $1,25(OH)_2D_3$ restores tight junction integrity upon injury induced by bacterial lipopolysaccharide in Caco-2 cells (Chen et al., 2015a). Additionally, $1,25(OH)_2D_3$ maintains mucosal barrier integrity by blocking intestinal cell apoptosis induced by TNF-α-NFκB-PUMA during inflammation (Liu et al., 2013; Liu et al., 2015).

$1,25(OH)_2D_3$ increases the expression and/or activity of several brush border enzymes that are considered differentiation markers in the small intestine, such as alkaline phosphatase and maltase, and enhances the formation of microvilli (Brehier and Thomasset, 1988; Halline et al., 1994; Chen et al., 1999). The effect on alkaline phosphatase seems to be mediated by the transcription factor activator protein-1 (Chen et al., 1999). In CRC cells $1,25(OH)_2D_3$ increases the expression of a plasma membrane calcium ATPase isoform associated with differentiation (Ribiczey et al., 2015), while the short-chain fatty acid butyrate enhances VDR expression and the prodifferentiation action of $1,25(OH)_2D_3$ evaluated by analyzing the activity of alkaline phosphatase (Gaschott and Stein, 2003). Likewise, 17β-estradiol and certain phytoestrogens increase VDR and CYP27B1 expression while they reduce that of CYP24A1, the enzyme that degrades $1,25(OH)_2D_3$, in cultured CRC cells and in the rodent colon. This may be one of the beneficial effects of estrogens in the gut (Schwartz et al., 2000; Gilad et al., 2006).

The importance of $1,25(OH)_2D_3$ in gut biology is further supported by its relation with the Caudal-related homeobox transcription factor 2 (CDX-2). CDX-2 is a

homeodomain transcription factor that mediates intestinal differentiation and has a key role in intestinal development and in homeostasis in adult life (Beck et al., 1999; Gao et al., 2009). CDX-2 expression is linked to the differentiation (polarization and adhesive phenotype) of human Caco-2 colon adenocarcinoma cells (Pereira et al., 2013). Although it is rarely mutated in CRC, *CDX-2* downregulation is frequent in CRC (and other neoplasias of the digestive tract) and is associated with poor tumor differentiation (Brabletz et al., 2004). Therefore, it is considered a tumor suppressor gene in CRC (Bonhomme et al., 2003). Interestingly, CDX-2 protein binds the human *VDR* gene promoter and induces its transcription in Caco-2 cells (Yamamoto et al., 1999). Moreover, a polymorphism (A>G) in the CDX-2 binding site of the *VDR* promoter (*Cdx2* polymorphism) affects *VDR* transcription and activity, and is associated with the risk of several cancers including CRC (Pulito et al., 2015; Serrano et al., 2016). These data indicate that $1,25(OH)_2D_3$/VDR may mediate part of the crucial effects of CDX-2 on gut biology.

4. Anti-inflammatory and anti-infectious actions. Inflammatory bowel diseases

In line with the expression of VDR in most types of immune cells, $1,25(OH)_2D_3$ regulates both the innate (positively) and the adaptive (negatively in situations of over/autoreactivity) immune response (Adams and Hewison, 2008) (see also the articles by J. White and by A. Verstuyf and C. Mathieu in this issue). In the gut, these actions of $1,25(OH)_2D_3$ translate into important anti-inflammatory and anti-infectious effects in experimental systems, including: synthesis of antimicrobial defensive peptides (cathelicidin/LL-37, β-defensin); regulation of the differentiation, trafficking and secretion of cytokines by immune cells; and preservation of the gut barrier function. Accordingly, a series of studies have proposed that vitamin D deficiency could be

associated with chronic inflammation and autoimmune disorders (Krishnan and Feldman, 2010; Cantorna et al., 2014; Meeker et al., 2014; Cai et al., 2015; van Harten-Gerritsen et al., 2015; Meeker et al., 2016). Epidemiological studies indicate that vitamin D deficiency is associated with, and deleterious to, clinical conditions linked to immune imbalances and chronic inflammation such as ulcerative colitis (most frequently affecting the rectum) and Crohn's disease (generally affecting the terminal ileum) (Yu et al., 2008; Meckel et al., 2016).

However, the causality of this association is a matter of debate (Autier et al., 2014; Mangin et al., 2014) and the protective effects of supplementation with vitamin D, $1,25(OH)_2D_3$ or other VDR agonists on inflammatory or autoinmmune diseases such as IBDs have not been demonstrated unequivocally. Hypovitamin D status has been linked to higher risk of CRC, and particularly to chronic inflammation-associated CRC, which may occur in long-term ulcerative colitis and Crohn's disease patients (Wada et al., 2009). In addition, vitamin D supplementation decreased symptoms in a pilot study in Crohn's patients (Yang et al., 2013), and higher predicted plasma 25(OH)D levels significantly reduced the risk of Crohn's disease (Ananthakrishnan et al., 2012). Moreover, as in other diseases, vitamin D deficiency has a negative effect on the action of other therapies (i.e., anti-TNF-α in ulcerative colitis patients) (Zator et al., 2014).

Deletion of *Vdr* or vitamin D deficiency in mice does not cause IBD but increases inflammation and predisposes to and enhances the severity of the disease. Concordantly, expression of human VDR in intestinal epithelial cells of mice defective for interleukin (IL)-10 attenuates the development of spontaneous colitis in these animals (Golan et al., 2015). Mice lacking *Vdr* display chronic inflammation, with reduced CD4/CD8αα intraepithelial lymphocytes and decreased homing of T cells to the gut (Yu et al., 2008). Interestingly, a recent study has shown that deletion of *Vdr* in

the epithelial intestine downregulates the expression of lysozyme and ATG16L1, which causes defective autophagy, impairs the antimicrobial function of Paneth cells and increases susceptibility to experimental colitis (Wu et al., 2015a).

In a number of systems, the anti-inflammatory action of $1,25(OH)_2D_3$ is based on its multilevel repressing effect on NFκB, a transcription factor that controls many genes involved in the immune response and inflammation. $1,25(OH)_2D_3$ decreases the expression of NFκB components (c-Rel, p50) and increases the expression of the NFκB inhibitor IκBα, which leads to the inhibition of NFκB nuclear translocation and transcriptional activity (Yu et al., 1995; Bao et al., 2006). A recent study has revealed that $1,25(OH)_2D_3$ suppresses NFκB function in HT-29 colon carcinoma cells as well as in other cancer cell types by enhancing the FBW7 E3 ligase-dependent turnover of several NFκB subunits (Fekrmandi et al., 2015) . As a consequence, $1,25(OH)_2D_3$ downregulates the expression of pro-inflammatory cytokines such as TNF-α and several interleukins (IL-1β, IL-6, IL-8 and others), chemokines (CCL2 and others), and cyclooxygenase-2, the enzyme responsible for prostaglandin synthesis. Consistently, this NFκB-induced program is enhanced in mice lacking *Vdr*, which are also more susceptible to bacterially and chemically induced colitis (Liu et al., 2013). In line with these data, a recent study has shown that $1,25(OH)_2D_3$-deficient mice due to the lack of the *Cyp27b1* gene display a colon inflammatory phenotype with shortened colon length, abnormal mucosal structure, and inflammatory cell infiltration accompanied by increased reactive oxidative species and DNA damage, cellular senescence and production of senescence-associated inflammatory cytokines (Liu et al., 2016).

Conversely, hypovitamin D status and low VDR expression may be a consequence of local inflammation or infection, rather than the cause (Mangin et al., 2014). Supporting this view, TNF-α reduces VDR levels by inducing microRNA (miR)-

346 that targets *VDR* mRNA (Chen et al., 2014), and patients with ulcerative colitis and Crohn's disease show reduced levels of VDR in the intestinal epithelium (Li et al., 2013). Conceivably, the resulting situation *in vivo* will depend on the intensity of the two opposite signals: the anti-inflammatory action of $1,25(OH)_2D_3$ and the inflammation-based effects that repress VDR expression and function.

In summary, $1,25(OH)_2D_3$/VDR modulates inflammation and infection, which are interrelated critical factors for intestinal homeostasis whose deregulation is implicated in the pathogenesis of IBDs. A deficient $1,25(OH)_2D_3$/VDR system does not cause IBDs *per se* but available data suggest that it may contribute to their development as an important regulator of the intestinal immune system and the epithelial barrier function.

5. Relation with the intestinal microbiota

Intestinal microbiota is another important factor in IBDs together with genetics, environment, and inflammation (Frank et al., 2007). Damaged intestinal mucosa may facilitate microbial translocation that in turn may alter the local immune system. Moreover, the intestinal microbiota may also affect systemic diseases (including diabetes and rheumatoid arthritis), CRC and the gut-brain axis (Qin et al., 2012; Nakatsu et al., 2015; Giloteaux et al., 2016; Obata and Pachnis, 2016; Sharon et al., 2016)

Mutual, ill-defined interactions exist between vitamin D and the normal and pathological microbiota. Vitamin D not only regulates the immune response to the microbiota but also changes the gut microbiota (dysbiosis); an effect that is mediated by the host as bacteria lack VDR (Ooi et al., 2013; Cantorna et al., 2014). In mice, vitamin D deficiency at birth causes lower numbers of colonic *Bacteroides* and *Prevotella* later

in life (Jahani et al., 2014). Mice lacking *Vdr* have reduced amounts of bacteria from the *Firmicutes* phylum and increased amounts from the *Bacteroides* and the *Proteobacteria* phyla in the feces (Ooi et al., 2013), and changes in several bacterial genus members including *Eubacterium*, *Bacteroides* and *Salmonella* are also observed between $Vdr^{-/-}$ and wild-type mice (Chen et al., 2015b). In addition, Jin *et al*. have reported that a lack of *Vdr* in mice not only causes dysbiosis, with depletion of *Lactobacillus* and enrichment of *Clostridium* and *Bacteroides* in the fecal stool and depletion of *Alistipes* and *Odoribacter* and enrichment of *Eggerthella* in the cecal stool, but also leads to changes in important pathways of the intestinal microbiota that together can potentially affect detoxification, infection, cancer and other diseases (Jin et al., 2015). Curiously, treatment with $1,25(OH)_2D_3$ has been proposed to increase *Citrobacter rodentium* load in the colon and spleen of mice probably through suppression of the Th17 response (Ryz et al., 2012), while a vitamin D-deficient diet aggravated barrier dysfunction, dysbiosis and inflammation following infection with bacteria (Assa et al., 2015), and a lack of *Vdr* augmented IL-22-producing innate lymphoid cells causing dysbiosis and resistance to colonization by *Citrobacter rodentium* (Chen et al., 2015b).

There is a dynamic interplay between bacteria and VDR expression in the gut. On the one hand, $Vdr^{-/-}$ mice express less IκBα and so have increased basal and bacteria-induced NFκB activity and inflammation, together with fewer Paneth cells in the ileum and higher bacterial load in the intestinal mucosa (see Wu et al., 2015a and references therein). Remarkably, immune alterations caused by *Vdr* deficiency in mice lead to IBD-like unregulated inflammation in response to normally non-pathogenic bacteria and increased sensitivity to *Salmonella*-induced colitis (Yu et al., 2008; Wu et al., 2015b). Moreover, probiotic treatment (*Lactobacillus rhamnosus* strain GG and *Lactobacillus plantarum*) increases VDR protein and the expression of target genes in

human and mouse intestinal epithelial cells, and confers protection against *Salmonella*-induced colitis in wild-type mice but not in *Vdr*$^{-/-}$ mice (Wu et al., 2015b). Based on these results, these authors suggest that the variable response to probiotics and clinical outcomes of IBD patients may partly be due to the status of their 1,25$(OH)_2D_3$/VDR system. Interestingly, butyrate, which is a fermentation product of gut microbes that increases intestinal VDR expression, attenuates basal and TNF-α- and lipopolysaccharide-induced NFκB activity in human HT-29 colon carcinoma cells and suppresses inflammation in a colitis model (Schwab et al., 2007). However, several studies have found that some pathogenic bacteria and other microorganisms downregulate Vdr expression (Mangin et al., 2014), while increased expression and relocation of Vdr in the colon by pathogenic bacteria has also been reported (Sun et al., 2008).

Data in humans are very scarce. In a double-blind, placebo-controlled, randomized, parallel-arm, multicenter study, oral supplementation with probiotic *Lactobacillus reuteri* NCIMB 30242 increased circulating 25(OH)D levels relative to placebo (Jones et al., 2013). Notably, in a study in African American males, the level of circulating 25(OH)D was associated with differences in stool microbial composition: a decrease in the relative abundance of *Roseburia*, *Blautia*, *Ruminococcus* and *Dorea* (*Firmicutes* phylum, *Clostridia* class) was found in individuals with higher 25(OH)D concentration (Ciubotaru et al., 2015). Remarkably, a genome-wide association analysis using two cohorts totaling 1,812 individuals has identified the *VDR* gene as a host factor influencing the gut microbiota (Wang et al., 2016). Clearly, much work is needed to define the crosstalk between the vitamin D system and intestinal microbiota. However, available data suggest a complex mutual relation that may contribute to intestinal homeostasis.

6. Intestinal fibroblasts

The effect of $1,25(OH)_2D_3$ on intestinal fibroblasts has only recently begun to be investigated. In a mouse model of chronic colitis, vitamin D inhibits intestinal fibrosis induced by administration of 2,4,6-trinitrobenzene through the reduction of the transforming growth factor (TGF)-β1-dependent stimulation of collagen I synthesis and extracellular matrix deposition by colonic subepithelial myofibroblasts (Tao et al., 2015). In humans, our group has reported that normal colon fibroblasts derived from healthy colonic tissue obtained from biopsies of CRC patients express VDR and respond to $1,25(OH)_2D_3$. Thus, $1,25(OH)_2D_3$ treatment regulates the expression of around one thousand genes in these cells that are involved in a large series of cellular functions: adhesion, migration, wound healing and matrix remodeling, chemokine production and inflammatory response (Ferrer-Mayorga et al., 2016). Moreover, $1,25(OH)_2D_3$ reduces the capacity of these cells to contract collagen gels and to induce the migration of colon carcinoma cells; two hallmarks of protumoral fibroblastic activation (Ferrer-Mayorga et al., 2016). These results indicate a homeostatic effect of $1,25(OH)_2D_3$ at the level of intestinal fibroblasts.

7. Detoxification

Global transcriptomic studies have revealed that $1,25(OH)_2D_3$ regulates the expression of several antioxidant (γ-glutamyl transpeptidase, glutathione peroxidase, Pi-class glutathione S-transferases, epoxide hydrolase), Phase I (CYP1A1, CYP3A1, CYP3A3, CYP4F5, CYP2B2, CYP3A9, 3α- and 17β-hydroxysteroid dehydrogenases) and Phase II (UDP-glucuronosyltransferases and sulfotransferases) enzymes, which are involved in the chemical modification and elimination of toxic compounds in the rat intestinal

lumen (Kutuzova and DeLuca, 2007) and in cultured human colon carcinoma cells (Pálmer et al., 2003; Tachibana et al., 2009).

Lithocholic acid (LCA) is a secondary bile acid that has deleterious effects in colonic cells, where it induces DNA damage and inhibits DNA repair enzymes. Remarkably, LCA binds (with lower affinity than $1,25(OH)_2D_3$) and activates VDR (Makishima et al., 2002). In intestinal cells, this induces the expression of CYP3A4, a drug-metabolizing enzyme; SULT2A1 sulfotransferase; and MRP3, a multi-drug resistance-associated protein (Chatterjee et al., 2005; Jurutka et al., 2005; McCarthy et al., 2005; Fukumori et al., 2007; Matsubara et al., 2008; Fan et al., 2009). As CYP3A4, SULT2A1 and MRP3 are involved in the elimination of LCA, this is a feedback mechanism to eliminate the highly toxic LCA from the colon.

Altogether, these results indicate that $1,25(OH)_2D_3$ is a xenobiotic sensor and contributes to the detoxification process in the intestine by controlling the expression of enzymes involved in the catabolism of xenobiotics, steroids, bile acids and other compounds.

8. Colorectal cancer

In concordance with its homeostatic actions in the healthy intestine, a large number of epidemiological and preclinical studies support a protective role of vitamin D against CRC. Thus, several meta-analyses indicate that vitamin D deficiency is associated with increased CRC incidence, progression, and/or mortality (Giovannucci, 2013; Tagliabue et al., 2015; Jacobs et al., 2016). Studies in animals (xenografted tumors, chemically-induced carcinogenesis, genetic models for CRC) and cultured cells show that $1,25(OH)_2D_3$ and other VDR agonists exert a variety of antitumor effects on colon carcinoma cells, including inhibition of proliferation and invasiveness, increase of

differentiation, sensitization to apoptosis, and inhibition of angiogenesis and metastasis (Pereira et al., 2012; Feldman et al., 2014; Ma et al., 2016) (Figure 3). Thus, high vitamin D diet prevents chemically-induced colonic preneoplastic lesions in mice and increases the expression of differentiation markers (sucrase isomaltase and alkaline phosphatase) in the colon of normal mice (Hummel et al., 2013; Aggarwal et al., 2016). Moreover, two studies have shown that *APC*^{min} mice lacking the *Vdr* gene develop bigger intestinal tumors (Larriba et al., 2011; Zheng et al., 2012).

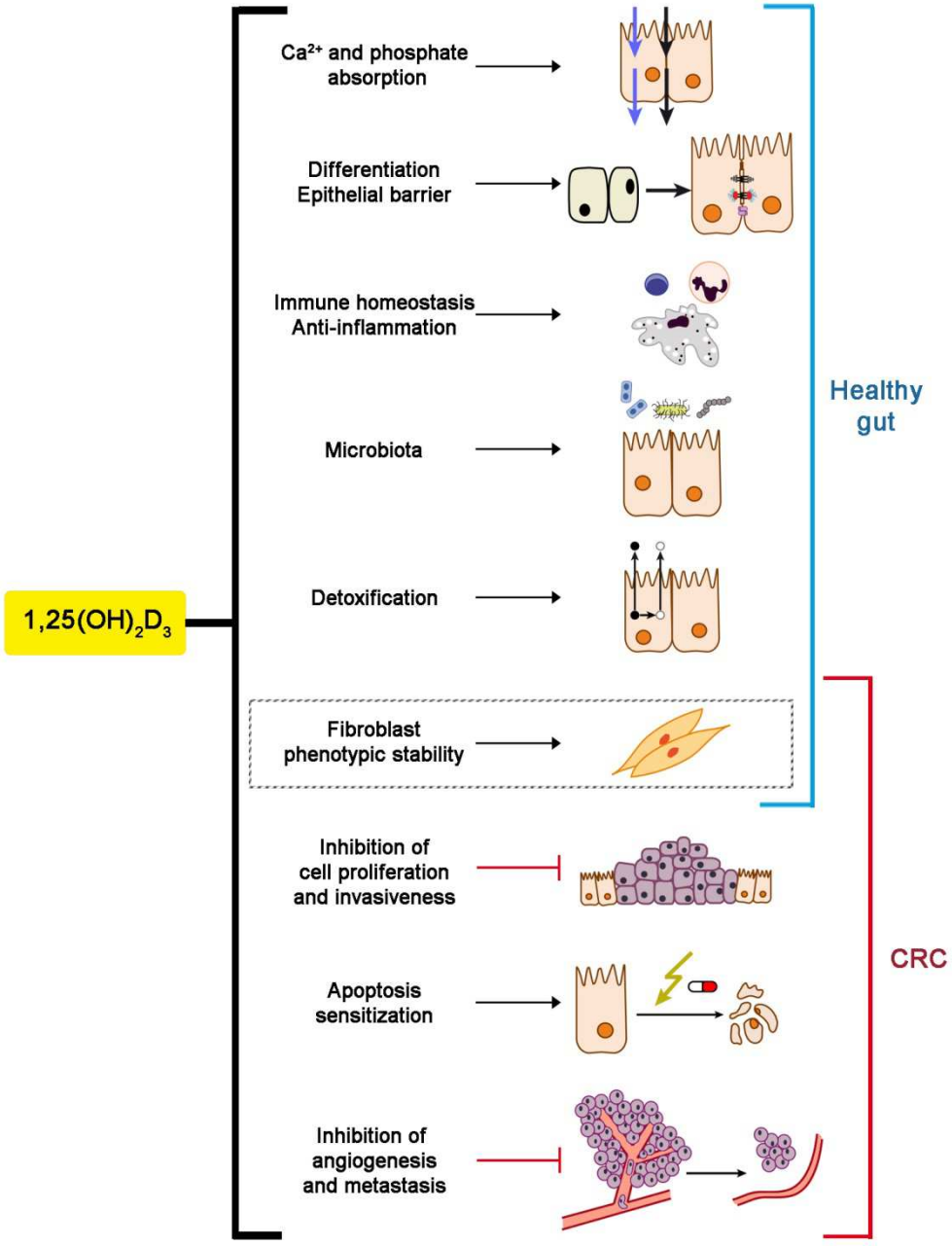

Fig. 3. Mechanisms and cellular processes controlled by $1,25(OH)_2D_3$ in the healthy gut and in colorectal cancer (CRC).

In addition, $1,25(OH)_2D_3$ inhibits the protumoral properties of CRC-associated fibroblasts and, accordingly, high VDR expression in these fibroblasts is associated with a favorable clinical outcome for metastatic CRC patients (Ferrer-Mayorga et al., 2016).

Mechanistically, these effects are based on regulation by $1,25(OH)_2D_3$ of crucial genes controlling the cell cycle *CDKN1A*/$p21^{CIP1}$, *CDKN1B*/$p27^{KIP1}$, c-*MYC*, and others), apoptosis (*BAX*, and others), invasion (metalloproteases, and others), the epithelial-to-mesenchymal transition (*CDH1*/E-cadherin, and others) and angiogenesis (Feldman et al., 2014; Larriba et al., 2016). In addition, $1,25(OH)_2D_3$ interferes with growth factor receptor-activated pathways, and antagonizes the Wnt/β-catenin signaling pathway (whose aberrant activation initiates and maintains CRC) in colon carcinoma and adenoma cells (Pálmer et al., 2001; Gröschel et al., 2016), a relevant finding that takes place at several levels by different mechanisms and has been extensively corroborated (Pereira et al., 2012; Larriba et al., 2013).

Further supporting a protective role of the vitamin D system against CRC, a number of studies have shown tumor-autonomous actions of $1,25(OH)_2D_3$ in this neoplasia (and others) that are variably disrupted at several levels. A series of studies have shown that the expression of VDR and CYP27B1 in colon epithelial cells increases at early steps of tumorigenesis (adenomas and well-differentiated carcinomas) as compared to that in normal cells but it strongly declines in advanced, poorly differentiated tumors (Cross et al., 2001; Bises et al., 2004; Matusiak et al., 2005). SNAIL1 and SNAIL2/SLUG, two transcription factors that are major inducers of epithelial-to-mesenchymal transition and become upregulated in a high proportion of

human CRC repress the *VDR* gene (Pálmer et al., 2004; Larriba et al., 2009). Thus, although rarely mutated, VDR and CYP27B1 are downregulated in a relatively high proportion of advanced colorectal tumors. Conversely, CYP24A1 is upregulated (Bareis et al., 2001; Cross et al., 2005; Höbaus et al., 2013)

Despite all these data suggesting that vitamin D has beneficial effects in CRC, results from interventional studies are unclear (Tagliabue et al., 2015). The few completed trials have limitations that make it difficult to draw conclusions, including low doses, short duration, false placebo groups, and a lack of adequate assessment of individual response. In addition, the optimal form of vitamin D to be administered and the most adequate treatment regimen still need to be defined. Contrarily, it has been proposed that CRC patients may have low serum 25(OH)D levels as a consequence of inadequate function of the vitamin D system due to the neoplastic process (the *reverse causality* hypothesis) (Autier et al., 2014). Against this, the coincident effects in terms of gene expression (increase in *CYP27B1*, *CDKN1A*/$p21^{CIP1}$, *BAX* and *CDH1*/E-cadherin, among others) that vitamin D has on normal colon tissue in a randomized, double-blind, placebo-controlled clinical trial and in preclinical studies strongly suggest that vitamin D has a protective, antitumor role in the human intestine (Bostick, 2015). Until new, well-designed, population-based studies and clinical trials have been carried out, it cannot be determined whether all these data form the basis for prevention strategies that avoid vitamin D deficiency and/or for the treatment of CRC patients with VDR agonists, preferentially in combination with other agents.

9. Conclusions & future perspectives

The gut is a major target organ of vitamin D, as shown by the expression of a high level of VDR in most cell types and the local synthesis of $1,25(OH)_2D_3$. In addition to endocrine action, this is suggestive of intracrine, autocrine and paracrine actions of

$1,25(OH)_2D_3$. Indeed, $1,25(OH)_2D_3$, and thus an appropriate vitamin D status, plays a crucial role in the control of gut homeostasis in experimental systems through an array of regulatory effects including calcium and phosphate absorption, maintenance of the epithelial barrier function, protection against infection, anti-inflammatory action and, probably, modulation of the gut microbiota. These effects result from regulation of the expression of a large number of target genes and signaling pathways and functions that they control in intestinal epithelial cells, fibroblasts and immune cells. In addition and in concordance with these actions, a deficient vitamin D system is associated with many abnormalities and a high risk of CRC, and protective effects of several VDR agonists against this neoplasia have been identified in preclinical settings. Future work is needed to elucidate how much of what we know about the actions of the vitamin D system in the gut can be translated to humans. Importantly, this includes the identification of effects on the differentiation and physiology of the several lineages of human intestinal cells and determination of the possibility of using vitamin D or any VDR agonist in the prevention and/or treatment of highly prevalent and serious diseases such as ulcerative colitis, Crohn's disease or CRC. To this purpose, large and well-designed, randomized trials using appropriate placebo groups and, ideally, proper analysis of individual sensitivity to VDR agonists (target genes, *VDR* polymorphisms) are desirable.

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Highlights

Gut expresses a high level of vitamin D receptor and is a main target of vitamin D

Vitamin D is a major responsible for intestinal Ca^{2+} and phosphate absorption

Vitamin D induces gut epithelial barrier function and defense against pathogens

Vitamin D deficiency is linked to inflammatory bowel diseases

Vitamin D deficiency is linked to an increased risk of colorectal cancer

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