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Review

Non-classical mechanisms of transcriptional regulation by the vitamin D receptor: Insights into calcium homeostasis, immune system regulation and cancer chemoprevention

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ABSTRACT

Hormonal 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] signals through the nuclear vitamin D receptor (VDR), a ligand-regulated transcription factor. Gene expression profiling studies have revealed that 1,25$(OH)_2$D signaling through the VDR can lead to activation or repression of target gene transcription in roughly equal proportions. Classically, transcriptional regulation by the VDR, similar to other nuclear receptors, has been characterized by its capacity to recognize high affinity cognate vitamin D response elements (VDREs), located in the regulatory regions of target genes. Several biochemical studies revealed that the VDRE-bound receptor recruits a series of coregulatory proteins, leading to transactivation of adjacent target genes. However, genome-wide and other analyses of VDR binding have revealed that a subset of VDR binding sites does not contain VDREs, and that VDREs are not associated with transcriptionally repressed VDR target genes. Work over the last ~20 years and in particular recent findings have revealed a diverse array of mechanisms by which VDR can form complexes with several other classes of transcriptional activators, leading to repression of gene transcription. Moreover, these efforts have led to several insights into the molecular basis for the physiological regulation of calcium homeostasis, immune system function and cancer chemoprevention by 1,25$(OH)_2$D/VDR signaling.

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Contents

1. Introduction

Vitamin D, obtained from supplements, limited dietary sources or photochemical and thermal conversion of 7-dehydrocholesterol in skin exposed to adequate solar ultraviolet B irradiation [1], must undergo two modifications to become biologically active. It is constitutively 25-hydroxylated in the liver to produce the major circulating form, 25-hydroxyvitamin D, which is then 1α-hydroxylated by the enzyme CYP27B1 to become hormonal 1,25$(OH)_2$D. CYP27B1 was originally characterized for its expression and function in the kidney, which is under control of calcium regulatory inputs such as parathyroid hormone (PTH). However, work in the past decade or so has revealed that CYP27B1 is widely expressed and its expression is under control of distinct physiological inputs in non-renal tissues [2,3]. These findings have

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accompanied a growing realization that $1,25(OH)_2D$ signaling has several physiological roles in addition to its calcium regulatory functions, notably in the immune system, and that it can function as a cancer chemopreventive agent [4–7]. The importance of the diverse physiological functions of $1,25(OH)_2D$ signaling have been bolstered by identification of target genes through gene expression profiling studies [8], and through several biochemical studies of the different mechanisms of transcriptional regulation by the VDR.

The VDR is a ligand-regulated transcription factor composed of an N-terminal DNA binding domain containing two zinc fingers, and a C-terminal ligand binding domain, which serves as a transcriptional regulatory domain [9,10]. It functions as a heterodimer with related retinoid X receptors (RXRs) and, similar to other nuclear receptors, VDR/RXR heterodimers can activate target gene transcription by binding to cognate DNA motifs called vitamin D response elements (VDREs). DNA-bound receptors recruit coactivators and other coregulatory complexes necessary for chromatin remodeling and RNA polymerase II recruitment. High affinity VDREs are composed of direct repeats of PuG(G/T)TCA half-sites separated by 3 bp (DR3 elements). Indeed, VDRE recognition by the VDR contributed to what was initially termed the 3-4-5 rule, wherein RXR heterodimerized with the VDR, thyroid hormone or retinoic acid receptors recognized related half sites separated by 3, 4 or 5 bp, respectively [11]. Subsequently, a series of elegant structural studies revealed the molecular basis for response element discrimination by RXRs heterodimerized with distinct partners, including the VDR [12,13].

The classical mechanism of ligand-induced dimerization and binding of VDR/RXRs to VDREs in target gene promoters has long been at the forefront of our understanding of $1,25(OH)_2D$-regulated gene transcription. However, evidence for alternative modes of gene regulation by the $1,25(OH)_2D$-stimulated VDR has been accumulating rapidly, notably in several recent studies describing the genome-wide association of VDR with chromatin [14–17]. Ramagopalan and colleagues cataloged VDR association with DNA genome-wide using ChIP coupled to massively parallel sequencing (ChIPseq) using lymphoblastoid cells [17]. Only 67% of all binding events contained apparent VDRE-like motifs, suggesting binding of VDR through tethering mechanisms to other sites. In particular, association of the receptor in unstimulated cells rarely occurred to DNA segments containing a VDRE, whereas this proportion increased in $1,25(OH)_2D$-treated cells. Stronger $1,25(OH)_2D_3$-induced VDR binding correlated with sites containing a VDRE. However, it should be noted that crosslinking may not efficiently capture the receptor tethered to DNA via another transcription factor, resulting in apparently weaker binding. In other ChIPseq studies, Heikkinen and colleagues [14], found that 80% of VDR-bound sites in unstimulated THP-1 monocytic cells and 10% of VDR binding events in $1,25(OH)_2D$-treated cells contained apparent non-VDRE motifs including those for SP1 (538 sites) and ETS transcription factors family member (274 sites). Evidence for the importance of the latter in mediating VDR-dependent *CYP24A1* gene expression is also provided in an earlier study [18]. Meyer et al. [16] observed in ChIPseq experiments on colon carcinoma cells an enrichment of binding sites for TCF4/β-catenin, CDX2 and C/EBPβ, which was also observed in several other reports [19–21]. Taken together, the reports of Heikkinen et al. [14] and Meyer et al. [16] also suggest, not surprisingly, that the DNA-bound factors to which the VDR tethers are cell-specific. In addition, studies in VDR-null mice with re-expressed wild-type or mutant human VDR reinforce the idea that receptor tethering could be an important mechanism of mediating $1,25(OH)_2D$-induced gene expression. Notably, DNA binding-incompetent VDR retained partial growth inhibition of mammary tumor cells similar to wild-type VDR [22]. Our goals here are to review the biochemical and molecular genetic evidence for the diverse non-classical mechanisms and the wide array of partners with which the VDR interacts to regulate gene transcription, and the insights these findings have provided into vitamin D physiology.

2. Molecular basis for negative feedback regulation of *PTH* gene transcription by $1,25(OH)_2D$

Some of the most strongly established negative feedback loops in vitamin D physiology target the calcium homeostatic signals that induce renal $1,25(OH)_2D$ production; specifically the inhibition by $1,25(OH)_2D$ of expression of *CYP27B1* in the kidney, and of the gene encoding PTH in the parathyroids, the principle inducer of renal CYP27B1 function. Transcriptional repression of these genes apparently occurs through similar mechanisms. Studies of the promoter of the *CYP27B1* gene in renal cells revealed the presence of tandem motifs of the E-box type (CANNTG) in the promoter that appeared to confer inhibition by $1,25(OH)_2D$ [23]. These motifs bind a factor designated VDR-interacting repressor (VDIR) [23], isolated by yeast one-hybrid assay using the *CYP27B1* tandem E-box motifs as a bait. VDIR [also known as transcription factor 3 (TCF3), immunoglobulin transcription factor (ITF1), or immunoglobulin enhancer-binding factor E2A] was originally identified as a transcription factor binding the immunoglobulin kappa chain enhancer [24]. It is a member of the bHLH family of transcriptional regulators and is homologous to transcription factors such as MYOD and c-MYC. Consistent with its identification as an enhancer factor, VDIR functions as a transactivator of the *CYP27B1* gene in the absence of the VDR [23]. However, addition of $1,25(OH)_2D$ leads to repression of gene transcription through VDR-VDIR protein-protein interactions. Subsequent work showed that the VDR also represses transcription from the *PTH* promoter through interacting with E-box-bound VDIR [25], although these experiments were not performed in parathyroid cells. Both studies showed that $1,25(OH)_2D$-induced interaction of the VDR with VDIR leads to dismissal of coactivators and recruitment of histone deacetylases and other cofactors associated with transcriptional repression [23,25].

3. Repression of transcription of the genes encoding interleukin-2 and granulocyte-macrophage colony-stimulating factor by the VDR

Two of the earliest studies demonstrating transcriptional repression by the VDR through interaction with other classes of transcription factors came from the group of Leonard Freedman, who was interested in determining how $1,25(OH)_2D$ suppressed T-lymphocyte proliferation. This was associated in part with cycloheximide-resistant suppression of interleukin-2 (*IL2*) and *GMCSF* gene transcription by $1,25(OH)_2D$ in T cells, consistent with direct repression by the VDR. Alroy et al. [26], found that the hormone-bound VDR repressed transcription of the *IL2* gene through a 40 bp region containing critical binding site for the transcription factor NFAT1 (nuclear factor of activated T cells) and AP-1 (activator protein 1). Repression required both the DNA and ligand-binding domains of the receptor, and *in vitro* biochemical experiments revealed that VDR/RXR heterodimers blocked NFAT1/AP1 complex formation and associated with the NFAT1 binding site of the *IL2* promoter, as measured by electrophoretic mobility shift assay. Later, Towers and Freedman [27] discovered that the VDR, in the absence of RXRs, was capable of inhibiting NFAT-1/AP-1 complex formation and DNA binding in a $1,25(OH)_2D$-dependent fashion. Interestingly, no recognizable VDRE was present at the VDR binding site in the promoter of the *GMCSF* gene. The role of AP-1 sites in $1,25(OH)_2D$-regulated gene expression is also exemplified by the studies of the rat

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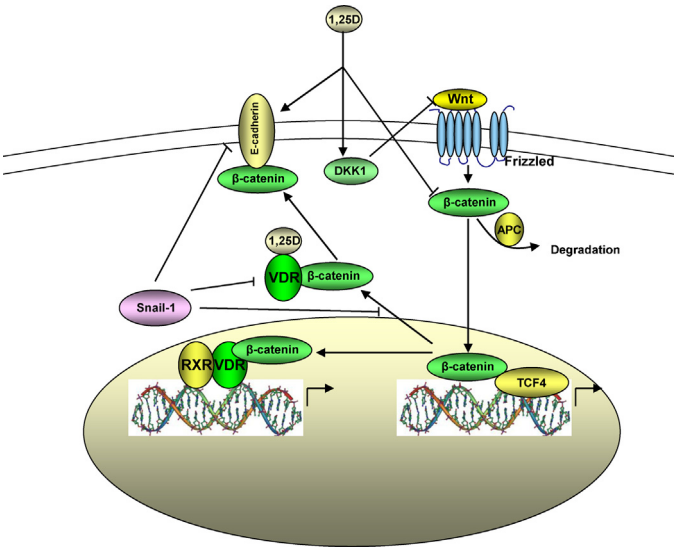

Fig. 1. Cross-talk between vitamin D and Wnt signaling pathways.

osteocalcin (OC) gene. Aslam et al. [28] showed that an internal AP-1 site within a VDRE in the OC gene promoter enhances VDR-mediated transcription and mutations in this sequence blocks the 1,25(OH)$_2$D-induced upregulation of the gene, suggesting a functional cooperation between VDR and AP1 factors.

4. Regulation by the VDR of oncogenic signaling pathways via protein–protein interactions

Dysregulated signaling of the Wnt pathway via β-catenin has been implicated in several malignancies, including prostate and colon cancers [29,30]. 1,25(OH)$_2$D signaling affects several components of the Wnt/β-catenin signaling pathway (Fig. 1). Notably, mutations in the genes encoding adenomatous polyposis coli (APC) and β-catenin that enhance β-catenin signaling through lymphoid enhancer-binding factor 1 (LEF1; also known as TCF1α) are predictors of cancer progression [31,32]. APC is a tumor suppressor and subunit of a multi-component cytoplasmic complex containing axin and GSK3β [33]. In the absence of Wnt ligands, the complex binds to β-catenin and promotes its phosphorylation, which eventually triggers its degradation [32]. It has been reported that 1,25(OH)$_2$D reduces β-catenin signaling by induction of *CDH1* (E-cadherin) gene expression [34,35]. E-cadherin is a transmembrane component of intercellular adherens junctions [36], which binds β-catenin, retains it in the cytoplasm and restricts its function in normal epithelial cells (Fig. 1) [37]. 1,25(OH)$_2$D also enhances binding of the VDR to β-catenin and promotes its nuclear export, an effect independent of E-cadherin [38]. Notably, while the ligand-bound VDR represses β-catenin function, there is evidence that β-catenin can also act as a coactivator of the VDR. β-catenin binds to the hormone-regulated activation function-2 (AF-2) in the ligand binding domain of the VDR and enhances transactivation by the DNA-bound VDR [38].

It is generally assumed that binding of β-catenin to members of the TCF/LEF family is cancer-promoting, although recent findings have suggested that TCF7L2/TCF4 may function as a transcriptional repressor that restricts breast and colorectal cancer cell growth [39]. This study proposed that 1,25(OH)$_2$D-induced TCF7L2 expression may have a protective role in colon cancer [39]. However, it should also be noted that function of β-catenin–TCF7L2 transcription complexes has been implicated in promoting epithelial to mesenchymal transition in cancer [40]. The inhibitory effects of the 1,25(OH)$_2$D-bound VDR on β-catenin signaling are antagonized by the transcription factor Snail1 (Fig. 1), which represses VDR expression and also abolishes the nuclear export of β-catenin induced by 1,25(OH)$_2$D in colon cancer cells [41]. In addition, Snail represses *CDH1* gene expression, thus enhancing β-catenin signaling [42,43].

1,25(OH)$_2$D also regulates differentially two genes encoding extracellular Wnt inhibitors DICKKOPF-1 and DICKKOPF-4 (*DKK1*, *DKK4*). 1,25(OH)$_2$D signaling indirectly stimulates expression of *DKK1* mRNA and protein. DKK-1 acts as a tumor suppressor in human colon cancer cells [44]. In contrast, *DKK4* gene expression was repressed by the hormone-bound VDR via recruitment of corepressor SMRT [45]. Although *DDK4* encodes an antagonist of the Wnt pathway, it is expressed in malignant, but not normal colon tissue. Moreover, ectopic DKK4 expression increased the migration and invasion properties of colon cancer cells [46].

Members of the Jun family, c-Jun, JunB and JunD, are components of AP-1 transcription factors. c-Jun is a *bona fide* oncoprotein and is overexpressed in a subgroup of undifferentiated, aggressive sarcomas. Recent work showed that the VDR binds to c-Jun and inhibits its function as a transactivator. In addition, c-Jun binds to the promoter of the *VDR* gene and induces its transcription [47], consistent with the VDR functioning in a negative feedback loop to inhibit Jun activity. In addition, two studies have linked regulation of c-Jun function by the VDR to 1,25(OH)$_2$D arrest of cell proliferation in breast cancer and osteosarcoma cells [48,49].

The VDR also interacts with p65, a subunit of the NF-kB transcription factor [50–52]. NF-kB regulates genes controlling inflammation, cell growth, apoptosis, cancer invasion/metastasis, and tumor promotion [53]. NF-kB functions as a dimer of different subunits, with the classic combination being the p65/p50 heterodimer, which is sequestered in the cytoplasm as an inactive complex with inhibitor IkB [54]. Several studies have suggested that p65 and the VDR are mutually repressive. P65 binding to the DNA-bound VDR inhibits coactivator recruitment and suppresses transactivation by the VDR [50]. Other work has suggested that the interaction between p65 and the VDR suppresses NF-κB function. In addition, 1,25(OH)$_2$D represses NF-kB signaling indirectly by increasing IkBα gene expression [51,55,56]. These studies are consistent with the observation that NF-κB activity is elevated in fibroblasts lacking the VDR [52]. However, the two transcription factor can collaborate under some conditions. Our work has suggested that the VDR and NF-κB can cooperatively induce expression of the *DEFB4*/*HBD2* antimicrobial peptide gene in human monocytes [57], providing a mechanism for stimulation of innate immune responses to infection.

There is also cross-talk between the VDR and the SMAD3 transcription factor. SMAD proteins (SMAD1–SMAD8) are transducers of signaling by the transforming growth factor-β (TGF-β) family [58,59]. TGF-β inhibits cell proliferation, induces apoptosis and mediates differentiation, suggesting that components of TGF-β signaling pathways have tumor-suppressor activity in epithelial tumors [60,61]. TGF-β is the prototypic member of a family of secreted proteins that includes TGF-β, activins and bone morphogenetic proteins (BMPs). TGF-β signals through type I receptor serine-threonine kinases and induces SMAD2 and SMAD3 function [62,63]. Phosphorylated SMADs bind to SMAD4, the common mediator of TGF-β pathways, and translocate into the nucleus where they regulate target genes either by interacting directly with DNA or with other transcription factors [63,64]. SMAD3 binds to the VDR via its MH1 domain and acts as a coactivator of 1,25(OH)$_2$D-induced transactivation by forming a complex with SRC1 [65] and the VDR, and SMAD3 can regulate synergistically common target genes containing tandem VDRE and SMAD-binding motifs [65,66]. In rat prostatic epithelial cells, 1,25(OH)$_2$D-induced expression of TGF-β target genes via activation of several components of the TGF-β signaling pathway [67]. 1,25(OH)$_2$D also induced expression of both TGF receptors and TGF-β ligand in promyelocytic

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HL-60 cells, and the antiproliferative activity of $1,25(OH)_2D$ was blocked by a TGF-β-neutralizing antibody [68]. $1,25(OH)_2D$ induces phosphorylation of SMAD3 and cell differentiation through induction of TGF-β1 protein expression [69].

5. The ligand-bound VDR as a regulator of Sirt1 and FoxO protein function

FoxO proteins FoxO1 (FKHR), FoxO3A (FKHRL1), FoxO4 (AFX) and FoxO6 regulate cell proliferation and differentiation. They are inhibited by PI3 kinase, which stimulates their Akt-dependent phosphorylation and nuclear export [70–74]. In cancer cells lacking PI3 kinase antagonist PTEN, FoxO proteins are cytoplasmic and inactive. FoxO3a phosphorylation can be reversed by phosphatases [75]. Notably, phosphorylated FoxOs are targeted to the proteasome by $p45^{SKP2}$ [75–77], and there is an inverse correlation between SKP2 and FoxO1 expression in cancer [76,77]. FoxO proteins are also regulated by acetylation, which can be reversed by the class III histone deacetylase Sirt1 [78]. Acetylation reduces DNA binding to certain target genes and enhances phosphorylation and inactivation [79]. FoxO protein function has been shown to control longevity in a range of organisms [70–74], and recent genome-wide association studies have linked variants in the human FoxO3a gene to extreme longevity in humans of Japanese and German ancestry [80,81]. Serial ablation in mice of genes encoding FoxO proteins demonstrated that they are *bona fide* tumor suppressors [75–77]. FoxO proteins can activate or repress transcription, and microarray studies revealed that gene repression by FoxO1 was particularly important for its inhibitory effects on the cell cycle [82]. FoxO repression of D-type cyclin gene transcription [79,82–84] is relieved by PI3 kinase induction, which is essential for stimulation by cMYC of cyclin D2 (CCND2) expression and transformation [84]. Moreover, FoxO and c-MYC target genes partially overlap, and FoxO factors repress a subset of cMYC-regulated genes [84,85].

Our previous analysis of $1,25(OH)_2D$-regulated expression profiles in head and neck squamous carcinoma cells [8], revealed a partial overlap of VDR and FoxO target genes [86], including *CCND2*. Vitamin D signaling can stimulate FoxO protein function by both rapid and longer-term mechanisms. Treatment of cancer cells with $1,25(OH)_2D$ suppresses expression of $p45^{SKP2}$, the ubiquitin ligase that regulates FoxO protein proteasomal turnover [77,87], leading to a graduate increase in FoxO protein levels in the presence of $1,25(OH)_2D$ [86]. Notably the VDR was shown to interact with transcription factor Sp1 in prostate cancer cells [88]. VDR/Sp1 complexes bound to the *SKP2* promoter *in vivo* in a partially $1,25(OH)_2D$-dependent manner, and $1,25(OH)_2D$-induced recruitment of HDAC1 and repression of *SKP2* expression.

Of more interest, we found that the VDR associated directly with FoxO proteins independent of $1,25(OH)_2D$, whereas addition of $1,25(OH)_2D$ enhanced recruitment of FoxO cofactor Sirt1 to the VDR (Fig. 2) [86]. The VDR also bound constitutively to the catalytic subunit of protein phosphatase 1 (PP1c), consistent with previous studies demonstrating the ligand-independent association of PP1c with the VDR in CaCo2 colon carcinoma cells [89]. This latter study also showed that $1,25(OH)_2D$ binding to the VDR stimulated associated phosphatase activity [89]. The interactions of the VDR with FoxO proteins and their cofactors had profound effects on FoxO protein function. $1,25(OH)_2D$ rapidly (<4 h) induced FoxO deacetylation and dephosphorylation, consistent with activation, which coincided with increased binding of FoxO proteins to promoters of target genes (Fig. 2) [86]. Chromatin immunoprecipitation assays revealed the presence of both the VDR and Sirt1 on FoxO target genes, and ablation of FoxO or Sirt1 expression attenuated or eliminated $1,25(OH)_2D$-dependent regulation of common VDR/FoxO target genes [86]. Most importantly, $1,25(OH)_2D$-dependent cell cycle arrest was blocked in FoxO3a-deficient cells, indicating that FoxO proteins are key downstream mediators of the antiproliferative actions of $1,25(OH)_2D$. Given the well-establish roles of FoxO proteins as tumor suppressors, these findings provide a molecular basis for the cancer chemopreventive actions of vitamin D.

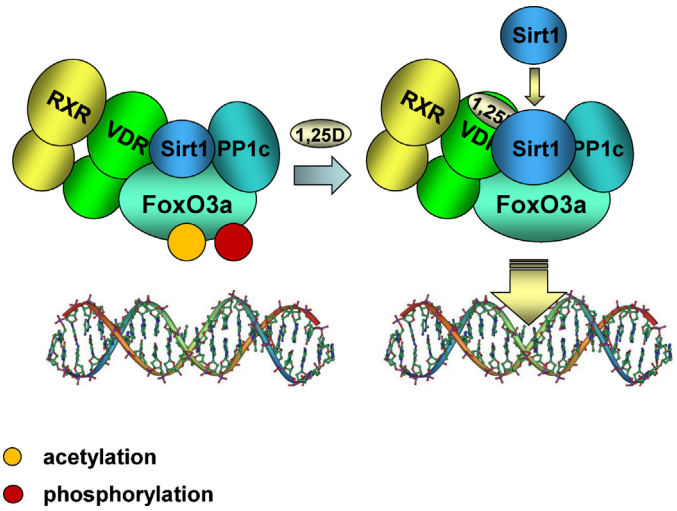

Fig. 2. Regulation of FoxO protein post-translational modification and DNA binding by the ligand-bound VDR.

It will be important to explore the regulation by vitamin D signaling of Sirt1 function in more detail. Other work has suggested that Sirt1 controls FoxO function selectively, enhancing the regulation of genes implicated in cell cycle arrest, but suppressing regulation of proapoptotic genes [78,90–92]. Initial data suggest that the VDR is a selective regulator of Sirt1 function. Sirt1-dependent deacetylation inhibits p53-dependent transactivation [93]. However, deacetylation of p53 was not observed in $1,25(OH)_2D$-treated cells [86]. $1,25(OH)_2D$-driven VDR function has been strongly implicated in colon cancer chemoprevention, and the VDR interacts directly with β-catenin and inhibits its activity [94]. Notably, Sirt1 suppressed intestinal tumorigenesis in a β-catenin-driven model of colon cancer in mice, and Sirt1 deacetylated β-catenin and promoted its nucleocytoplasmic shuttling [95]. $1,25(OH)_2D$ may thus stimulate Sirt1-induced β-catenin deacetylation. Similarly, Sirt1 and $1,25(OH)_2D$ signaling through the VDR inhibit NF-κB function [96,97], suggesting that $1,25(OH)_2D$ may enhance NF-κB deacetylation though its interaction with Sirt1.

Sirt1 has emerged as somewhat of a double-edged sword in cancer prevention and tumorigenesis [93,98,99]. Sirt1-null embryos display impaired DNA damage responses and genomic instability and Sirt+/−; p53+/− mice spontaneously develop multiple tumors [100]. As mentioned above, Sirt1 may suppress β-catenin-driven colon tumorigenesis [95]. Moreover, a survey of recent experiments assessing cancer susceptibility of genetically modified mouse models concluded that there was no evidence for Sirt1 function contributing to tumor development and that the bulk of the evidence supported a role in cancer prevention. However, Sirt1 is overexpressed in a number of tumors, can block cellular senescence and differentiation, and Sirt1 inhibitors have anticancer properties in animal models [99]. It will therefore be of interest to investigate further the extent of the regulation of Sirt1 function by the hormone-bound VDR.

6. Regulation of the c-MYC/MXD1 network by the hormone-bound VDR

The transcription factor c-MYC is a critical driver of cell cycle progression, and elevated or deregulated expression of c-MYC

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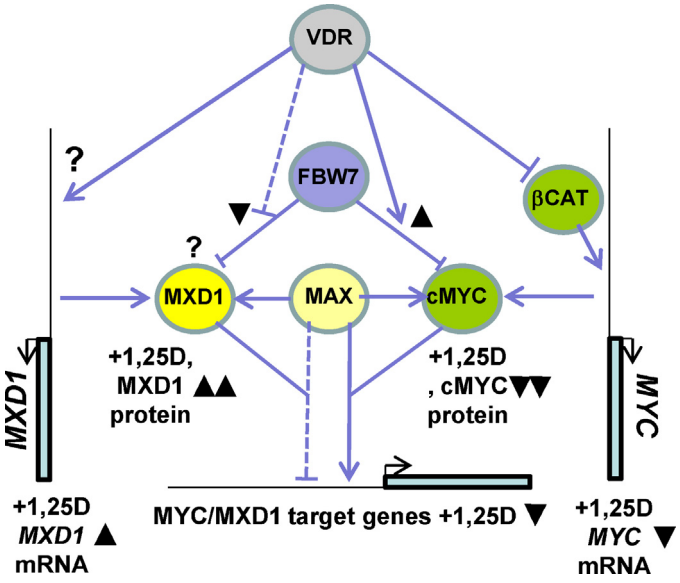

Fig. 3. Schematic representation of the regulation of the c-MYC/MXD1/MAX network by 1,25D and the VDR. See text for details. Note that the mechanisms of downregulation of *MXD1* gene expression and the regulation by FBW7 of MXD1 proteasomal turnover have yet to be determined.

is widespread in cancer [101]. Similar to VDIR/E2A discussed above, c-MYC is a bHLH-domain-containing protein that binds cognate E-box motifs (CACGTG) as a heterodimer with DNA binding partner MAX to induce expression of cell cycle regulatory genes such as *CCND2* and *CDK4*. It also represses expression of cyclin-dependent kinase (CDK) inhibitors through interaction with MIZ-1 [101]. c-MYC is antagonized by the transcriptional repressor MAD1/MXD1, which also heterodimerizes with MAX and recruits corepressors to inhibit c-MYC target gene transcription [102]. c-MYC is highly regulated post-translationally and its turnover is controlled largely via the SCF ubiquitin ligase complex containing the E3 ligase F-box protein FBW7 (FBXW7/Sel-10/Ago/hCDC4), which recognizes and ubiquitinates Ser62/Thr58 doubly phosphorylated c-MYC [103].

The first hints that $1,25(OH)_2D$ signaling comprehensively regulated c-MYC function came from the observation that $1,25(OH)_2D$ treatment over 24–48 h suppressed expression of $p45^{SKP2}$ [104], which, while functioning as an inducer of FoxO protein turnover (see above), acts as a coactivator of c-MYC [105,106]. More recent work has revealed that $1,25(OH)_2D$ regulates several aspects of the expression and function of both c-MYC and MXD1 [107] (summarized in Fig. 3). $1,25(OH)_2D$ treatment of SCC25 human head and neck squamous carcinoma cells, primary human keratinocytes or promyelocytic HL60 cells led to a dramatic drop in c-MYC protein levels. Significantly, coimmunoprecipitation experiments revealed a rapid $1,25(OH)_2D$-dependent association of the VDR with c-MYC, consistent with the effects of $1,25(OH)_2D$ on c-MYC driving cell cycle arrest rather than being a product of it. The sharp drop in total c-MYC protein was brought about by a combination of effects of $1,25(OH)_2D$ on *MYC* transcription and enhanced c-MYC turnover, whereas expression of c-MYC heterodimeric partner MAX was unaffected. $1,25(OH)_2D$-induced c-MYC turnover was largely attenuated by ablation of either VDR, or FBW7 expression, suggesting that $1,25(OH)_2D$ regulates c-MYC turnover largely through FBW7 [107]. Strikingly, $1,25(OH)_2D$ signaling had exactly the opposite effects on MXD1 expression and stability, with a rapid $1,25(OH)_2D$-dependent association of MXD1 with the VDR, leading to a dramatic increase in its expression. MXD1 turnover was also reduced, although not abolished, in FBW7-deficient cells, revealing that FBW7 regulates both the activator and repressor arms of the c-MYC/MXD1 network.

$1,25(OH)_2D$ treatment led to a dramatic loss of DNA-bound c-MYC and a corresponding increase in DNA-bound MXD1 and associated cofactors HDAC2 and mSIN3A [107]. Moreover, the VDR was directly associated with DNA-bound c-MYC or MXD1, as confirmed by reChIP experiments. It is not clear whether hormone-dependent binding of the VDR to c-MYC and MXD1 induced dissociation of c-MYC and binding of MXD1 to DNA, or whether the exchange was a consequence of the effects of $1,25(OH)_2D$ on c-MYC and MXD1 expression and turnover. It is noteworthy that reanalysis at identical stringencies of the distribution of VDR and c-MYC peaks in published ChIPseq data sets from related lymphoblastoid cell lines [17,108] revealed a substantial overlap between a subset of VDR binding sites detected in the absence or presence of $1,25(OH)_2D$ and high fidelity c-MYC sites [107], consistent with the hormone-bound VDR regulating c-MYC/MXD1 signaling at numerous genomic sites.

In addition to the above, c-MYC protein levels were elevated in VDR-deficient cells [107]. This arose from elevated β-catenin-driven expression of *MYC* transcription in the absence of the VDR, consistent with the roles described above of $1,25(OH)_2D$ and the VDR in regulating Wnt signaling. These observations were highly intriguing as loss of epidermal VDR leads to elevated β-catenin signaling in *vdr*−/− mice [109], which also display a hyperproliferative phenotype in colonic epithelia [109]. *Vdr* null mice or patients with vitamin D-resistant rickets due to inactivating *VDR* mutations [109,110] develop alopecia due to dysregulated epidermal differentiation. Regulated c-MYC expression is critical for normal epidermal differentiation as its overexpression depletes epidermal stem cells [111–113], and, remarkably, disrupts hair follicle development and increases sebaceous activity, very similar to *vdr* knockout [109]. In addition, β-catenin function and c-MYC expression are often elevated in colon cancer [114,115]. Consistent with the above, substantially elevated c-MYC was found in both skin and colon of null mice, with colonic overexpression visible in epithelial crypt cells. Moreover, topical $1,25(OH)_2D$ suppressed expression of c-MYC and the product of its epidermal target gene, *setd8* [116] in mouse skin, and increased MXD1 levels in the skin of wild-type but not *vdr*−/− mice [107]. These results are thus consistent with c-MYC overexpression contributing to alopecia and to colonic epithelial hyperproliferation observed in *vdr*−/− mice, and provide fundamental insights into the roles of VDR signaling in cancer prevention, as well as in control of epidermal differentiation.

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