HHS Public Access

Author manuscript

Biochem J. Author manuscript; available in PMC 2015 September 16.

Published in final edited form as:

Biochem J. 2012 January 1; 441(1): 61–76. doi:10.1042/BJ20110744.

Vitamin D and Cancer: A review of molecular mechanisms

James C. Fleet[1,5], **Marsha DeSmet**[4], **Robert Johnson**[2], and **Yan Li**[3]

[1]Department of Nutrition Science, Purdue University, West Lafayette, IN 47907-2059

[2]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907-2059

[3]Interdepartmental Nutrition Program, Purdue University, West Lafayette, IN 47907-2059

[4]Purdue University Interdisciplinary Life Sciences Program, Purdue University, West Lafayette, IN 47907-2059

[5]Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907-2059

Synopsis

The population-based association between low vitamin D status and increased cancer risk can be inconsistent but is now generally accepted. These relationships link low serum 25 hydroxyvitamin D levels to cancer while cell-based studies show that the metabolite 1,25 dihydroxyvitamin D is the biologically active metabolite that works through vitamin D receptor to regulate gene transcription. Here we review the literature relevant to the molecular events that may account for the beneficial impact of vitamin D on cancer prevention or treatment. This data shows that while vitamin D-induced growth arrest and apoptosis of tumor cells or their non-neoplastic progenitors are plausible mechanisms, other chemoprotective mechanisms are also worthy of consideration. These alternative mechanisms include enhancing DNA repair, antioxidant protection, and immunomodulation. In addition, other cell targets such as the stromal cells, endothelial cells, and cells of the immune system may be regulated by 1,25 dihydroxyvitamin D and contribute to vitamin D mediated cancer prevention.

Keywords

1,25 dihydroxyvitamin D; 25 hydroxyvitamin D; proliferation; apoptosis; transcription; vitamin D receptor

Introduction

Over the past decade, researchers have generated data demonstrating that vitamin D and its metabolites have actions that may be useful for the prevention or treatment of various cancers. This is a new role for vitamin D that is distinct from the traditional role it has in the control of calcium and bone metabolism [1].

Address correspondence to: James C. Fleet, Ph.D., Department of Foods and Nutrition, Purdue University, 700 West State St., West Lafayette, IN 47906-2059, fleet@purdue.edu, (O) 1-765-494-0302, (F) 1-765-494-0906.

Disclosures

JCF has acted as a scientific consultant for Johnson & Johnson

The first suggestion that vitamin D might influence cancer came in 1980 when Garland and Garland proposed that the high rate of colon cancer seen in the Northern US compared to the Southern US was due to the UV light-induced production of vitamin D in the skin [2]. Ecological studies have since extended the "sunlight" hypothesis to 18 different types of cancer [3]. Although ecological studies like these are the weakest forms of scientific evidence, several other lines of evidence suggest that vitamin D or its metabolites have a direct inhibitory action on the development and progression of various cancers. Some population-based studies show that low serum 25 hydroxyvitamin D (25OH D) levels are associated with increased risk of cancers of the colon, [4], breast [5], and prostate [6] [7] as well as other cancers [8]. Studies in animals have shown that severe vitamin D deficiency [9–11] or deletion of the vitamin D receptor (VDR) gene [12–14] increase cancer risk. In addition there are a number of studies that show a reduction in cancer (tumor incidence or tumor size) in animals injected with chemical analogs of the vitamin D hormone 1,25 dihydroxyvitamin D ($1,25(OH)_2$ D) [15–20]. While there is a growing consensus that vitamin D and its metabolites are important for the control of various cancers, the mechanistic foundation for this protection is still being determined. In this review we summarize the current thinking on the mechanisms used by vitamin D metabolites to influence the development of cancer.

Brief review of Vitamin D metabolism and the cellular actions of 1,25 dihydroxyvitamin D ($1,25(OH)_2$ D)

Vitamin D_3 can be produced from 7-dehydrocholesterol when skin is exposed to UVB light. However, regardless of whether vitamin D comes from the skin or the diet, vitamin D_3 is transported in the circulation by the Vitamin D Binding Protein (DBP) [21]. Once delivered to the liver, vitamin D is hydroxylated on its side chain to form 25 hydroxyvitamin D (25OH D). This is a stable metabolite whose serum levels are used to assess vitamin D status. The kidney is the primary site where the active form of vitamin D, $1,25(OH)_2$ D, is produced. The circulating levels of $1,25(OH)_2$ D result from 1 alpha hydroxylase (CYP27B1) mediated conversion of 25OH D in the proximal tubule of the kidney [22]. Renal CYP27B1 gene expression is activated by PTH and suppressed by $1,25(OH)_2$ D and the serum levels of these hormones are inversely related to dietary calcium intake [23]. Once produced in the proximal tubule, $1,25(OH)_2$ D is released into the serum and acts as an endocrine hormone on the intestine, bone, and kidney to control calcium metabolism. Although serum $1,25(OH)_2$ D levels do not associate with cancer risk in human studies, this is the metabolite responsible for the anticancer actions of vitamin D at the cellular level. In addition to the 1 alpha hydroxylase activity found in the kidney, low levels of CYP27B1 protein and message have been observed in many tissues, including cells of the skin, lymph nodes, colon, pancreas, adrenal medulla, brain, placenta [24], in primary prostate epithelial cells [25] in the MCF-7 breast cancer cell line [26], and in the colon cancer cell lines HT-29 and Caco- [27]. Extrarenal production of $1,25(OH)_2$ D is proposed to be driven by the serum 25OH D level, thereby accounting for why serum levels of this inactive metabolite could associate with cancer risk. This is consistent with studies that show CYP27B1 operates well below its Km [28], the fact that $1,25(OH)_2$ D production is seen in cultured non-renal cells treated with 25OH D [29;30], and the observation that targeted disruption of the CYP27B1 gene in RAS-transformed keratinocytes blocks the antiproliferative and prodifferentiating effects of

25OH D *in vitro* [31]. However, the weakness of this hypothesis is that no direct evidence currently exists to prove that meaningful local production occurs *in vivo*.

Molecular mechanism of 1,25(OH)$_2$ D signaling—The primary molecular action of 1,25(OH)$_2$ D is to initiate or suppress gene transcription by binding to the vitamin D receptor (VDR), a member of the steroid hormone receptor superfamily of ligand-activated transcription factors [32] (Figure 1A). The VDR can be found in both the cytoplasm and nucleus of vitamin D target cells but in many cells it is predominantly a nuclear protein. Binding of 1,25(OH)$_2$ D to the VDR promotes association of VDR with the retinoid X receptor (RXR) and this interaction is essential for VDR transcriptional activity. Some data demonstrates that heterodimerization is required for migration of the RXR-VDR-ligand complex from the cytoplasm to the nucleus [33–36] where the 1,25(OH)$_2$ D-VDR-RXR complex binds to vitamin D response elements (VDRE) in DNA to initiate gene transcription [32]. However, recent data from studies using chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) to identify VDR binding sites throughout the genome show that RXR can be bound to VDR binding sites in DNA prior to VDR recruitment to those sites [37]. ChIP-seq analysis conducted in osteoblasts [37] and in lymphoblastoid cells [38] also reveal that the great majority of genomic sites occupied by VDR after 1,25(OH)$_2$ D treatment are likely acting as enhancer elements – only 13 to 23% of VDR binding sites are within the classical promoter regions of genes just proximal to the transcription start site (Figure 1B). Regardless, once bound to DNA the VDR-RXR heterodimer recruits protein complexes that alter chromatin structure. For transcriptional activation these proteins form a complex with histone acetyl transferase (HAT) activity (e.g. CBP/p300, SRC1 [39;40]) as well as ATP-dependent remodeling activity (e.g. the BAF57 subunit of SWI/SNF directly interacts with SRC1 and steroid hormone receptors [41]) to release higher-order chromatin structure that limits gene transcription. After chromosomal unwinding, the VDR-RXR dimer recruits the mediator complex to the promoter and utilizes it to recruit and activate the basal transcription unit containing RNA polymerase II [42]. For transcriptional repression the VDR-RXR dimer recruits co-repressors like NCoR1, NCoR2/SMRT, and Alien that then recruit histone deacetylases and DNA methyltransferases that alter histone tails in ways that lead to a more compact chromatin structure [43] (Figure 1A). For those interested in more details regarding the molecular activation of VDR and its role in gene transcription, the subject was recently reviewed by Pike et al. [37;44].

In addition to the transcriptional regulation mediated through the VDR, there is some evidence that 1,25(OH)$_2$ D may work in other novel ways. These mechanisms are less well described but, where relevant, they will be presented in the following sections.

Effects of 1,25(OH)$_2$ D on Cell Proliferation and Apoptosis

Many believe that the target cells for the anti-cancer action of vitamin D are tumor cells and the normal cell types within tissues that transform into tumor cells. In this context the best-studied role of 1,25(OH)$_2$ D is its growth inhibitory effects on proliferating epithelial cells. Colston et al. first showed a dose-dependent decrease in growth rate of melanoma cells treated with 1,25(OH)$_2$ D [45]. The growth inhibitory property of 1,25(OH)$_2$ D has since been reported in tumor-derived cells from other tissues including the colon [46], breast [47]

and prostate [48]. The VDR is essential for 1,25$(OH)_2$ D-mediated growth inhibition. In both mouse epidermal keratinocytes [49] and in SaOS-2 osteosarcoma cells [50] 1,25$(OH)_2$ D-induced growth arrest is lost when VDR is absent. Also, antisense-oligonucleotides that reduce cell VDR levels can block 1,25$(OH)_2$ D -induced growth arrest in ALVA-31 prostate cancer cells [51] and over-expression of VDR enhanced 1,25$(OH)_2$ D induced growth arrest in several prostate cancer cell lines [52;53]. Recently we reported that prostate epithelial cell (PEC)-specific deletion of VDR in the mouse increased PEC proliferation and reduced PEC apoptosis [54]. In contrast, Costa et al. [55] found that although siRNA mediated VDR knockdown was nearly complete in MCF7 cells (90% lower), the growth inhibitory effect of 1,25$(OH)_2$ D was not significantly reduced by anti-VDR siRNA, suggesting there also may be VDR independent mechanisms for this action.

Cell cycle regulators

Many investigators have looked for direct effects of 1,25$(OH)_2$ D on the expression of genes that control cell growth. An example of this is 1,25$(OH)_2$ D-mediated transcriptional regulation of the gene encoding the cyclin-dependent kinase inhibitor p21 in the myelomonocytic cell line U937 [56]. A number of other studies have shown that cyclin-dependent kinase inhibitors like p21 or p27 increase, and that cell cycle regulatory proteins like cyclins decrease, coincident with 1,25$(OH)_2$ D-induced growth arrest [57–60]. In addition, 1,25$(OH)_2$ D-mediated growth arrest of prostate cancer cell lines can be inhibited by antisense RNA or siRNA against p21 [58;61]. In contrast, 1,25$(OH)_2$ D has a minimal effect on p21 mRNA levels in MCF-7 cells even though the cells growth arrest in response to treatment [62]. In addition, 1,25$(OH)_2$ D increased p21 protein but not p21 mRNA in LNCaP cells [63]. This suggests the growth inhibitory effect of 1,25$(OH)_2$ D is not mediated by VDR-mediated transcription activation of the p21 gene promoter in some cell types. However, Thorne et al. [64] recently found that histone modification patterns at three distinct VDRE containing sites in the p21 gene promoter (at −7 kb, −4.5 kb, and −2.1 kb) are important for vitamin D-regulated expression of this gene in immortalized, but non-transformed RWPE1 prostate epithelial cells. In particular, they found that histone signatures associated with vitamin D-mediated gene activation were enriched in G1 and S phase cells, suggesting a more robust role for induction of p21 in the early phases of cell cycle.

FoxO proteins are tumor suppressors that control cell proliferation [65]. The function of several FoxO family members is inhibited by MAPK-mediated phosphorylation. Recently An et al. [66] showed that 1,25$(OH)_2$ D treatment regulates binding of FoxO3a and FoxO4 to DNA regulatory regions by stimulating a direct interaction between VDR, FoxO3a or FoxO4, and the FoxO regulators Sirt1 (a class III histone deacetylase) and protein phosphatase 1. Sirt1 and protein phosphatase 1 promote nuclear retention of FoxO proteins by counteracting MAPK-mediated phosphorylation. Consistent with an essential role for these interactions, 1,25$(OH)_2$ D-mediated growth arrest is blocked in SCC25 cells treated with siRNA against FoxO3a.

Microarray analyses have revealed other potential vitamin D gene targets related to cell cycle control. In primary prostate cancer cells, expression of CDK1 mRNA (encoding a

protein required for cell cycle progression) was suppressed after 24 hr of $1,25(OH)_2$ D treatment [67] and transcripts for cell cycle inhibitor proteins like RBL2 (Rb-like protein p130) and RBBP6 (Rb binding protein 6) were up-regulated by $1,25(OH)_2$D in the MCF-7 and MDA-MB-231 breast cancer cell lines [68]. A number of transcripts encoding cell cycle control proteins appear to be indirectly regulated by $1,25(OH)_2$D. For example, in RWPE1 prostate epithelial cells, cell cycle control genes generally do not change until 24 or 48 after treatment [69]. Consistent with this, cyclin A, cyclin B, and cyclin F mRNA levels were downregulated in SCC25 cells [70] and cyclin F mRNA was reduced in SW480-ADH cells only after 48 hr of treatment with $1,25(OH)_2$ D or the vitamin D analog EB1089.

Insulin-like Growth Factor (IGF) Signaling

$1,25(OH)_2$ D may also indirectly influence the growth rate of cells by interfering with the action of growth factors that stimulate proliferation or by increasing the production of those that promote cell differentiation. In MCF-7 cells, insulin-like growth factor 1 (IGF1)-stimulated cell growth was inhibited by vitamin D analogs and this effect was associated with increased release of IGF binding protein 3 (IGFBP3) into the medium [71]. IGFBP3 is known to limit the pro-proliferative, anti-apoptotic actions of IGF1 and IGF2 by binding to them and limiting their ability to interact with cell surface receptors. $1,25(OH)_2$ D and vitamin D analogs also induce accumulation of IGFBP3 in prostate cancer cells and primary prostate epithelial cells and this subsequently inhibits IGF2-action [72;73]. Upregulation of IGFBP3 mRNA levels by $1,25(OH)_2$ D has also been seen in microarray analysis of LNCaP prostate cancer cells [74] and the immortalized prostate epithelial cell line RWPE1 [69]. A putative VDRE was identified in the IGFBP3 gene promoter and characterized by EMSA and ChIP assays, indicating that the $1,25(OH)_2$ D-induced increase in IGFBP3 levels is likely to be direct [75]. Further evidence that IGFBP3 is a critical mediator of the growth inhibitory properties of $1,25(OH)_2$ D comes from studies showing that antisense oligonucleotides against IGFBP3 abolish $1,25(OH)_2$ D-mediated growth arrest in LNCaP cells [76]. Nickerson and Huynh [77] have also shown that a 14-day treatment with the vitamin D analog EB1089 increased the prostate expression of many IGFBP isoforms, including IGFBP3, and this was associated with reduced prostate size in rats. In addition to an effect on IGFBP3 mRNA levels, microarray analyses of $1,25(OH)_2$ D treated SW480-ADH cells also show upregulation of IGFBP2 and IGFBP6 transcripts but the functional impact of this regulation has not been explored [78].

Transforming Growth Factor Beta (TGFβ) Signaling Pathway

TGFβ2 is essential for the maintenance of tissue homeostasis and is an anti-proliferative factor in normal epithelial cells and at early stages of carcinogenesis [79]. For example, the TGFβ-SMAD4 signaling axis constrains prostate cancer growth and metastatic progression in Pten-null mice [80]. Short term $1,25(OH)_2$ D or vitamin D analog treatment (< 12 h) increased expression of TGFβ2 mRNA in MCF-7 cells, MDA-MB-231 cells [68], MCF10CA1a cells [81], and primary prostate cancer cells [67]. Consistent with this induction, Wu et al. [82] used deletion/mutation analysis in reporter gene assays and EMSA to identify and characterize two VDREs in the TGFβ2 promoter.

In addition to TGFβ2 expression, 1,25(OH)$_2$ D and its analog EB1089 induce expression of TGFβ1 and TGFβ receptors in MCF7 breast cancer cells and immortalized mammary epithelial cells (185A1 cells) through a mechanism that appears to require SMAD3 as a co-activator [83]. Also, negative regulators of TGFβ availability, LTBP1 (latent TGFβ binding protein 1) and LTBP2 were significantly suppressed by 1,25(OH)$_2$ D-treatment in OVCAR3 cells [84] and primary prostate cancer cells, respectively [67]. The early response of these genes to 1,25(OH)$_2$ D suggests that some genes whose protein products control TGFβ signaling may be direct targets of 1,25(OH)$_2$D/VDR.

Another TGFβ superfamily member is growth differentiation factor 15 (GDF15). Forced expression of GDF15 in PC-3 cells decreased cell proliferation, soft agar clone formation, and xenograft tumor growth [85]. GDF15 mRNA level is upregulated by 1,25(OH)$_2$ D in LNCaP cells [74] and GDF15 has been shown to be a direct VDR target gene that is indispensible for 1,25(OH)$_2$ D-mediated growth inhibition [85]. In contrast, the effects of 1,25(OH)$_2$ D on TGFβ family member mRNA levels (e.g. TGFBR1, SMAD6, TGFβ1) are only seen after prolonged treatment in other cell types and this suggests that the effect may be indirect [70;78].

Bone morphogenic proteins (BMP) are another group of growth factors belonging to the TGFβ superfamily that play pivotal roles in regulating tissue morphogenesis; BMP signaling is often dysregulated in cancer (e.g. colon cancer [86]). The mRNA level of several BMP forms are regulated by 1,25(OH)$_2$ D or vitamin D analog treatment in primary prostate cancer cells (BMP6 [67]), MCF10AT1 cells, (BMP2 and BMP6, [81], and squamous cell carcinoma lines (TGFβ1 and BMP2A [70]).

Wnt-β Catenin Signaling

An alternative hypothesis has emerged to explain how vitamin D mediates cell growth arrest - disruption of β-catenin function, the terminal mediator of Wnt signaling. In the cytoplasm, β-catenin is found in association with APC. Activation of Wnt signaling leads to the accumulation of β-catenin and its release from APC. This free β-catenin translocates to the nucleus, binds with the transcription factor TCF4 on DNA, and activates transcription of genes whose protein products control proliferation (e.g. c-myc and cyclin D1) [87]. Mutations in the APC gene that disrupt APC-β-catenin interactions are common in colon cancer [88]. 1,25(OH)$_2$ D can block β-catenin-mediated gene transcription in cultured SW480-ADH [89], Caco-2, and HT-29 colon cancer cells [90] by inducing binding of VDR to β-catenin, an event that subsequently reduces the formation of the TCF4/β-catenin transcriptional complex [89]. Consistent with these cell-based observations, Xu et al. [91] found that thrice weekly injections of 1,25(OH)$_2$ D and 1,25(OH)$_2$ D analogs for 12 weeks reduced polyp number and load in APC^{min} mice and this was associated with reduced expression of β-catenin target genes in small intestine and colon. Shah et al. used a mammalian two-hybrid assay in HEK293 kidney cells to show that the AF-2 domain of VDR interacts with the C-terminus of β-catenin; this interaction may also be enhanced by acetylation of lysines 671/672 on β-catenin [92]. 1,25(OH)$_2$ D-mediated events may also indirectly influence β-catenin function through increased production of E-cadherin, a membrane protein that can bind β-catenin and prevent its nuclear accumulation. However,

$1,25(OH)_2$ D treatment can repress β-catenin-mediated gene transcription even in SKBR-3 cells lacking the E-cadherin gene [92]. Thus, these data demonstrate that E-cadherin up-regulation is not the only mechanism for $1,25(OH)_2$ D-mediated repression of β-catenin signaling.

In addition to its impact on VDR-β-catenin interactions, $1,25(OH)_2$ D can also influence expression of known regulators of Wnt-signaling: e.g. inhibition of the Wnt activator dickkopf-4 (DKK-4) [93] and upregulation of the Wnt antagonist dickkopf-1 (DKK-1) [94]. Evidence for crosstalk between vitamin D and the Wnt signaling pathway has also been observed *in vivo*. In $Apc^{Min/+}$ mice $1,25(OH)_2$ D injections decreased nuclear β-catenin, TCF1, CD44, and c-Myc levels in tumor-free colonic and small intestine tissue [91].

Collectively these data suggest that vitamin D may decrease colon tumor cell proliferation by interfering with the Wnt signaling pathway. Still, many gaps exist in our understanding of this VDR-mediated growth arrest mechanism. For example, it is not clear whether $1,25(OH)_2$ D can interfere with β-catenin function through VDR in cells other than colonocytes and mammary gland cells lines [95]. In addition, it is not clear if all β-catenin-DNA binding sites are equally inhibited by VDR. Recent ChIP-chip studies show that at some TCF4/β-catenin binding sites, CDX2 is required for TCF4 binding and activation of gene transcription [96]. This shows that there are subgroups of β-catenin binding sites in DNA but it is not clear whether binding of β-catenin to these different types of sites are equally affected by $1,25(OH)_2$ D and VDR.

Apoptosis

Several groups have reported that $1,25(OH)_2$ D influences apoptosis in MCF-7 breast cancer cells [97] and a variety of colon cancer cell lines [98]. Pan et al.[99] found that $1,25(OH)_2$ D treatment promoted apoptosis in the undifferentiated gastric cancer cell line HGC-27 through a mechanism that depends upon VDR-mediated PTEN upregulation. PTEN is a tumor suppressor gene that negatively regulates the anti-apoptotic Akt signaling pathway. Also, in the colorectal cancer cell line MIP101, $1,25(OH)_2$ D treatment increased basal and chemotherapy-induced apoptosis by a mechanism that was sensitive to SPARC (secreted protein acidic and rich in cysteine)-induced VDR synthesis [100], suggesting the regulation may be transcriptionally mediated. Blutt et al observed that 6 days of $1,25(OH)_2$ D treatment induced apoptosis in LNCaP cells and this was accompanied by downregulation of the anti-apoptotic proteins Bcl-2 and Bcl-XL[101].

Consistent with an effect of $1,25(OH)_2$ D on the regulation of pro- and anti-apoptotic proteins, a number of studies have found that transcripts for genes encoding proteins that control apoptosis are regulated by $1,25(OH)_2$ D treatment. GoS2 is a pro-apoptotic protein whose expression is frequently suppressed in cancer [102]. Expression of GoS2 mRNA was induced after $1,25(OH)_2$ D treatment in SW480-ADH colon cancer cells [78] and by the $1,25(OH)_2$ D analog EB1089 in SCC25 squamous carcinoma cells [70]. In the chronic myeloid leukemia cell line K562 $1,25(OH)_2$ D-induced growth arrest and apoptosis is accompanied by lower expression of Bcl2 and Bcl-XL mRNA (anti-apoptotic) and increased Bax mRNA levels (pro-apoptotic)[103] while in SW480-ADH cells, $1,25(OH)_2$ D increased mRNA levels for the pro-apoptotic proteins Baxα, Baxγ and Baxδ [78]. Transcript profiling

studies show that the mRNA levels for the pro-apoptotic proteins DAP-3 (Death associated protein 3), CFKAR (CASP8 and FADD-like apoptosis regulator), and a number of caspases (e.g. Caspase 3, 4, 6 and 8) were induced in $1,25(OH)_2$ D treated MCF-7 and/or MDA-MB-231 cells [68], while in MCF10AT1 and MCF10CA1a cells, the vitamin D analog Ro3582 induced PDCD4 (programmed cell death 4) mRNA levels [81]. While these transcript level changes suggest that $1,25(OH)_2$ D induces apoptosis by transcriptionally activating or repressing various genes, such direct regulation has not yet been reported for most of these genes.

Novel Molecular Events Regulated by $1,25(OH)_2$ D that May Contribute to its Anti-Cancer Activity

In the past decade $1,25(OH)_2$ D has been shown to regulate a much wider array of cellular events than previously thought possible. Below we will discuss several of these processes and relate them to the prevention of cancer.

Autophagy

Autophagy is a process used by cells to degrade cytosolic macromolecules and organelles in lysosomes. While autophagy is generally considered a survival tactic to protect cells during stress (e.g. starvation, pro-oxidant conditions), this process can also be used to trigger the death of cancer cells and to block tumor growth [104]. The first suggestion that $1,25(OH)_2$ D may induce autophagy was indirect. Mathiasen et al. [105] found that $1,25(OH)_2$ D induced cell growth arrest and cell death by a caspase and p53 independent pathway that could be inhibited by the anti-apoptotic protein Bcl-2. Hoyer-Hansen [106] later found that the vitamin D analog EB1089 induced autophagy in MCF-7S1 cells that could be enhanced by the Atg protein beclin-1. In HL-60 leukemia cells $1,25(OH)_2$ D treatment suppresses anti-autophagic mTOR protein and activity levels as well as increases the level of the pro-autophagic protein beclin-1. This treatment also increases the interactions between beclin-1 and PI3K (a pro-autophagic event) or the anti-apoptotic protein Bcl-XL (leading to reduced apoptosis). Vitamin D-induced autophagy may require a complex interplay with cyclin-dependent kinase inhibitors [107]; in p19-deficient SCC25 cells $1,25(OH)_2$ D can induce autophagy that can be suppressed by suppression of p27. Despite these promising mechanistic relationships, there is currently no *in vivo* research to directly connect activation of autophagy to the anti-cancer actions of vitamin D compounds. However, the logic of the argument in favor of vitamin D-induced autophagy as a mechanism for cancer treatment or prevention was recently discussed in an opinion piece by Hoyer-Hansen et al. [108].

Antioxidant Defense and DNA Repair

Oxidative stress-induced damage of DNA and loss of DNA repair mechanisms contribute to carcinogenesis [109] but these effects can be prevented by induction of antioxidant defense mechanisms that reduce the biological impact of reactive oxygen species. Oxidative DNA damage (measured by the level of 8 hydroxy-2'deoxyguanosine) is elevated in the distal colonic epithelium of VDR knockout mice [110] and is reduced in the colon epithelium of humans receiving a daily supplement of 800 IU vitamin D_3 [111]. $1,25(OH)_2$ D has been shown to induce the expression of several enzymes involved in the antioxidant defense

system. In primary prostate cancer cells, SW480-ADH, MCF-7, MDA-MB-231, and MCF10AT1 cells, $1,25(OH)_2$ D or vitamin D analogs induce the expression of TXNRD1 (thioredoxin reductase 1) a protein that keeps thioredoxin in the reduced state needed for its role as an antioxidant [67;68;78;81]. In addition, mRNA levels for the essential antioxidant proteins SOD1 and SOD2 (superoxide dismutase) are induced by $1,25(OH)_2$ D in primary prostate epithelial cells [67] and LNCaP cells [85], respectively. $1,25(OH)_2$ D-induced SOD1 activity has also been seen in the liver of diethylnitrosamine-treated rats and is associated with reduced DNA damage (assessed by comet assay) [112]. $1,25(OH)_2$ D induced G6PD (glucose-6-phosphate dehydrogenase) after treatment in ovarian cancer cells [84], in RWPE1 cells [69], and in cells from benign prostatic hypertrophy, but not in malignant prostate cancer cells (DU 145, CWR22R) [113]. G6PD is an enzyme involved in maintaining reduced glutathione levels in cells. Consistent with a critical role for G6PD in vitamin D-mediated antioxidant protection, Bao et al. [113] showed that G6PD expression is controlled by $1,25(OH)_2$ D in prostate epithelial cells through a VDRE located in the first intron of the gene, that $1,25(OH)_2$ D protected RWPE1 cells against H_2O_2-induced apoptosis, and this protection was lost in the presence of a non-competitive G6PD inhibitor. It is also possible that vitamin D-mediated protection from pro-oxidant stress is indirect due to the induction of nuclear factor (erythroid-derived 2)-like 2 (NFE2L2), a transcription factor that controls expression of genes for many antioxidant enzyme systems [114]. NFE2L2 expression is down-regulated in prostate cancer and suppression of NFE2L2 promotes prostate tumor development in TRAMP mice [115]. Consistent with a role for NFE2L2 in vitamin D-mediated cancer prevention, a number of NFE2L2 target genes were increased in RWPE1 cells after $1,25(OH)_2$ D treatment, e.g. GPX3, HMOX1, AKR1C2, and TXNRD1 [69]. Finally, GPX1 (glutathione peroxidase) was induced by $1,25(OH)_2$ D in SW480-ADH cells [78] and by EB1089 in SCC25 cells [70].

There is some evidence that $1,25(OH)_2$ D regulates genes for proteins that protect the genome. Akutsu et al [116] found that the $1,25(OH)_2$ D analog EB 1089 up-regulated Growth Arrest and DNA-Damage-inducible alpha (GADD45α) mRNA and protein levels in SSC cells. GADD45α is a p53 target gene whose product is involved in DNA repair. It was later shown that the GADD45 gene contains an exonic enhancer element that binds VDR after $1,25(OH)_2$ D treatment leading to increased GADD45 mRNA levels in ovarian cancer cells [117]. $1,25(OH)_2$ D-mediated G2/M arrest in ovarian cancer cells is lost upon deletion of GADD45, suggesting the critical importance of GADD45 induction in vitamin D effects [117]. Other $1,25(OH)_2$ D regulated transcripts whose protein products may contribute to DNA-repair and pro-apoptotic effects of vitamin D have been revealed by microarray analyses. In MCF-7 cells, $1,25(OH)_2$ D induced the expression of mRNAs for p53, RAD23B (RAD23 homolog B), PCNA [68] and DAP-1α (45) [78]. Taken together, it is possible that $1,25(OH)_2$ D directly regulates the expression of a variety of genes whose protein products are involved in DNA damage repair and programmed cell death, thereby offering protection against carcinogenesis.

Prostaglandin Metabolism and Action

A variety of studies have shown that prostaglandin signaling stimulates cancer cell growth and cancer progression [118–121]. In this context, cyclooxygenases 1 and 2 (COX1 and

COX2) are the rate limiting enzymes in prostaglandin synthesis. In particular, COX-2 expression is induced by a variety of mitogens, cytokines, and tumor promoters [122] making this pathway a drug target for cancer treatment, e.g. drugs specifically targeting COX-2 have been shown to lower the risk of prostate cancer [120]. $1,25(OH)_2$ D has been proposed as a negative regulator of prostaglandin synthesis and signaling and this is supported by research on the advanced prostate cancer cell lines, LNCaP and PC-3 [123]. In these cells, $1,25(OH)_2$ D suppressed COX2 expression, reduced expression of the prostaglandin receptors EP2 and FP, and induced expression of 15-PGDH (hydroxyprostaglandin dehydrogenase 15-NAD), the enzyme that inactivates prostaglandins [74;123]. More importantly, $1,25(OH)_2$ D reduced prostaglandin E2 levels, blocked prostaglandin-mediated induction of c-fos mRNA, and reduced the growth stimulatory effects of prostaglandins and prostaglandin precursors in LNCaP cells [124]. These results suggest the direct regulation of the prostaglandin metabolism and signaling by $1,25(OH)_2$ D and the subsequent attenuation of their signaling activities. In contrast to the findings from advanced prostate cancer cells, COX2 mRNA was induced by $1,25(OH)_2$D in the immortalized but non-tumorigenic RWPE1 cell line and neither 15-PGDH nor prostaglandin receptor mRNA levels were altered [69]. Consistent with this observation, Moreno et al. [124] found that COX2 mRNA was suppressed and 15-PGDH mRNA was increased in some, but not all, primary prostate epithelial cell lines.

Are Adult or Cancer Stem Cells Targets of Vitamin D Action?—In the past decade adult stem cells have been discussed as potential target cells for accumulating mutations that contribute to carcinogenesis and therefore as cells that would benefit from exposure to cancer prevention agents [125]. Adult stem cells in tissues serve the primary function of replacing cells lost during the normal lifespan of an organ or following tissue injury. The hallmarks of the adult stem cell are its ability for self-renewal and multipotency. Unfortunately, this capacity for self-renewal means that adult stem cells can accumulate first-hit mutations that, while not harmful themselves, could be combined with subsequent mutations to cause cancer. Trosko argued that rather than the induction of “immortalization” of a normal differentiated cell, we should view carcinogenesis as a process that begins by blocking “mortalization” (or the ability to growth arrest and differentiate) of an adult stem cell [126].

In addition to adult stem cells, a small subset of cells within tumors has the ability to recapitulate the morphologic diversity of a neoplasm when isolated from the parent neoplasm and xenotransplanted into nude mice. These cells have been called “cancer stem cells” and are thought to be the cells from which primary cancers arise or which survive cytotoxic treatments and cause tumor recurrence. Cancer stem cells are also attractive targets for cancer prevention and treatment [127].

The question to be asked in the context of vitamin D signaling and carcinogenesis is “Are adult stem cells or cancer stem cells targets for $1,25(OH)_2$ D action?” As discussed above, $1,25(OH)_2$ D regulates a number of important biological processes. These effects would be beneficial for protecting an adult stem cell (e.g. DNA repair and protection from oxidative cellular injury) or for limiting the expansion of cancer stem cells (e.g. cell cycle arrest, apoptosis). To date, the vast majority of the experimental data exploring the molecular

function of $1,25(OH)_2$ D has been collected from *in vitro* cell culture systems using cancer cell lines or primary cell cultures. Only recently have researchers begun to directly evaluate the impact of $1,25(OH)_2$ D on stem cells. For example, as part of a team headed by Dr. Scott Cramer (Wake Forest University) we have shown that the growth of mouse prostate stem cells [128] is suppressed by $1,25(OH)_2$ D through a VDR-dependent induction of IL-1α production [129]. In the mouse colon, VDR message is predominantly found in the differentiated, luminal colonocytes [130]. However, Fedirko *et al*. recently found that hTERT labeling in the upper part of the colon crypt was suppressed in people given vitamin D_3 and calcium supplements [131]. hTERT is the catalytic subunit of telomerase that marks a slowly cycling population of intestinal stem cells [132], thus vitamin D may suppress the expansion of this cell population and protect them from potential cancer-causing gene mutations. Finally, $1,25(OH)_2$ D and its analogues have been show to regulate the expression of a putative cancer stem cell marker (CD44) in MCF10DCIS.com human breast cancer cells *in vitro* and when they are implanted into immunodeficient mice [133]. Research on the impact of vitamin D on adult and cancer stem cells is certain to expand in the future.

Are Non-epithelial Cells the Targets of Vitamin D-Mediated Cancer Prevention?

Although most cell-based vitamin D research has focused on the impact of $1,25(OH)_2$ D on either tumor cells or their non-neoplastic progenitors, there is also evidence that other cells that exist in the tissue or tumor microenvironment are targets of vitamin D action. For example, in prostate the communication between epithelial cells and the stromal cells surrounding them is critical for the progression of cancer in that organ [134]. Lou et al. [135] reported that $1,25(OH)_2$ D can suppress the growth of prostate stromal cell lines but it is not yet clear if it can alter stromal-epithelial cell communication. In the next section we will discuss two additional examples of non-epithelial cell targets of vitamin D action – vascular cells and immune cells.

Inhibition of Angiogenesis

Angiogenesis is essential for the expansion of tumor growth and for tumor cell metastasis. It is a multistep, multicellular process that depends upon a variety of pro-angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet derived growth factor BB homodimer (PDGF BB). $1,25(OH)_2$ D may inhibit the development of the tumor vasculature that is required for the progression of solid tumors and this may occur due to effects on either endothelial or epithelial cells [136–140]. $1,25(OH)_2$ D can directly inhibit the proliferation of aortic and tumor-derived endothelial cells and can stop endothelial cell sprouting and elongation induced by VEGF [136;141]. Consistent with a direct role of vitamin D signaling in endothelial cells, Chung et al. [138] found that prostate tumors from TRAMP mice grew larger, had a greater vascular volume, and had larger vessels when implanted into VDR knockout mice. Effects of vitamin D on epithelial cells may also influence angiogenesis. One proposed mechanism for the anti-angiogenic effects of $1,25(OH)_2$ D is that it suppresses expression of VEGF family members, the major pro-angiogenic cytokines. $1,25(OH)_2$ D reduces expression of VEGF in

normal prostate epithelial cells [142], reduces VEGF mRNA and increases mRNA levels for the anti-angiogenic protein thrombospondin-1 in SW480-ADH colon cancer cells [140], reduces hypoxia-induced VEGF expression in a variety of cancer cell lines [143], and suppresses mRNA levels for VEGFC and the VEGF receptors KDR and NRP1 in RWPE1, a prostate epithelial cell line [69]. The impact of $1,25(OH)_2$ D is partially due to reduced levels and activity of the transcription factor hypoxia inducible factor-1 (HIF-1); the ability of $1,25(OH)_2$ D to suppress VEGF expression is lost in HCT116 colon cancer cells lacking HIF-1α [143]. VEGF signaling can be suppressed by competitive binding of semaphorins to the VEGF receptor NRP1. In RWPE1 cells, $1,25(OH)_2$ D induced expression of several semaphorin isoforms including SEMA3B, 3F, and 6D and VDR binding to the SEMA3B gene promoter was enriched after $1,25(OH)_2$ D treatment [69]. In prostate cancer cells $1,25(OH)_2$ D can also reduce the mRNA level of the pro-angiogenic cytokine IL-8 by reducing translocation of the p65 subunit of NFkB into the nucleus, thereby limiting NFkB −mediated IL-8 gene transcription [139]. However, the impact of $1,25(OH)_2$ D on VEGF gene regulation has not been entirely consistent. In mouse embryo fibroblasts and human vascular smooth muscle cells $1,25(OH)_2$ D induces VEGFA expression through a VDRE in its promoter [144;145].

Regulation of Immune Cell Function by $1,25(OH)_2$ D

The first line of protection against pathogens and environmental insults is the barrier epithelium of the lung, skin, and intestine. There is evidence that $1,25(OH)_2$ D signaling through the VDR helps maintain intestinal barrier function. In VDR knockout mice, but not wild-type mice, dextran sulfate sodium (DSS) treatment causes the redistribution of the tight junction proteins occludin and zona occludin-1 away from the membrane and severely disrupts tight junctions in the colon [146]. This is consistent with earlier work showing that prolonged treatment of SW480-ADH colon cancer cells with $1,25(OH)_2$ D (100 nM, 2–7 days) induced expression of tight junction and adhesion proteins (E-cadherin, occludin, ZO-1, vinculin) and promoted translocation of ZO-1 to the plasma membrane [89]. In vitamin D deficient rats, microarray analysis revealed that $1,25(OH)_2$ D treatment increased the mRNA level of junction proteins like claudin 3, claudin 17, and RhoA [147]. Ordonez-Moran et al. [148] found that $1,25(OH)_2$ D treatment increased cytosolic calcium leading to the activation of RhoA in SW480-ADH cells. By using dominant negative RhoA, they were subsequently able to block $1,25(OH)_2$ D-induced localization of occludin to the plasma membrane. Collectively, these data support a mechanism whereby vitamin D signaling regulates the level and localization of tight junction proteins leading to a more secure intestinal barrier. It is not clear whether similar protections are present in other barrier tissues like skin and lung nor is it clear what level of vitamin D status is necessary to protect this function.

There are three distinct roles for the immune system in cancer prevention [149]. First, it protects the host against viral infection and suppresses virus-induced tumors. Second, it suppresses inflammation that facilitates tumorigenesis by effectively eliminating pathogens and limiting the period of inflammation that can promote carcinogenesis [150;151]. Finally, it performs immunosurveillance that eliminates nascent tumor cells in tissues by activating receptors on innate immune cells and lymphocytes of the adaptive immune system. As a

result of these functions, the cells of the immune system may be important targets for limiting cancer. However, in the context of cancer, the role of the immune system is not straightforward. Interactions can occur between cancer cells and host immune cells in the tumor microenvironment to create an immunosuppressive network that promotes tumor growth and protects the tumor from immune attack [152]. Thus, agents that can suppress inflammation and immune responses prior to tumor formation may be harmful in established tumors where tumor-associated immunosuppression already exists.

A variety of studies have shown that $1,25(OH)_2$ D regulates cells of both the innate and adaptive immune system [153]. However, few of these studies have been conducted in the context of cancer. As a result, it remains to be shown that the effects of vitamin D on immunity are effective in the prevention of cancer or in the tumor environment. However, non-cancer examples give us insight into the impact that vitamin D could have on inflammation and immunity in carcinogenesis.

The innate immune system interacts with vitamin D in several interesting ways. First, a primary role of macrophages is to engulf and kill bacteria. Invading microbes can induce inflammation when barrier tissues become leaky, e.g. during the intestinal condition Crohn's disease or experimental colitis induced by DSS [154]. $1,25(OH)_2$ D can regulate a number of genes in cells of the innate immune system whose protein products are crucial for autophagy and anti-microbial actions. The human cathelicidin and beta defensin genes are activated by $1,25(OH)_2$ D treatment through vitamin D response elements (VDRE) in their promoters but these VDREs are not conserved in mice [155;156]. Beta defensin can also be up-regulated by an NFkB-mediated transcriptional event after NFkB has been activated through the pattern recognition receptor NOD2 (nucleotide-binding oligomerization domain 2). $1,25(OH)_2$ D can stimulate expression of the NOD2 gene in primary human monocytic cells through a VDRE in a distal enhancer [157]. In addition, the mRNA levels for the antimicrobial protein angiogenin-4 are significantly reduced in the colon of vitamin D deficient mice and this may increase the severity of DSS-induced colitis [158].

Several cytokines can modulate vitamin D metabolism in macrophages, monocytes, and dendritic cells. Proinflammatory cytokines like IFNγ [159] and TNFα [160] stimulate production of $1,25(OH)_2$ D by increasing CYP27B1 expression in monocytes. Inflammatory cytokines and toll like receptor agonists also increase CYP27B1 and VDR expression in dendritic cells [161]. In contrast, IL-4 produced by Th2 cells activates CYP24 expression in monocytes leading to formation of the inactive metabolite 24, 25 dihydroxyvitamin D [159]. These regulatory events can influence local vitamin D metabolite levels and these vitamin D metabolites may then modulate the function other cells in the microenvironment. For example, *in vitro* $1,25(OH)_2$ D reprograms dendritic cells to become tolerogenic [161].

In the adaptive immune system, many T-lympocyte subpopulations express the VDR and are vitamin D target cells [162]. However, mice lacking VDR have no gross abnormalities in either the number or type of T cells present [163] nor is the function of their mature T-cells strongly influenced by VDR deletion [164;165]. This suggests that VDR does not have a primary function for normal T-cell function but it does not preclude the possibility that $1,25(OH)_2$ D may be a modulator of T-cell mediated immune responses. Consistent with the

"modulator" hypothesis, 1,25(OH)$_2$D promotes T-cell profiles reflective of immunotolerence and immunosuppression. *In vivo*, VDR deficient non-obese diabetic (NOD) mice develop a more aggressive form of autoimmune prostatitis with more severe lesions, a greater lymphoproliferative response against prostate antigen, higher levels of INFγ secretion, and higher mononuclear cell infiltration into the prostate gland [166]. Similarly, VDR is necessary in CD4+ cells for 1,25(OH)$_2$ D to prevent autoimmune T-cell responses that cause experimental autoimmune encephalomyelitis in mice [167]. *In vitro*, 1,25(OH)$_2$ D can suppress NFkB signaling necessary for T helper cell activation [168], increase the activity of regulatory T cells necessary for immunosuppression [169], and block development of Th17 and Th9 cells implicated in the pathogenesis of different types of autoimmunity and inflammatory diseases [170]. The effect of 1,25(OH)$_2$ D on Th9 cell development is mediated through IL-10, a cytokine that can be induced by 1,25(OH)$_2$ D in B cells [171] and naïve T-cells [170]. Treatment of Th17 cells with 1,25(OH)$_2$ D suppresses IL-17 production indirectly by inducing the expression of C/EBP homologous protein (CHOP), a molecule involved in endoplasmic reticulum stress and translational inhibition.

Other cells outside of the traditional immune system have the capacity to respond to and produce immuno-modulatory factors. CD14 mRNA is strongly up-regulated by 1,25(OH)$_2$ D in the prostate epithelial cell line RWPE1 [69], keratinocytes [172], U937 monocytes [173] and the myeloid cell line HL-60 [174]. CD14 and toll-like receptor 4 (TLR4) are important for detection of pathogens that have lipopolysaccharide on their cell surface [175] and CD14 has been identified as a crucial factor for vitamin D induced expression of the antimicrobial peptide cathelicidin in human keratinocytes [172]. Nonn et al. have shown that in normal prostate epithelial cells, 1,25(OH)$_2$ D inhibits TNFα-induced IL-6 production through a mechanism that requires direct transcriptional regulation of the MAPK phosphatase 5 gene (DUSP-10) [176].

Although these data suggest that vitamin D should suppress immune and inflammatory conditions that promote cancer, very little data explicitly supports this hypothesis, particularly as it relates to the ranges of vitamin D status being discussed as relevant to cancer prevention in humans. However, injections of the vitamin D analog Ro26-2198 delayed the onset of clinical colitis and reduced the number of dysplastic foci present in mice treated with azoxymethane and DSS [177].

1,25(OH)$_2$ D Action May not be Uniform Across all Stages of Cancer

Although we generally focus on the impact that vitamin D has on the development of cancer, it is just as important to understand the impact that cancer has on vitamin D action. In this section we will review studies that indicate vitamin D signaling is altered in various cancers. This concept is summarized in Figure 2.

VDR

There are conflicting findings regarding the impact of cancer on VDR protein levels. For example, using immunohistochemistry (IHC) Matusiak et al. [27] found that VDR protein levels decline as a function of colon tumor dedifferentiation while Kure et al. [178] observed that VDR protein levels were overexpressed in colorectal cancer tumors with KRAS

mutations. Unfortunately, a recent study highlights the inadequacy of many anti-VDR antibodies for IHC [179], and therefore IHC-based studies should be view with caution. None-the less, using IHC VDR levels have been reported to decline with the development of cancer in pigmented skin lesions [180], invasive breast tumors [181], and ovarian cancer [182] while high VDR is associated with reduced risk of lethal prostate cancer [183] and improved overall survival for non-small cell lung carcinoma [184]. Consistent with the hypothesis that cancer reduces VDR protein levels, Xu et al. [91] showed that VDR mRNA and protein expression (by Western blot analysis) were lower in the small intestine and colon tissue of $Apc^{Min/+}$ mice compared to wild-type mice. Other mutations that cause cancer can also lead to reduced VDR expression; H-Ras transformation in HC-11 mammary cells [185], SV40 large T antigen in HME human mammary epithelial cells [186], and in KRAS transformation in RWPE-2 human prostate epithelial cells [187].

A number of factors have been proposed to influence VDR expression in cancer. First, over-expression of SNAIL transcription factors can reduce VDR gene expression and blunt $1,25(OH)_2$ D or vitamin D analog-induced cell growth arrest and/or gene expression of colon cancer cell lines (e.g. SW-480-ADH, HCT116, Caco-2, LS174T, HT29) [188–190]. SNAIL1 is absent in normal tissue and expressed in colon tumors while SNAIL2 is expressed in normal tissue but its levels are increased in colon tumors [190;191]. In colon cancer cell lines and breast cancer cell lines SNAIL1 and 2 can bind to E-boxes in the proximal promoter of the VDR gene and increase recruitment of co-repressors that down-regulate VDR gene expression [190;192]. Another protein regulating VDR gene expression is p53. Over-expression of wild-type p53 in Saos-2 and H1299 cells (both p53 null) increases VDR protein levels by enhancing p53 association with conserved p53 binding sites within intron 1 of the VDR gene [193]. In addition to suppression of VDR gene expression, the mutant form of p53 found in many tumors can interact with VDR, be recruited to VDR-regulated genes, and modulate the expression of these genes in breast and lung cancer cell lines [194]. In this context, mutant p53 converts $1,25(OH)_2$ D treatment from a pro-apoptotic agent into an anti-apoptotic agent and suggesting in cancers with p53 mutations the therapeutic potential of vitamin D or vitamin D analogs could be limited.

There is also some evidence that the VDR gene may be subject to epigenetic silencing in cancer. Smirnoff et al. [195] first observed that in colonic tissue from ovariectomized rats treated with dimethylhydrazine (DMH), CpG island methylation in the VDR gene increased. This was associated with reduced VDR protein levels; both CpG island methylation in the VDR gene and reduced VDR expression could be prevented by estradiol treatment. In breast tumors and in breast cancer cell lines CpG island methylation in the −700 bp VDR promoter region was associated with lower VDR expression [196]. Demethylation of the VDR promoter with 5-azacytidine increased both VDR protein levels and potentiated the growth inhibitory response to $1,25(OH)_2$ D in normal and breast cancer cell lines.

VDR mRNA levels are also a target of the microRNA miR125b [197]. Over-expression of miR125b reduced VDR protein levels and attenuated $1,25(OH)_2$ D-induced expression of CYP24 in MCF7 cells [197]. Similarly, melanoma cell lines with low miR125b levels have higher VDR levels and are more sensitive to $1,25(OH)_2$ D-induced growth suppression than cells with high miR125b levels [198]. miR125b levels are upregulated in patients with

metastatic prostate cancer [199] but are downregulated in subjects with HER2-overexpressing breast cancers [200] but it remains to be determined whether miR125b expression can influence the therapeutic potential of vitamin D for specific cancers.

In addition to the negative impact of cancer on VDR levels, there is also evidence that Ras activation, a common mutation in many cancers, can impair vitamin D-mediated transcriptional activity. This was first shown by Solomon et al. [201] who found that H-Ras transformed kerotinocytes have reduced VDR transcriptional activity due phosphorylation of the VDR heterodimeric partner RXRα on serine 260. In the K-Ras transformed human prostate epithelial cell line RWPE2, we have shown that $1,25(OH)_2$ D mediated transcription is blunted due to phosphorylation of RXRα within the AF-1 domain [187], a process that impairs the ability of the coactivator SRC-1 to bind to RXRα. Collectively, this suggests that the development of cancer may lead to lower responses to $1,25(OH)_2$ D by impairing signaling through the VDR.

CYP27B1

The current hypothesis for how high vitamin D status reduces cancer risk is that $1,25(OH)_2$ D is produced and acts locally, i.e. the CYP27B1 enzyme known to produce $1,25(OH)_2$ D from 25OH D in the kidney is present and active in cancer target tissues [202]. Several groups have used cultured cells to show that CYP27B1 activity, and the ability to produce $1,25(OH)_2$ D from 25OH D, is lost as cells develop a more severe cancer phenotype. Hsu [25] found that CYP27B1 was present in normal prostate epithelial cells but that its activity was reduced in cells isolated from subjects with benign prostatic hypertrophy and nearly absent in cells isolated from subjects with prostate cancer. As a result, while normal cells could respond to treatment with 25OH D by growth arresting, cancer cells with low CYP27B1 expression could not. This observation was confirmed by Chen et al. [30] who also showed that transgenic expression of CYP27B1 confers a 25OH D-mediated growth inhibitory response to LNCaP cells with low CYP27B1 activity. CYP27B1 expression is also absent in metastases from colon tumors in humans [27;203]. However, cancer-associated reductions in CYP27B1 levels are not uniformly observed for all cancers. Friedrich et al. [26] found that CYP27B1 mRNA was increased in breast cancer as compared to normal tissue. More recently Lopes [181] analyzed CYP27B1 protein levels by immunohistochemistry in breast tissue samples and found that CYP27B1 expression did not significantly change between normal and cancer tissue.

CYP24

Some have also hypothesized that the enzyme responsible for the degradation of vitamin D metabolites, 25 hydroxyvitamin D-24 hydroxylase (CYP24), is influenced by cancer. CYP24 has been proposed as a putative oncogene that is amplified in breast tumors [204]. Consistent with this, Anderson et al. [205] reported that CYP24 mRNA levels are higher in colorectal cancer than in adjacent normal colon tissue. Matusiak and Benya [203] subsequently found that CYP24 protein was present in the nuclei of normal tissue, that CYP24 levels were increased in aberrant crypt foci and polyps, and that CYP24 protein was shifted to the cytoplasm in tumors and metastatic colon cancer. Overexpression of CYP24 has also been reported in ovarian, cervical, lung, cutaneous basal cell, and squamous cell

carcinoma [205–207]. This suggests that increased $1,25(OH)_2$ D metabolism may be a feature of advanced cancer. This is supported by studies showing that KTZ, a CYP24 inhibitor, can restore $1,25(OH)_2$ D-mediated growth arrest to prostate cancer cells that are insensitive to the action of $1,25(OH)_2$ D [208]. Recently, Komagata et al. [209] found that CYP24 protein levels are post-transcriptionally suppressed by the miR-125b, a microRNA that can bind to the 3' UTR of the CYP24 mRNA. In addition, they found that CYP24 levels were inversely related to miR-125b levels in breast cancer, suggesting that the loss of this regulatory RNA may account for elevated CYP24 levels in cancer. In contrast to other cancers, a microarray dataset in the ONCOMINE database shows that CYP24 expression can be lower in prostate cancer (www.oncomine.org). Consistent with this, CYP24 mRNA levels are inversely correlated with CYP24 promoter methylation in prostate cancer cell lines with the promoter being unmethylated, and vitamin D induction of CYP24 expression being greatest, in DU145 cells [210].

The overall impact of these cancer-induced changes to vitamin D metabolism and signaling could affect cancer prevention in several ways. The protection provided by high serum 25OH D levels depends upon the level of CYP27B1 in cells. Therefore, if CYP27B1 activity is lost, so will the protection due to high vitamin D status. On the other hand, when CYP24 activity is elevated and/or when VDR level/signaling is reduced, higher cellular levels of $1,25(OH)_2$ D will be needed to induce chemoprotective molecular events. However, it isn't clear whether local production of $1,25(OH)_2$ D is sufficient to overcome this cancer-associated insensitivity. Because of the strong effect it has on calcium metabolism, increases in serum $1,25(OH)_2$ D levels are not likely to be an effective means to correct tumor associated vitamin D resistance. $1,25(OH)_2$ D-treatment-associated hypercalcemia is a major motivation to produce vitamin D analogs that separate the calcemic and non-calcemic effects of $1,25(OH)_2$ D. However, even the effectiveness of vitamin D analogs will be limited in cancers with reduced VDR expression.

Conclusions

There is now a large amount of population-based evidence showing that higher vitamin D status can protect against a variety of cancers. Unfortunately, the mechanisms supporting this association have not yet been resolved. None-the-less, mechanistic explanations for how vitamin D can reduce cancer risk or disrupt the biology of transformed cells are increasing. While the role of $1,25(OH)_2$ D in cancer has traditionally been linked to the suppression of proliferation and the stimulation of apoptosis, there is now compelling evidence that $1,25(OH)_2$ D regulates other cancer-relevant cellular processes. For example, several lines of evidence suggest that the immunoregulatory actions of vitamin D metabolites are worth exploring for cancer prevention. The breadth of potential mechanisms used by vitamin D to prevent or suppress cancer is given in Figure 3. Still, many gaps remain in our understanding of how vitamin D regulates cell biology relevant to cancer prevention and treatment. Many of the mechanisms proposed for vitamin D and cancer prevention have been studied only in the context of one tissue or one type of cancer and so further studies must be conducted to determine if these mechanisms can be generalized (e.g. VDR-mediated inhibition of β catenin function has been studied primarily in colon). Also, while many studies show that $1,25(OH)_2$ D can alter various transcript levels, it is not clear which genes are direct targets

for VDR-mediated regulation. Recent advances in global profiling of transcription factor binding sites with techniques like ChIP-seq should overcome this uncertainly and help researchers differentiate between the direct and indirect effects of vitamin D in the context of cancer.

Another feature of the vitamin D/cancer paradigm that requires additional investigation is that vitamin D metabolism and signaling through the VDR may be disrupted as cancers progress. Until we understand this relationship more clearly, our ability to use vitamin D or vitamin D analogs for cancer prevention or treatment will be limited. Similarly, few controlled animal studies exist that confirm the validity of proposed molecular mechanisms for vitamin D-mediated cancer prevention using the ranges of vitamin D status that are observed in human populations. The lack of such “proof of principle” studies has hampered a broader acceptance of the vitamin D/cancer relationship in the research and medical community. For example, while higher serum 25OH D is associated with reduced cancer risk, and CYP27B1 is expressed in non-renal cells, there is no evidence that the low-level expression of CYP27B1 in tissues leads to meaningful local production of $1,25(OH)_2$ D, and that this mediates the protection from cancer provided by high vitamin D status. Still, even with all of these gaps in our understanding, this is an exciting time for research on vitamin D and cancer.

Acknowledgements

This work was supported by NIH award CA10113 and American Institute for Cancer Research award 09A098 to JCF.

Reference List

1. Fleet, JC. In Calcium in Human Health. Weaver, CM.; Heaney, RP., editors. Totowa, NJ: Humana Press; 2006. p. 163-190.
2. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int.J.Epidemiol. 1980; 9:227–231. [PubMed: 7440046]
3. Grant WB, Mohr SB. Ecological studies of ultraviolet B, vitamin D and cancer since 2000. Ann Epidemiol. 2009; 19:446–454. [PubMed: 19269856]
4. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet. 1989; 2:1176–1178. [PubMed: 2572900]
5. Engel P, Fagherazzi G, Boutten A, Dupre T, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F. Serum 25(OH) vitamin D and risk of breast cancer: a nested case-control study from the French E3N cohort. Cancer Epidemiol Biomarkers Prev. 2010; 19:2341–2350. [PubMed: 20826834]
6. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control. 2000; 11:847–852. [PubMed: 11075874]
7. Tretli S, Hernes E, Berg JP, Hestvik UE, Robsahm TE. Association between serum 25(OH)D and death from prostate cancer. Br.J Cancer. 2009; 100:450–454. [PubMed: 19156140]
8. Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, Willett WC. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J.Natl.Cancer Inst. 2006; 98:451–459. [PubMed: 16595781]
9. Sitrin MD, Halline AG, Abrahams C, Brasitus TA. Dietary calcium and vitamin D modulate 1,2-dimethylhydrazine-induced colonic carcinogenesis in the rat. Cancer Res. 1991; 51:5608–5613. [PubMed: 1913678]

10. Llor X, Jacoby RF, Teng BB, Davidson NO, Sitrin MD, Brasitus TA. K-ras mutations in 1,2-dimethylhydrazine-induced colonic tumors: effects of supplemental dietary calcium and vitamin D deficiency. Cancer Res. 1991; 51:4305–4309. [PubMed: 1868452]
11. Tangpricha V, Spina C, Yao M, Chen TC, Wolfe MM, Holick MF. Vitamin D deficiency enhances the growth of MC-26 colon cancer xenografts in Balb/c mice. J.Nutr. 2005; 135:2350–2354. [PubMed: 16177194]
12. Mordan-McCombs S, Brown T, Wang WL, Gaupel AC, Welsh J, Tenniswood M. Tumor progression in the LPB-Tag transgenic model of prostate cancer is altered by vitamin D receptor and serum testosterone status. J Steroid Biochem Mol Biol. 2010; 121:368–371. [PubMed: 20347977]
13. Zinser GM, Suckow M, Welsh J. Vitamin D receptor (VDR) ablation alters carcinogen-induced tumorigenesis in mammary gland, epidermis and lymphoid tissues. J Steroid.Biochem Mol Biol. 2005; 97:153–164. [PubMed: 16111884]
14. Zheng W, Wong KE, Zhang Z, Dougherty U, Mustafi R, Kong J, Deb DK, Zheng H, Bissonnette M, Li YC. Inactivation of the vitamin D receptor in APC(min/+) mice reveals a critical role for the vitamin D receptor in intestinal tumor growth. Int J Cancer e-pub ahead of print. 2011
15. Chen TC, Holick MF, Lokeshwar BL, Burnstein KL, Schwartz GG. Evaluation of vitamin D analogs as therapeutic agents for prostate cancer. Recent.Results.Cancer Res. 2003; 164:273–288. [PubMed: 12899529]
16. Oades GM, Senaratne SG, Clarke IA, Kirby RS, Colston KW. Nitrogen containing bisphosphonates induce apoptosis and inhibit the mevalonate pathway, impairing Ras membrane localization in prostate cancer cells. J Urol. 2003; 170:246–252. [PubMed: 12796698]
17. Huerta S, Irwin RW, Heber D, Go VL, Koeffler HP, Uskokovic MR, Harris DM. 1alpha,25-(OH)(2)-D(3) and its synthetic analogue decrease tumor load in the Apc(min) Mouse. Cancer Res. 2002; 62:741–746. [PubMed: 11830528]
18. Mehta RG. Stage-specific inhibition of mammary carcinogenesis by 1alpha-hydroxyvitamin D5. Eur.J Cancer. 2004; 40:2331–2337. [PubMed: 15454260]
19. Otoshi T, Iwata H, Kitano M, Nishizawa Y, Morii H, Yano Y, Otani S, Fukushima S. Inhibition of intestinal tumor development in rat multi-organ carcinogenesis and aberrant crypt foci in rat colon carcinogenesis by 22-oxa-calcitriol, a synthetic analogue of 1 alpha, 25-dihydroxyvitamin D3. Carcinogenesis. 1995; 16:2091–2097. [PubMed: 7554059]
20. Seubwai W, Wongkham C, Puapairoj A, Okada S, Wongkham S. 22-oxa-1,25-dihydroxyvitamin D3 efficiently inhibits tumor growth in inoculated mice and primary histoculture of cholangiocarcinoma. Cancer. 2010; 116:5535–5543. [PubMed: 20681031]
21. White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab. 2000; 11:320–327. [PubMed: 10996527]
22. Hewison M, Zehnder D, Bland R, Stewart PM. 1alpha-Hydroxylase and the action of vitamin D. J Mol.Endocrinol. 2000; 25:141–148. [PubMed: 11013342]
23. Song Y, Peng X, Porta A, Takanaga H, Peng JB, Hediger MA, Fleet JC, Christakos S. Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. Endocrinology. 2003; 144:3885–3894. [PubMed: 12933662]
24. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin.Endocrinol Metab. 2001; 86:888–894. [PubMed: 11158062]
25. Hsu JY, Feldman D, McNeal JE, Peehl DM. Reduced 1alpha-hydroxylase activity in human prostate cancer cells correlates with decreased susceptibility to 25-hydroxyvitamin D3-induced growth inhibition. Cancer Res. 2001; 61:2852–2856. [PubMed: 11306457]
26. Friedrich M, Diesing D, Cordes T, Fischer D, Becker S, Chen TC, Flanagan JN, Tangpricha V, Gherson I, Holick MF, Reichrath J. Analysis of 25-hydroxyvitamin D3-1alpha-hydroxylase in normal and malignant breast tissue. Anticancer Res. 2006; 26:2615–2620. [PubMed: 16886671]
27. Matusiak D, Murillo G, Carroll RE, Mehta RG, Benya RV. Expression of vitamin D receptor and 25-hydroxyvitamin D3-1{alpha}-hydroxylase in normal and malignant human colon. Cancer Epidemiol.Biomarkers Prev. 2005; 14:2370–2376. [PubMed: 16214919]

28. Vieth R, McCarten K, Norwich KH. Role of 25-hydroxyvitamin D3 dose in determining rat 1,25-dihydroxyvitamin D3 production. Am J Physiol. 1990; 258:E780–E789. [PubMed: 2185661]
29. Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino ACW, O'Loughlin PD, Morris HA. Metabolism of vitamin D-3 in human osteoblasts: Evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D-3. Bone. 2007; 40:1517–1528. [PubMed: 17395559]
30. Chen TC, Wang L, Whitlatch LW, Flanagan JN, Holick MF. Prostatic 25-hydroxyvitamin D-1alpha-hydroxylase and its implication in prostate cancer. J Cell Biochem. 2003; 88:315–322. [PubMed: 12520532]
31. Huang DC, Papavasiliou V, Rhim JS, Horst RL, Kremer R. Targeted disruption of the 25-hydroxyvitamin D3 1alpha-hydroxylase gene in ras-transformed keratinocytes demonstrates that locally produced 1alpha,25-dihydroxyvitamin D3 suppresses growth and induces differentiation in an autocrine fashion. Mol.Cancer Res. 2002; 1:56–67. [PubMed: 12496369]
32. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J.Bone Miner.Res. 1998; 13:325–349. [PubMed: 9525333]
33. Barsony J, Pike JW, DeLuca HF, Marx SJ. Immunocytology with microwave-fixed fibroblasts shows 1 alpha,25-dihydroxyvitamin D3-dependent rapid and estrogen-dependent slow reorganization of vitamin D receptors. J.Cell Biol. 1990; 111:2385–2395. [PubMed: 2177476]
34. Prufer K, Barsony J. Retinoid X receptor dominates the nuclear import and export of the unliganded vitamin D receptor. Mol.Endocrinol. 2002; 16:1738–1751. [PubMed: 12145331]
35. Barsony J, Renyi I, McKoy W. Subcellular distribution of normal and mutant vitamin D receptors in living cells. J.Biol.Chem. 1997; 272:5774–5782. [PubMed: 9038191]
36. Prufer K, Racz A, Lin GC, Barsony J. Dimerization with retinoid X receptors promotes nuclear localization and subnuclear targeting of vitamin D receptors. J Biol.Chem. 2000; 275:41114–41123. [PubMed: 11001945]
37. Pike JW, Meyer MB, Martowicz ML, Bishop KA, Lee SM, Nerenz RD, Goetsch PD. Emerging regulatory paradigms for control of gene expression by 1,25-dihydroxyvitamin D3. J Steroid Biochem Mol Biol. 2010; 121:130–135. [PubMed: 20214983]
38. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, Handunnetthi L, Handel AE, Disanto G, Orton SM, Watson CT, Morahan JM, Giovannoni G, Ponting CP, Ebers GC, Knight JC. A ChIP-seq defined genome-wide map of vitamin D receptor binding: Associations with disease and evolution. Genome Res. 2010; 20:1352–1360. [PubMed: 20736230]
39. Freedman LP. Increasing the complexity of coactivation in nuclear receptor signaling. Cell. 1999; 97:5–8. [PubMed: 10199396]
40. Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell. 1999; 98:675–686. [PubMed: 10490106]
41. Belandia B, Orford RL, Hurst HC, Parker MG. Targeting of SWI/SNF chromatin remodelling complexes to estrogen-responsive genes. EMBO J. 2002; 21:4094–4103. [PubMed: 12145209]
42. Rachez C, Lemon BD, Suldan Z, Bromleigh V, Gamble M, Naar AM, Erdjument-Bromage H, Tempst P, Freedman LP. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. Nature. 1999; 398:824–828. [PubMed: 10235266]
43. Battaglia S, Maguire O, Campbell MJ. Transcription factor co-repressors in cancer biology: roles and targeting. Int J Cancer. 2010; 126:2511–2519. [PubMed: 20091860]
44. Pike JW, Zella LA, Meyer MB, Fretz JA, Kim S. Molecular actions of 1,25-dihydroxyvitamin D3 on genes involved in calcium homeostasis. J Bone Miner Res. 2007; 2(22 Suppl):V16–V19. [PubMed: 18290714]
45. Colston K, Colston MJ, Feldman D. 1,25-dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. Endocrinology. 1981; 108:1083–1086. [PubMed: 6257495]
46. Lointier P, Wargovich MJ, Saez S, Levin B, Wildrick DM, Boman BM. The role of vitamin D3 in the proliferation of a human colon cancer cell line in vitro. Anticancer Res. 1987; 7:817–821. [PubMed: 2823686]

47. Gross M, Kost SB, Ennis B, Stumpf W, Kumar R. Effect of 1,25-dihydroxyvitamin D3 on mouse mammary tumor (GR) cells: evidence for receptors, cellular uptake, inhibition of growth and alteration in morphology at physiologic concentrations of hormone. J Bone Miner Res. 1986; 1:457–467. [PubMed: 2845731]

48. Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. Endocrinology. 1993; 132:1952–1960. [PubMed: 7682937]

49. Eelen G, Verlinden L, Van Camp M, Van Hummelen P, Marchal K, De Moor B, Mathieu C, Carmeliet G, Bouillon R, Verstuyf A. The effects of 1alpha,25-dihydroxyvitamin D3 on the expression of DNA replication genes. J Bone Miner.Res. 2004; 19:133–146. [PubMed: 14753745]

50. Wu W, Zhang X, Zanello LP. 1alpha,25-Dihydroxyvitamin D(3) antiproliferative actions involve vitamin D receptor-mediated activation of MAPK pathways and AP-1/p21(waf1) upregulation in human osteosarcoma. Cancer Lett. 2007; 254:75–86. [PubMed: 17412493]

51. Hedlund TE, Moffatt KA, Miller GJ. Vitamin D receptor expression is required for growth modulation by 1 alpha,25-dihydroxyvitamin D3 in the human prostatic carcinoma cell line ALVA-31. J Steroid Biochem Mol Biol. 1996; 58:277–288. [PubMed: 8836163]

52. Zhuang SH, Schwartz GG, Cameron D, Burnstein KL. Vitamin D receptor content and transcriptional activity do not fully predict antiproliferative effects of vitamin D in human prostate cancer cell lines. Mol Cell Endocrinol. 1997; 126:83–90. [PubMed: 9027366]

53. Hedlund TE, Moffatt KA, Miller GJ. Stable expression of the nuclear vitamin D receptor in the human prostatic carcinoma cell line JCA-1: Evidence thqt the antiproliferative effects of 1 alpha, 25-dihydroxyvitamin D3 are mediated exclusively through the genomic signaling pathway. Endocrinology. 1996; 137:1554–1561. [PubMed: 8612485]

54. Kovalenko PL, Zhang Z, Yu JG, Li Y, Clinton SK, Fleet JC. Dietary vitamin D and vitamin D receptor level modulate epithelial cell proliferation and apoptosis in the prostate. Cancer Prevention Research. 2011 In Press.

55. Costa JL, Eijk PP, van de Wiel MA, ten BD, Schmitt F, Narvaez CJ, Welsh J, Ylstra B. Anti-proliferative action of vitamin D in MCF7 is still active after siRNA-VDR knock-down. BMC Genomics. 2009; 10:499. [PubMed: 19863778]

56. Liu M, Lee MH, Cohen M, Bommakanti M, Freedman LP. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev. 1996; 10:142–153. [PubMed: 8566748]

57. Hager G, Formanek M, Gedlicka C, Thurnher D, Knerer B, Kornfehl J. 1,25(OH)2 vitamin D3 induces elevated expression of the cell cycle-regulating genes P21 and P27 in squamous carcinoma cell lines of the head and neck. Acta Otolaryngol. 2001; 121:103–109. [PubMed: 11270487]

58. Moffatt KA, Johannes WU, Hedlund TE, Miller GJ. Growth inhibitory effects of 1alpha, 25-dihydroxyvitamin D(3) are mediated by increased levels of p21 in the prostatic carcinoma cell line ALVA-31. Cancer Res. 2001; 61:7122–7129. [PubMed: 11585744]

59. Kawa S, Nikaido T, Aoki Y, Zhai Y, Kumagai T, Furihata K, Fujii S, Kiyosawa K. Vitamin D analogues up-regulate p21 and p27 during growth inhibition of pancreatic cancer cell lines. Br J Cancer. 1997; 76:884–889. [PubMed: 9328147]

60. Hager G, Kornfehl J, Knerer B, Weigel G, Formanek M. Molecular analysis of p21 promoter activity isolated from squamous carcinoma cell lines of the head and neck under the influence of 1,25(OH)2 vitamin D3 and its analogs. Acta Otolaryngol. 2004; 124:90–96. [PubMed: 14977084]

61. Rao A, Coan A, Welsh JE, Barclay WW, Koumenis C, Cramer SD. Vitamin D receptor and p21/WAF1 are targets of genistein and 1,25-dihydroxyvitamin D3 in human prostate cancer cells. Cancer Res. 2004; 64:2143–2147. [PubMed: 15026355]

62. Narvaez CJ, Welsh J. Differential effects of 1,25-dihydroxyvitamin D3 and tetradecanoylphorbol acetate on cell cycle and apoptosis of MCF-7 cells and a vitamin D3-resistant variant. Endocrinology. 1997; 138:4690–4698. [PubMed: 9348195]

63. Zhuang SH, Burnstein KL. Antiproliferative effect of 1alpha,25-dihydroxyvitamin D3 in human prostate cancer cell line LNCaP involves reduction of cyclin-dependent kinase 2 activity and persistent G1 accumulation. Endocrinology. 1998; 139:1197–1207. [PubMed: 9492054]

64. Thorne JL, Maguire O, Doig CL, Battaglia S, Fehr L, Sucheston LE, Heinaniemi M, O'Neill LP, McCabe CJ, Turner BM, Carlberg C, Campbell MJ. Epigenetic control of a VDR-governed feed-forward loop that regulates p21(waf1/cip1) expression and function in non-malignant prostate cells. Nucleic Acids Res. 2011; 39:2045–2056. [PubMed: 21088000]

65. Burgering BM. A brief introduction to FOXOlogy. Oncogene. 2008; 27:2258–2262. [PubMed: 18391968]

66. An BS, Tavera-Mendoza LE, Dimitrov V, Wang X, Calderon MR, Wang HJ, White JH. Stimulation of Sirt1-regulated FoxO protein function by the ligand-bound vitamin D receptor. Mol Cell Biol. 2010; 30:4890–4900. [PubMed: 20733005]

67. Peehl DM, Shinghal R, Nonn L, Seto E, Krishnan AV, Brooks JD, Feldman D. Molecular activity of 1,25-dihydroxyvitamin D3 in primary cultures of human prostatic epithelial cells revealed by cDNA microarray analysis. J Steroid Biochem Mol.Biol. 2004; 92:131–141. [PubMed: 15555907]

68. Swami S, Raghavachari N, Muller UR, Bao YP, Feldman D. Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray. Breast.Cancer Res Treat. 2003; 80:49–62. [PubMed: 12889598]

69. Kovalenko PL, Zhang Z, Cui M, Clinton SK, Fleet JC. 1,25 dihydroxyvitamin D-mediated orchestration of anticancer, transcript-level effects in the immortalized, non-transformed prostate epithelial cell line, RWPE1. BMC.Genomics. 2010; 11:26. [PubMed: 20070897]

70. Lin R, Nagai Y, Sladek R, Bastien Y, Ho J, Petrecca K, Sotiropoulou G, Diamandis EP, Hudson TJ, White JH. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol. 2002; 16:1243–1256. [PubMed: 12040012]

71. Colston KW, Perks CM, Xie SP, Holly JM. Growth inhibition of both MCF-7 and Hs578T human breast cancer cell lines by vitamin D analogues is associated with increased expression of insulin-like growth factor binding protein-3. J Mol Endocrinol. 1998; 20:157–162. [PubMed: 9513092]

72. Huynh H, Pollak M, Zhang JC. Regulation of insulin-like growth factor (IGF) II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1,25(OH)2D3 and its analog EB1089. Int J Oncol. 1998; 13:137–143. [PubMed: 9625815]

73. Sprenger CC, Peterson A, Lance R, Ware JL, Drivdahl RH, Plymate SR. Regulation of proliferation of prostate epithelial cells by 1,25- dihydroxyvitamin D3 is accompanied by an increase in insulin-like growth factor binding protein-3. J Endocrinol. 2001; 170:609–618. [PubMed: 11524241]

74. Krishnan AV, Shinghal R, Raghavachari N, Brooks JD, Peehl DM, Feldman D. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. Prostate. 2004; 59:243–251. [PubMed: 15042599]

75. Peng LH, Malloy PJ, Feldman D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. Molecular Endocrinology. 2004; 18:1109–1119. [PubMed: 14963110]

76. Boyle BJ, Zhao XY, Cohen P, Feldman D. Insulin-like growth factor binding protein-3 mediates 1 alpha,25- dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. J Urol. 2001; 165:1319–1324. [PubMed: 11257709]

77. Nickerson T, Huynh H. Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. J Endocrinol. 1999; 160:223–229. [PubMed: 9924191]

78. Palmer HG, Sanchez-Carbayo M, Ordonez-Moran P, Larriba MJ, Cordon-Cardo C, Munoz A. Genetic signatures of differentiation induced by 1alpha,25-dihydroxyvitamin D3 in human colon cancer cells. Cancer Res. 2003; 63:7799–7806. [PubMed: 14633706]

79. Buschke S, Stark HJ, Cerezo A, Pratzel-Wunder S, Boehnke K, Kollar J, Langbein L, Heldin CH, Boukamp P. A decisive function of transforming growth factor-{beta}/Smad signaling in tissue morphogenesis and differentiation of human HaCaT keratinocytes. Mol Biol Cell. 2011; 22:782–794. [PubMed: 21289094]

80. Ding Z, Wu CJ, Chu GC, Xiao Y, Ho D, Zhang J, Perry SR, Labrot ES, Wu X, Lis R, Hoshida Y, Hiller D, Hu B, Jiang S, Zheng H, Stegh AH, Scott KL, Signoretti S, Bardeesy N, Wang YA, Hill DE, Golub TR, Stampfer MJ, Wong WH, Loda M, Mucci L, Chin L, DePinho RA. SMAD4-

dependent barrier constrains prostate cancer growth and metastatic progression. Nature. 2011; 470:269–273. [PubMed: 21289624]

81. Lee HJ, Liu H, Goodman C, Ji Y, Maehr H, Uskokovic M, Notterman D, Reiss M, Suh N. Gene expression profiling changes induced by a novel Gemini Vitamin D derivative during the progression of breast cancer. Biochem Pharmacol. 2006; 72:332–343. [PubMed: 16737686]
82. Wu Y, Craig TA, Lutz WH, Kumar R. Identification of 1 alpha,25-dihydroxyvitamin D3 response elements in the human transforming growth factor beta 2 gene. Biochemistry. 1999; 38:2654–2660. [PubMed: 10052935]
83. Yang L, Yang J, Venkateswarlu S, Ko T, Brattain MG. Autocrine TGFbeta signaling mediates vitamin D3 analog-induced growth inhibition in breast cells. J Cell Physiol. 2001; 188:383–393. [PubMed: 11473365]
84. Zhang X, Li P, Bao J, Nicosia SV, Wang H, Enkemann SA, Bai W. Suppression of death receptor-mediated apoptosis by 1,25-dihydroxyvitamin D3 revealed by microarray analysis. J Biol Chem. 2005; 280:35458–35468. [PubMed: 16093247]
85. Lambert JR, Kelly JA, Shim M, Huffer WE, Nordeen SK, Baek SJ, Eling TE, Lucia MS. Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth. J Cell Physiol. 2006; 208:566–574. [PubMed: 16741990]
86. Kodach LL, Wiercinska E, de Miranda NF, Bleuming SA, Musler AR, Peppelenbosch MP, Dekker E, van den Brink GR, van Noesel CJ, Morreau H, Hommes DW, Ten DP, Offerhaus GJ, Hardwick JC. The bone morphogenetic protein pathway is inactivated in the majority of sporadic colorectal cancers. Gastroenterology. 2008; 134:1332–1341. [PubMed: 18471510]
87. Macleod K. Tumor suppressor genes. Curr.Opin.Genet Dev. 2000; 10:81–93. [PubMed: 10679386]
88. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut. 2007; 56:417–425. [PubMed: 16840506]
89. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Munoz A. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol. 2001; 154:369–387. [PubMed: 11470825]
90. Egan JB, Thompson PA, Vitanov MV, Bartik L, Jacobs ET, Haussler MR, Gerner EW, Jurutka PW. Vitamin D receptor ligands, adenomatous polyposis coli, and the vitamin D receptor FokI polymorphism collectively modulate beta-catenin activity in colon cancer cells. Mol Carcinog. 2010; 49:337–352. [PubMed: 20043299]
91. Xu H, Posner GH, Stevenson M, Campbell FC. Apc(MIN) modulation of vitamin D secosteroid growth control. Carcinogenesis. 2010; 31:1434–1441. [PubMed: 20488884]
92. Shah S, Islam MN, Dakshanamurthy S, Rizvi I, Rao M, Herrell R, Zinser G, Valrance M, Aranda A, Moras D, Norman A, Welsh J, Byers SW. The molecular basis of vitamin D receptor and beta-catenin crossregulation. Mol Cell. 2006; 21:799–809. [PubMed: 16543149]
93. Pendas-Franco N, Garcia JM, Pena C, Valle N, Palmer HG, Heinaniemi M, Carlberg C, Jimenez B, Bonilla F, Munoz A, Gonzalez-Sancho JM. DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D(3). Oncogene. 2008
94. Aguilera O, Pena C, Garcia JM, Larriba MJ, Ordonez-Moran P, Navarro D, Barbachano A, Lopez dSI, Ballestar E, Fraga MF, Esteller M, Gamallo C, Bonilla F, Gonzalez-Sancho JM, Munoz A. The Wnt antagonist DICKKOPF-1 gene is induced by 1alpha,25-dihydroxyvitamin D3 associated to the differentiation of human colon cancer cells. Carcinogenesis. 2007; 28:1877–1884. [PubMed: 17449905]
95. Beildeck ME, Islam M, Shah S, Welsh J, Byers SW. Control of TCF-4 expression by VDR and vitamin D in the mouse mammary gland and colorectal cancer cell lines. PLoS ONE. 2009; 4:e7872. [PubMed: 19924301]
96. Verzi MP, Hatzis P, Sulahian R, Philips J, Schuijers J, Shin H, Freed E, Lynch JP, Dang DT, Brown M, Clevers H, Liu XS, Shivdasani RA. TCF4 and CDX2, major transcription factors for intestinal function, converge on the same cis-regulatory regions. Proceedings of the National Academy of Sciences. 2010; 107:15157–15162.

97. Simboli-Campbell M, Gagnon AM, Franks DJ, Welsh JE. 1,25-Dihydroxyvitamin D3 Translocates Protein Kinase Cß to Nucleus and Enhances Plasma Membrane Association of Protein Kinase C-alpha in Renal Epithelial Cells. The Journal of Biological Chemistry. 1994; 269:3257–64. [PubMed: 8106362]

98. Diaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. Cancer Res. 2000; 60:2304–2312. [PubMed: 10786699]

99. Pan L, Matloob AF, Du J, Pan H, Dong Z, Zhao J, Feng Y, Zhong Y, Huang B, Lu J. Vitamin D stimulates apoptosis in gastric cancer cells in synergy with trichostatin A /sodium butyrate-induced and 5-aza-2'-deoxycytidine-induced PTEN upregulation. FEBS J. 2010; 277:989–999. [PubMed: 20089040]

100. Taghizadeh F, Tang MJ, Tai IT. Synergism between vitamin D and secreted protein acidic and rich in cysteine-induced apoptosis and growth inhibition results in increased susceptibility of therapy-resistant colorectal cancer cells to chemotherapy. Mol Cancer Ther. 2007; 6:309–317. [PubMed: 17237289]

101. Blutt SE, McDonnell TJ, Polek TC, Weigel NL. Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2. Endocrinology. 2000; 141:10–17. [PubMed: 10614618]

102. Welch C, Santra MK, El-Assaad W, Zhu X, Huber WE, Keys RA, Teodoro JG, Green MR. Identification of a protein, G0S2, that lacks Bcl-2 homology domains and interacts with and antagonizes Bcl-2. Cancer Res. 2009; 69:6782–6789. [PubMed: 19706769]

103. Kizildag S, Ates H, Kizildag S. Treatment of K562 cells with 1,25-dihydroxyvitamin D(3) induces distinct alterations in the expression of apoptosis-related genes BCL2, BAX, BCL(XL), and p21. Ann Hematol. 2009

104. Singletary K, Milner J. Diet, autophagy, and cancer: a review. Cancer Epidemiol Biomarkers Prev. 2008; 17:1596–1610. [PubMed: 18628411]

105. Mathiasen IS, Lademann U, Jaattela M. Apoptosis induced by vitamin D compounds in breast cancer cells is inhibited by Bcl-2 but does not involve known caspases or p53. Cancer Res. 1999; 59:4848–4856. [PubMed: 10519395]

106. Hoyer-Hansen M, Bastholm L, Mathiasen IS, Elling F, Jaattela M. Vitamin D analog EB1089 triggers dramatic lysosomal changes and Beclin 1-mediated autophagic cell death. Cell Death Differ. 2005; 12:1297–1309. [PubMed: 15905882]

107. Tavera-Mendoza L, Wang TT, Lallemant B, Zhang R, Nagai Y, Bourdeau V, Ramirez-Calderon M, Desbarats J, Mader S, White JH. Convergence of vitamin D and retinoic acid signalling at a common hormone response element. EMBO Rep. 2006; 7:180–185. [PubMed: 16322758]

108. Hoyer-Hansen M, Nordbrandt SP, Jaattela M. Autophagy as a basis for the health-promoting effects of vitamin D. Trends Mol Med. 2010; 16:295–302. [PubMed: 20488750]

109. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions. 2006; 160:1–40. [PubMed: 16430879]

110. Kallay E, Pietschmann P, Toyokuni S, Bajna E, Hahn P, Mazzucco K, Bieglmayer C, Kato S, Cross HS. Characterization of a vitamin D receptor knockout mouse as a model of colorectal hyperproliferation and DNA damage. Carcinogenesis. 2001; 22:1429–1435. [PubMed: 11532865]

111. Fedirko V, Bostick RM, Long Q, Flanders WD, McCullough ML, Sidelnikov E, Daniel CR, Rutherford RE, Shaukat A. Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. Cancer Epidemiol.Biomarkers Prev. 2010; 19:280–291. [PubMed: 20056649]

112. Banakar MC, Paramasivan SK, Chattopadhyay MB, Datta S, Chakraborty P, Chatterjee M, Kannan K, Thygarajan E. 1alpha, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital. World J Gastroenterol. 2004; 10:1268–1275. [PubMed: 15112340]

113. Bao BY, Ting HJ, Hsu JW, Lee YF. Protective role of 1 alpha, 25-dihydroxyvitamin D-3 against oxidative stress in nonmalignant human prostate epithelial cells. International Journal of Cancer. 2008; 122:2699–2706.
114. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. Journal of Biological Chemistry. 1999; 274:33627–33636. [PubMed: 10559251]
115. Frohlich DA, Mccabe MT, Arnold RS, Day ML. The role of Nrf2 in increased reactive oxygen species and DNA damage in prostate tumorigenesis. Oncogene. 2008; 27:4353–4362. [PubMed: 18372916]
116. Akutsu N, Lin R, Bastien Y, Bestawros A, Enepekides DJ, Black MJ, White JH. Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. Mol.Endocrinol. 2001; 15:1127–1139. [PubMed: 11435613]
117. Jiang F, Li PF, Fornace AJ, Nicosia SV, Bai WL. G(2)/M arrest by 1,25-dihydroxyvitamin D-3 in ovarian cancer cells mediated through the induction of GADD45 via an exonic enhancer. Journal of Biological Chemistry. 2003; 278:48030–48040. [PubMed: 14506229]
118. Badawi AF. The role of prostaglandin synthesis in prostate cancer. BJU.Int. 2000; 85:451–462. [PubMed: 10691825]
119. Hussain T, Gupta S, Mukhtar H. Cyclooxygenase-2 and prostate carcinogenesis. Cancer Lett. 2003; 191:125–135. [PubMed: 12618325]
120. Nithipatikom K, Isbell MA, Lindholm PF, Kajdacsy-Balla A, Kaul S, Campell WB. Requirement of cyclooxygenase-2 expression and prostaglandins for human prostate cancer cell invasion. Clin Exp Metastasis. 2002; 19:593–601. [PubMed: 12498388]
121. Muller-Decker K, Furstenberger G. The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. Mol Carcinog. 2007; 46:705–710. [PubMed: 17546626]
122. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, Kaidi A. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009; 30:377–386. [PubMed: 19136477]
123. Moreno J, Krishnan AV, Feldman D. Molecular mechanisms mediating the anti-proliferative effects of Vitamin D in prostate cancer. J Steroid Biochem.Mol.Biol. 2005; 97:31–36. [PubMed: 16024246]
124. Moreno J, Krishnan AV, Swami S, Nonn L, Peehl DM, Feldman D. Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. Cancer Res. 2005; 65:7917–7925. [PubMed: 16140963]
125. Maund SL, Cramer SD. The tissue-specific stem cell as a target for chemoprevention. Stem Cell Rev. 2011; 7:307–314. [PubMed: 21086069]
126. Trosko JE. Dietary modulation of the multistage, multimechanisms of human carcinogenesis: effects on initiated stem cells and cell-cell communication. Nutr Cancer. 2006; 54:102–110. [PubMed: 16800778]
127. Alison MR, Lim SM, Nicholson LJ. Cancer stem cells: problems for therapy? J Pathol. 2011; 223:147–161. [PubMed: 21125672]
128. Barclay WW, Axanova LS, Chen W, Romero L, Maund SL, Soker S, Lees CJ, Cramer SD. Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. Stem.Cells. 2008; 26:600–610. [PubMed: 18055450]
129. Maund SL, Barclay WW, Hover LD, Axanova LS, Sui G, Hipp JD, Fleet JC, Thorburn A, Cramer SD. Interleukin-1{alpha} Mediates the Antiproliferative Effects of 1,25-Dihydroxyvitamin D3 in Prostate Progenitor/Stem Cells. Cancer Res. 2011; 71:5276–5286. [PubMed: 21653679]
130. Modica S, Gofflot F, Murzilli S, D'Orazio A, Salvatore L, Pellegrini F, Nicolucci A, Tognoni G, Copetti M, Valanzano R, Veschi S, Mariani-Costantini R, Palasciano G, Schoonjans K, Auwerx J, Moschetta A. The intestinal nuclear receptor signature with epithelial localization patterns and expression modulation in tumors. Gastroenterology. 2010; 138:636–648. 648. [PubMed: 19818784]

131. Fedirko V, Bostick RM, Flanders WD, Long Q, Sidelnikov E, Shaukat A, Daniel CR, Rutherford RE, Woodard JJ. Effects of Vitamin D and Calcium on Proliferation and Differentiation In Normal Colon Mucosa: a Randomized Clinical Trial. Cancer Epidemiology Biomarkers & Prevention. 2009; 18:2933–2941.

132. Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S, Breault DT. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. Proc Natl Acad Sci U S A. 2011; 108:179–184. [PubMed: 21173232]

133. So JY, Lee HJ, Smolarek AK, Paul S, Wang CX, Maehr H, Uskokovic M, Zheng X, Conney AH, Cai L, Liu F, Suh N. A Novel Gemini Vitamin D Analog Represses the Expression of a Stem Cell Marker CD44 in Breast Cancer. Mol Pharmacol. 2011; 79:360–367. [PubMed: 21115634]

134. Hill R, Song Y, Cardiff RD, Van Dyke T. Selective Evolution of Stromal Mesenchyme with p53 Loss in Response to Epithelial Tumorigenesis. Cell. 2005; 123:1001–1011. [PubMed: 16360031]

135. Lou YR, Laaksi I, Syvala H, Blauer M, Tammela TL, Ylikomi T, Tuohimaa P. 25-hydroxyvitamin D3 is an active hormone in human primary prostatic stromal cells. FASEB J. 2004; 18:332–334. [PubMed: 14657005]

136. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. Circ.Res. 2000; 87:214–220. [PubMed: 10926872]

137. Tosetti F, Ferrari N, De Flora S, Albini A. Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. FASEB J. 2002; 16:2–14. [PubMed: 11772931]

138. Chung I, Han G, Seshadri M, Gillard BM, Yu WD, Foster BA, Trump DL, Johnson CS. Role of vitamin D receptor in the antiproliferative effects of calcitriol in tumor-derived endothelial cells and tumor angiogenesis in vivo. Cancer Res. 2009; 69:967–975. [PubMed: 19141646]

139. Bao BY, Yao J, Lee YF. 1alpha, 25-dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. Carcinogenesis. 2006; 27:1883–1893. [PubMed: 16624828]

140. Fernandez-Garcia NI, Palmer HG, Garcia M, Gonzalez-Martin A, del Rio M, Barettino D, Volpert O, Munoz A, Jimenez B. 1alpha,25-Dihydroxyvitamin D3 regulates the expression of Id1 and Id2 genes and the angiogenic phenotype of human colon carcinoma cells. Oncogene. 2005; 24:6533–6544. [PubMed: 16007183]

141. Bernardi RJ, Johnson CS, Modzelewski RA, Trump DL. Antiproliferative effects of 1alpha,25-dihydroxyvitamin D(3) and vitamin D analogs on tumor-derived endothelial cells. Endocrinology. 2002; 143:2508–2514. [PubMed: 12072382]

142. Campbell CL, Savarese DMF, Quesenberry PJ, Savarese TM. Expression of multiple angiogenic cytokines in cultured normal human prostate epithelial cells: Predominance of vascular endothelial growth factor. International Journal of Cancer. 1999; 80:868–874.

143. Ben Shoshan M, Amir S, Dang DT, Dang LH, Weisman Y, Mabjeesh NJ. 1alpha,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. Mol Cancer Ther. 2007; 6:1433–1439. [PubMed: 17431122]

144. Levine MJ, Teegarden D. 1alpha,25-dihydroxycholecalciferol increases the expression of vascular endothelial growth factor in C3H10T1/2 mouse embryo fibroblasts. J Nutr. 2004; 134:2244–2250. [PubMed: 15333711]

145. Cardus A, Panizo S, Encinas M, Dolcet X, Gallego C, Aldea M, Fernandez E, Valdivielso JM. 1, 25-Dihydroxyvitamin D3 regulates VEGF production through a vitamin D response element in the VEGF promoter. Atherosclerosis. 2008

146. Kong J, Zhang Z, Musch MW, Ning G, Sun J, Hart J, Bissonnette M, Li YC. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. Am J Physiol Gastrointest Liver Physiol. 2008; 294:G208–G216. [PubMed: 17962355]

147. Kutuzova GD, DeLuca HF. Gene expression profiles in rat intestine identify pathways for 1,25-dihydroxyvitamin D(3) stimulated calcium absorption and clarify its immunomodulatory properties. Arch Biochem Biophys. 2004; 432:152–166. [PubMed: 15542054]

148. Ordonez-Moran P, Alvarez-Diaz S, Valle N, Larriba MJ, Bonilla F, Munoz A. The effects of 1,25-dihydroxyvitamin D3 on colon cancer cells depend on RhoA-ROCK-p38MAPK-MSK signaling. J Steroid Biochem Mol Biol. 2010; 121:355–361. [PubMed: 20223287]

149. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–1570. [PubMed: 21436444]

150. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–867. [PubMed: 12490959]

151. Haverkamp J, Charbonneau B, Ratliff TL. Prostate inflammation and its potential impact on prostate cancer: A current review. J Cell Biochem. 2007

152. de Souza AP, Bonorino C. Tumor immunosuppressive environment: effects on tumor-specific and nontumor antigen immune responses. Expert Rev Anticancer Ther. 2009; 9:1317–1332. [PubMed: 19761435]

153. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr. 2004; 80:1717S–1720S. [PubMed: 15585793]

154. Westbrook AM, Szakmary A, Schiestl RH. Mechanisms of intestinal inflammation and development of associated cancers: lessons learned from mouse models. Mutat.Res. 2010; 705:40–59. [PubMed: 20298806]

155. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S, White JH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004; 173:2909–2912. [PubMed: 15322146]

156. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J. 2005; 19:1067–1077. [PubMed: 15985530]

157. Wang TT, Dabbas B, Laperriere D, Bitton AJ, Soualhine H, Tavera-Mendoza LE, Dionne S, Servant MJ, Bitton A, Seidman EG, Mader S, Behr MA, White JH. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. J Biol Chem. 2010; 285:2227–2231. [PubMed: 19948723]

158. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyang Y, McLachlan SM, Adams JS, Hewison M. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. Endocrinology. 2010; 151:2423–2432. [PubMed: 20392825]

159. Edfeldt K, Liu PT, Chun R, Fabri M, Schenk M, Wheelwright M, Keegan C, Krutzik SR, Adams JS, Hewison M, Modlin RL. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. Proc Natl Acad Sci U S A. 2010; 107:22593–22598. [PubMed: 21149724]

160. Pryke AM, Duggan C, White CP, Posen S, Mason RS. Tumor necrosis factor-alpha induces vitamin D-1-hydroxylase activity in normal human alveolar macrophages. J.Cell Physiol. 1990; 142:652–656. [PubMed: 1690216]

161. Szeles L, Keresztes G, Torocsik D, Balajthy Z, Krenacs L, Poliska S, Steinmeyer A, Zuegel U, Pruenster M, Rot A, Nagy L. 1,25-dihydroxyvitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. J Immunol. 2009; 182:2074–2083. [PubMed: 19201860]

162. Cantorna MT. Why do T cells express the vitamin D receptor? Ann N Y.Acad Sci. 2011; 1217:77–82. [PubMed: 21114675]

163. Mathieu C, van EE, Gysemans C, Decallonne B, Kato S, Laureys J, Depovere J, Valckx D, Verstuyf A, Bouillon R. In vitro and in vivo analysis of the immune system of vitamin D receptor knockout mice. J Bone Miner Res. 2001; 16:2057–2065. [PubMed: 11697802]

164. Yu S, Bruce D, Froicu M, Weaver V, Cantorna MT. Failure of T cell homing, reduced CD4/CD8alphaalpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. Proc Natl Acad Sci U S A. 2008; 105:20834–20839. [PubMed: 19095793]

165. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Molecular Endocrinology. 2003; 17:2386–2392. [PubMed: 14500760]

166. Motrich RD, van EE, Depovere J, Riera CM, Rivero VE, Mathieu C. Impact of vitamin D receptor activity on experimental autoimmune prostatitis. J Autoimmun. 2009; 32:140–148. [PubMed: 19269787]

167. Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. Eur J Immunol. 2011; 41:822–832. [PubMed: 21287548]

168. Griffin MD, Dong X, Kumar R. Vitamin D receptor-mediated suppression of RelB in antigen presenting cells: a paradigm for ligand-augmented negative transcriptional regulation. Arch Biochem Biophys. 2007; 460:218–226. [PubMed: 17367745]

169. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp Biol Med (Maywood.). 2004; 229:1136–1142. [PubMed: 15564440]

170. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, Weaver CT. Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. J Biol Chem. 2011; 286:997–1004. [PubMed: 21047796]

171. Heine G, Niesner U, Chang HD, Steinmeyer A, Zugel U, Zuberbier T, Radbruch A, Worm M. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. Eur J Immunol. 2008; 38:2210–2218. [PubMed: 18651709]

172. Schauber J, Oda Y, Buchau AS, Yun QC, Steinmeyer A, Zugel U, Bikle DD, Gallo RL. Histone acetylation in keratinocytes enables control of the expression of cathelicidin and CD14 by 1,25-dihydroxyvitamin D3. J Invest Dermatol. 2008; 128:816–824. [PubMed: 17943182]

173. Liu HZ, Gong JP, Wu CX, Peng Y, Li XH, You HB. The U937 cell line induced to express CD14 protein by 1,25-dihydroxyvitamin D3 and be sensitive to endotoxin stimulation. Hepatobiliary.Pancreat.Dis.Int. 2005; 4:84–89. [PubMed: 15730927]

174. Li C, Wang Y, Gao L, Zhang J, Shao J, Wang S, Feng W, Wang X, Li M, Chang Z. Expression of toll-like receptors 2 and 4 and CD14 during differentiation of HL-60 cells induced by phorbol 12-myristate 13-acetate and 1 alpha, 25-dihydroxy-vitamin D(3). Cell Growth Differ. 2002; 13:27–38. [PubMed: 11801529]

175. Leung TF, Tang NL, Wong GW, Fok TF. CD14 and toll-like receptors: potential contribution of genetic factors and mechanisms to inflammation and allergy. Curr Drug Targets.Inflamm.Allergy. 2005; 4:169–175. [PubMed: 15853738]

176. Nonn L, Peng L, Feldman D, Peehl DM. Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. Cancer Res. 2006; 66:4516–4524. [PubMed: 16618780]

177. Fichera A, Little N, Dougherty U, Mustafi R, Cerda S, Li YC, Delgado J, Arora A, Campbell LK, Joseph L, Hart J, Noffsinger A, Bissonnette M. A vitamin D analogue inhibits colonic carcinogenesis in the AOM/DSS model. J Surg.Res. 2007; 142:239–245. [PubMed: 17574271]

178. Kure S, Nosho K, Baba Y, Irahara N, Shima K, Ng K, Meyerhardt JA, Giovannucci EL, Fuchs CS, Ogino S. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18:2765–2772. [PubMed: 19789368]

179. Wang Y, Becklund BR, DeLuca HF. Identification of a highly specific and versatile vitamin D receptor antibody. Archives of Biochemistry and Biophysics. 2010; 494:166–177. [PubMed: 19951695]

180. Brozyna AA, Jozwicki W, Janjetovic Z, Slominski AT. Expression of vitamin D receptor decreases during progression of pigmented skin lesions. Hum.Pathol. 2011; 42:618–631. [PubMed: 21292298]

181. Lopes N, Sousa B, Martins D, Gomes M, Vieira D, Veronese L, Milanezi F, Paredes J, Costa J, Schmitt F. Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions Vitamin D pathways unbalanced in breast lesions. BMC Cancer. 2010; 10:483. [PubMed: 20831823]

182. Thill M, Fischer D, Kelling K, Hoellen F, Dittmer C, Hornemann A, Salehin D, Diedrich K, Friedrich M, Becker S. Expression of vitamin D receptor (VDR), cyclooxygenase-2 (COX-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in benign and malignant ovarian tissue and 25-hydroxycholecalciferol (25(OH2)D3) and prostaglandin E2 (PGE2) serum level in ovarian cancer patients. J Steroid Biochem Mol Biol. 2010; 121:387–390. [PubMed: 20304053]

183. Hendrickson WK, Flavin R, Kasperzyk JL, Fiorentino M, Fang F, Lis R, Fiore C, Penney KL, Ma J, Kantoff PW, Stampfer MJ, Loda M, Mucci LA, Giovannucci E. Vitamin D receptor protein expression in tumor tissue and prostate cancer progression. J Clin Oncol. 2011; 29:2378–2385. [PubMed: 21537045]

184. Srinivasan M, Parwani AV, Hershberger PA, Lenzner DE, Weissfeld JL. Nuclear vitamin D receptor expression is associated with improved survival in non-small cell lung cancer. J Steroid Biochem Mol Biol. 2011; 123:30–36. [PubMed: 20955794]

185. Escaleira MT, Brentani MM. Vitamin D3 receptor (VDR) expression in HC-11 mammary cells: regulation by growth-modulatory agents, differentiation, and Ha-ras transformation. Breast Cancer Res Treat. 1999; 54:123–133. [PubMed: 10424403]

186. Kemmis CM, Welsh J. Mammary epithelial cell transformation is associated with deregulation of the vitamin D pathway. J Cell Biochem. 2008; 105:980–988. [PubMed: 18767073]

187. Zhang Z, Kovalenko P, Cui M, DeSmet M, Clinton SK, Fleet JC. Constitutive activation of the mitogen-activated protein kinase pathway impairs vitamin D signaling in human prostate epithelial cells. Journal of Cellular Physiology. 2010; 224:433–442. [PubMed: 20432439]

188. Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, Pena C, Peiro S, Puig I, Rodriguez R, de la FR, Bernad A, Pollan M, Bonilla F, Gamallo C, de Herreros AG, Munoz A. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. Nat Med. 2004; 10:917–919. [PubMed: 15322538]

189. Larriba MJ, Valle N, Palmer HG, Ordonez-Moran P, Alvarez-Diaz S, Becker KF, Gamallo C, de Herreros AG, Gonzalez-Sancho JM, Munoz A. The inhibition of Wnt/beta-catenin signalling by 1alpha,25-dihydroxyvitamin D3 is abrogated by Snail1 in human colon cancer cells. Endocr.Relat.Cancer. 2007; 14:141–151. [PubMed: 17395983]

190. Larriba MJ, Martin-Villar E, Garcia JM, Pereira F, Pena C, de Herreros AG, Bonilla F, Munoz A. Snail2 cooperates with Snail1 in the repression of vitamin D receptor in colon cancer. Carcinogenesis. 2009; 30:1459–1468. [PubMed: 19502595]

191. Pena C, Garcia JM, Silva J, Garcia V, Rodriguez R, Alonso I, Millan I, Salas C, de Herreros AG, Munoz A, Bonilla F. E-cadherin and vitamin D receptor regulation by SNAIL and ZEB1 in colon cancer: clinicopathological correlations. Hum.Mol Genet. 2005; 14:3361–3370. [PubMed: 16203744]

192. Mittal MK, Myers JN, Misra S, Bailey CK, Chaudhuri G. In vivo binding to and functional repression of the VDR gene promoter by SLUG in human breast cells. Biochemical and Biophysical Research Communications. 2008; 372:30–34. [PubMed: 18485278]

193. Maruyama R, Aoki F, Toyota M, Sasaki Y, Akashi H, Mita H, Suzuki H, Akino K, Ohe-Toyota M, Maruyama Y, Tatsumi H, Imai K, Shinomura Y, Tokino T. Comparative genome analysis identifies the vitamin D receptor gene as a direct target of p53-mediated transcriptional activation. Cancer Res. 2006; 66:4574–4583. [PubMed: 16651407]

194. Stambolsky P, Tabach Y, Fontemaggi G, Weisz L, Maor-Aloni R, Sigfried Z, Shiff I, Kogan I, Shay M, Kalo E, Blandino G, Simon I, Oren M, Rotter V. Modulation of the Vitamin D3 Response by Cancer-Associated Mutant p53. Cancer Cell. 2010; 17:273–285. [PubMed: 20227041]

195. Liel Y, Shany S, Smirnoff P, Schwartz B. Estrogen increases 1,25-dihydroxyvitamin D receptors expression and bioresponse in the rat duodenal mucosa. Endocrinology. 1999; 140:280–285. [PubMed: 9886836]

196. Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, Stearns V, Umbricht CB. DNA methylation-related vitamin D receptor insensitivity in breast cancer. Cancer Biol Ther. 2010; 10:44–53. [PubMed: 20431345]

197. Mohri T, Nakajima M, Takagi S, Komagata S, Yokoi T. MicroRNA regulates human vitamin D receptor. Int J Cancer. 2009

198. Essa S, Denzer N, Mahlknecht U, Klein R, Collnot EM, Tilgen W, Reichrath J. VDR microRNA expression and epigenetic silencing of vitamin D signaling in melanoma cells. J Steroid Biochem Mol Biol. 2010; 121:110–113. [PubMed: 20153427]

199. Pang Y, Young CY, Yuan H. MicroRNAs and prostate cancer. Acta Biochim.Biophys.Sin. (Shanghai). 2010; 42:363–369. [PubMed: 20539944]

200. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol Cancer. 2006; 5:24. [PubMed: 16784538]

201. Solomon C, White JH, Kremer R. Mitogen-activated protein kinase inhibits 1,25-dihydroxyvitamin D3- dependent signal transduction by phosphorylating human retinoid X receptor alpha. J Clin.Invest. 1999; 103:1729–1735. [PubMed: 10377179]

202. Cross HS, Nittke T, Peterlik M. Modulation of vitamin D synthesis and catabolism in colorectal mucosa: a new target for cancer prevention. Anticancer Res. 2009; 29:3705–3712. [PubMed: 19667168]

203. Matusiak D, Benya RV. CYP27A1 and CYP24 expression as a function of malignant transformation in the colon. J Histochem.Cytochem. 2007; 55:1257–1264. [PubMed: 17875655]

204. Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW, Pinkel D. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat.Genet. 2000; 25:144–146. [PubMed: 10835626]

205. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. Cancer Chemother.Pharmacol. 2006; 57:234–240. [PubMed: 16180015]

206. Friedrich M, Rafi L, Mitschele T, Tilgen W, Schmidt W, Reichrath J. Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. Recent Results Cancer Res. 2003; 164:239–246. [PubMed: 12899526]

207. Mitschele T, Diesel B, Friedrich M, Meineke V, Maas RM, Gartner BC, Kamradt J, Meese E, Tilgen W, Reichrath J. Analysis of the vitamin D system in basal cell carcinomas (BCCs). Lab Invest. 2004; 84:693–702. [PubMed: 15077124]

208. Muindi JR, Nganga A, Engler KL, Coignet LJ, Johnson CS, Trump DL. CYP24 splicing variants are associated with different patterns of constitutive and calcitriol-inducible CYP24 activity in human prostate cancer cell lines. J Steroid.Biochem Mol Biol. 2007; 103:334–337. [PubMed: 17368180]

209. Komagata S, Nakajima M, Takagi S, Mohri T, Taniya T, Yokoi T. Human CYP24 catalyzing the inactivation of calcitriol is post-transcriptionally regulated by miR-125b. Mol Pharmacol. 2009; 76:702–709. [PubMed: 19570947]

210. Luo W, Karpf AR, Deeb KK, Muindi JR, Morrison CD, Johnson CS, Trump DL. Epigenetic regulation of vitamin D 24-hydroxylase/CYP24A1 in human prostate cancer. Cancer Res. 2010; 70:5953–5962. [PubMed: 20587525]

211. Meyer MB, Goetsch PD, Pike JW. Genome-wide analysis of the VDR/RXR cistrome in osteoblast cells provides new mechanistic insight into the actions of the vitamin D hormone. J Steroid Biochem Mol Biol. 2010; 121:136–141. [PubMed: 20171278]

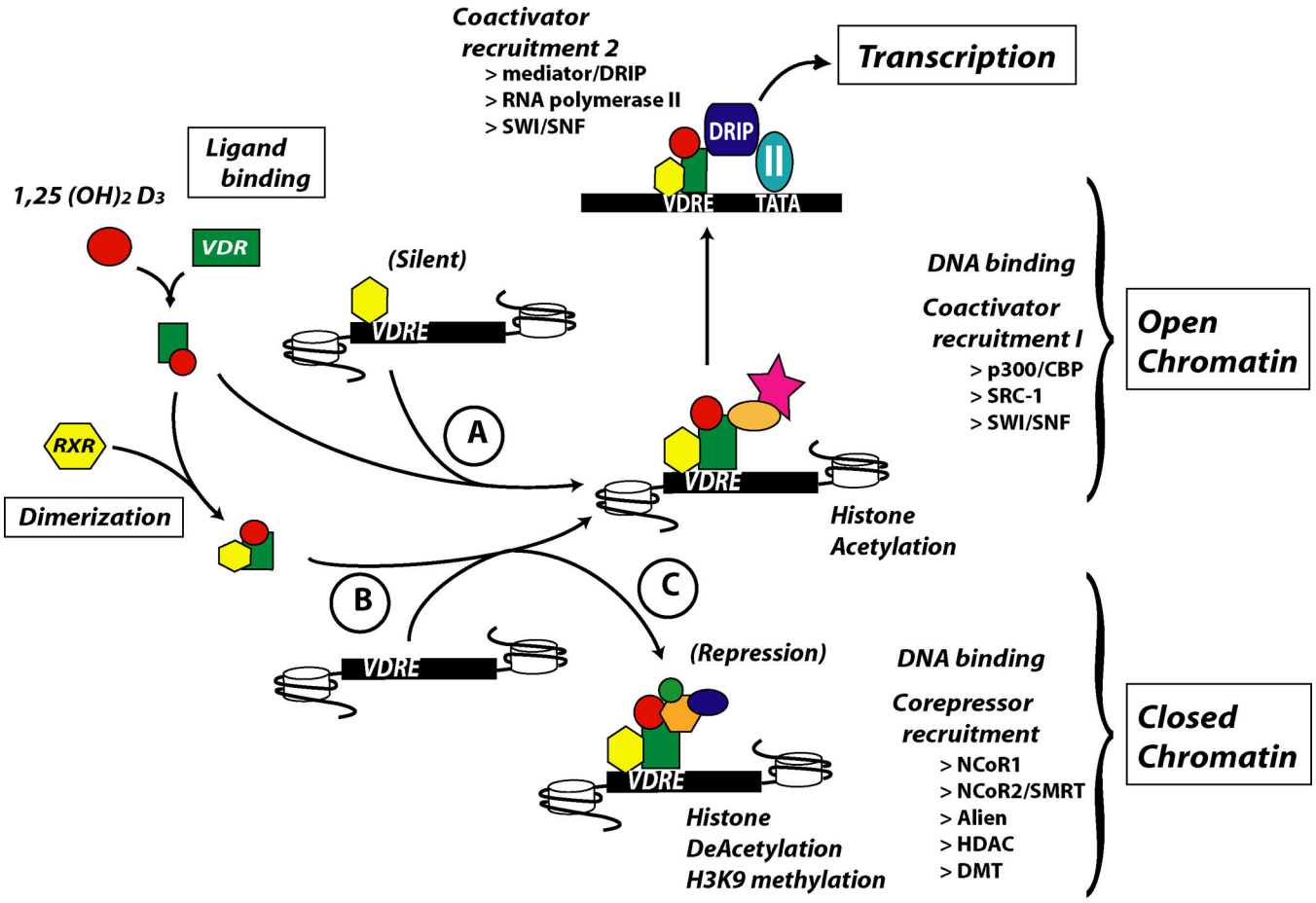

Figure 1.

Summary of vitamin D mediated gene transcription through the vitamin D receptor (VDR). (A) Schematic showing alternative models for binding of VDR-RXR dimers to DNA and the regulation of gene transcription by vitamin D. In model A, RXR (yellow hexagon) is resident. VDR (green rectangle) binds after being activated by ligand, releasing any co-repressor complexes if they are present. In model B, VDR and RXR dimerize then bind to DNA. Both model A and B lead to the recruitment of additional co-activators, e.g. p300/CBP (star) and SRC-1 (orange oval) for histone acetylation, ATP-dependent chromatin remodeling factors like SWI/SNF, the mediator complex (dark purple rectangle) for recruitment and activation of RNA polymerase II (light blue oval). Model C shows that the vitamin D-VDR complex can also recruit co-repressor complexes with histone deacetylase activity (purple oval) and DNA methyl transferase activity (green circle) to gene promoters. This will suppress gene transcription. (B) Schematic showing the distribution of VDR binding sites in the genome of osteoblasts [211] and lymphoblastoid cells [38] relative to the transcription start site (TSS) of a gene. Values are given as a percentage of total VDR binding sites and were determined by chromatin immunoprecipitation coupled to DNA arrays (ChIP-chip) or next-generation sequencing (ChIP-seq).

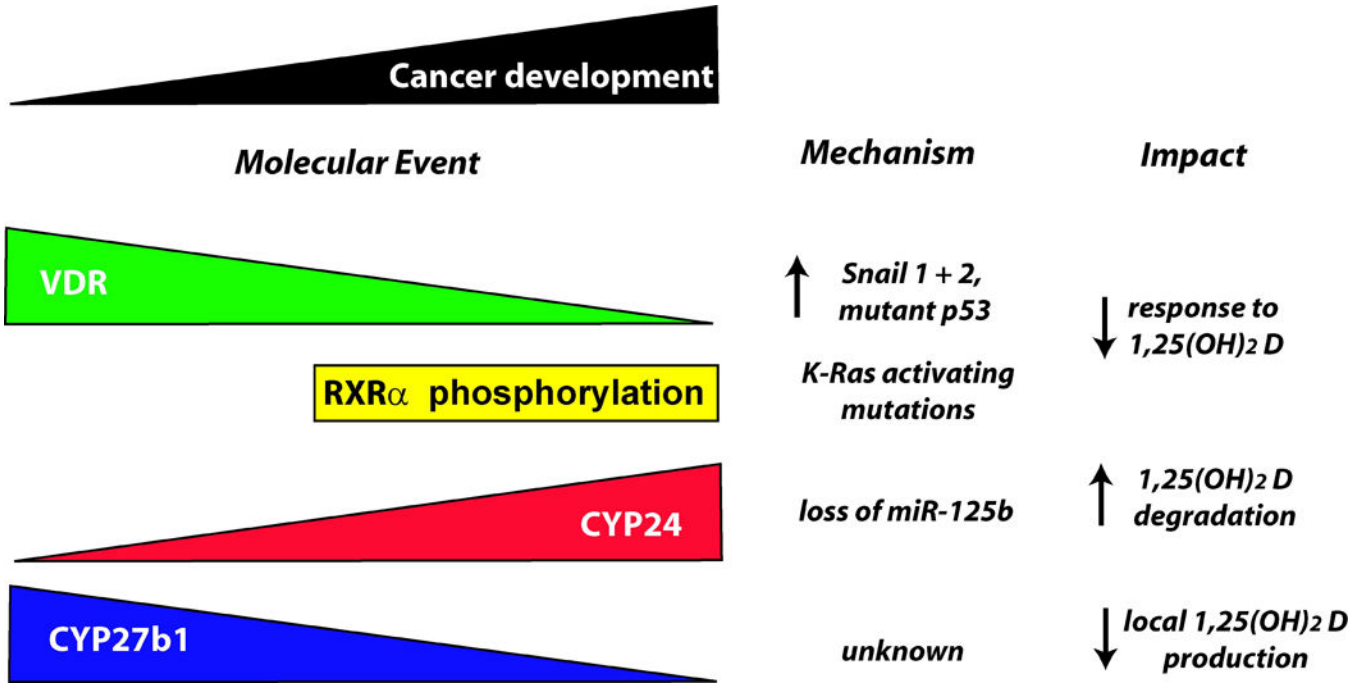

Figure 2.
A summary of the negative effects of cancer on vitamin D metabolism and action.

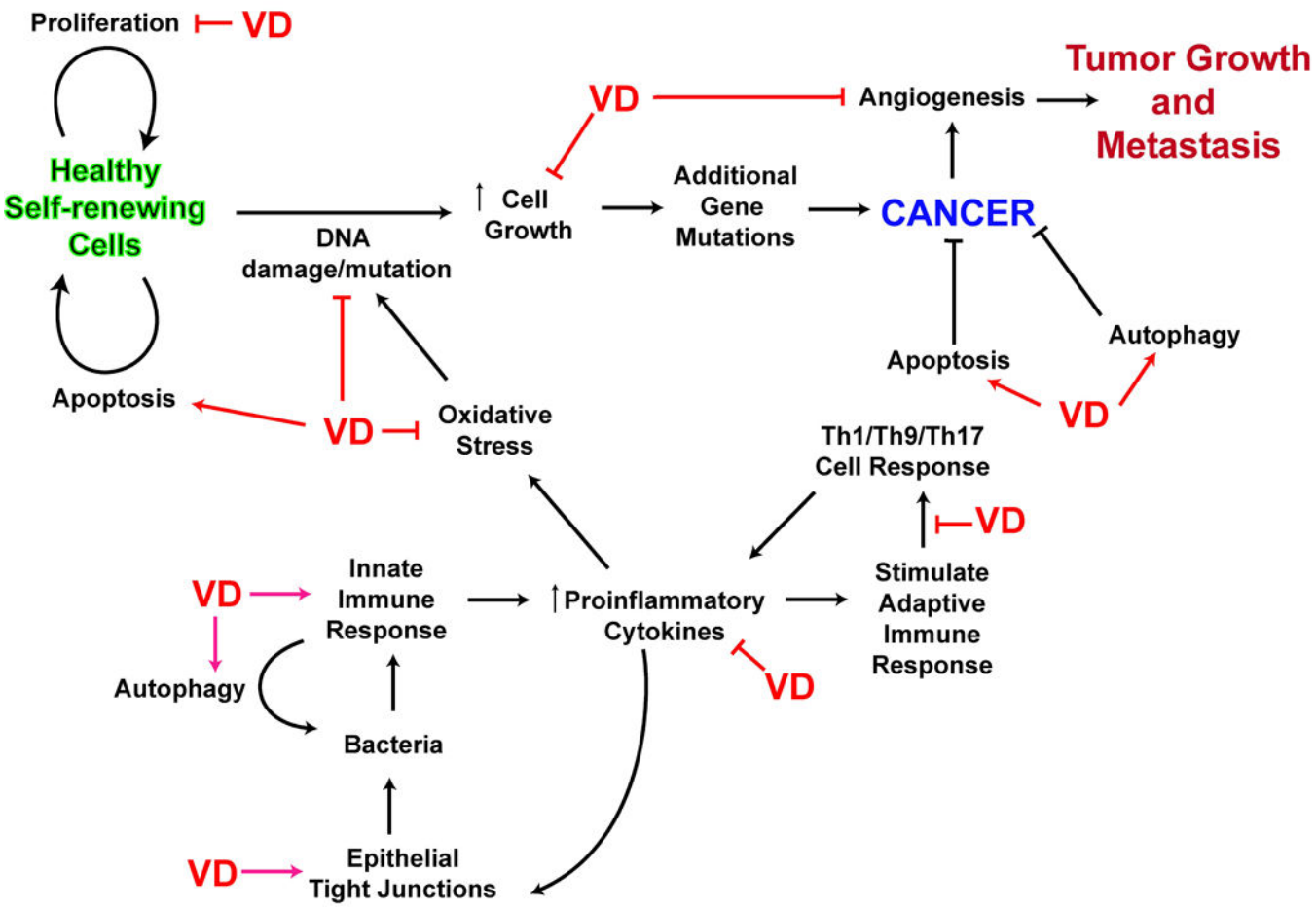

Figure 3.

A summary of the potential molecular events regulated by 1,25$(OH)_2$ D (VD) relevant to cancer.