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REVIEW

New insights into vitamin D anticancer properties: focus on miRNA modulation

Katarina Zeljic[1] · Gordana Supic[2,3] · Zvonko Magic[2,3]

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Abstract Vitamin D anticancer properties are well known and have been demonstrated in many in vitro and in vivo studies. Mechanistic insights have given an explanation on how vitamin D exerts antineoplastic functions, which are mainly conducted via the canonical vitamin D receptor (VDR)–vitamin D response elements (VDRE) pathway. Numerous findings indicate that dietary components, including vitamin D, could exert chemopreventive effects through alterations of microRNA (miRNA) expression. As miRNAs have important roles in regulating diverse and vital cellular processes, it has been speculated that vitamin D's non-classical effects, including anticancer effects, could be mediated through alterations of miRNA expression level. The current review focuses on up-to-date experimental data on modulation of miRNA expression by vitamin D treatment in cancer, obtained in a cell culture system, animal models and human cohorts. Reported findings in the review show that vitamin D modulates expression of numerous and diverse miRNAs specific for cancer types. Even in its early phases, with many questions remaining to be answered, dissecting the molecular pathways of vitamin D miRNA modulation is an emerging area of science. The complete unraveling of vitamin D molecular mechanisms will emphasize the vitamin D dietary component as a potential chemopreventive agent in cancer and personalized nutrition.

Keywords Vitamin D · miRNA · Cancer

Introduction

Numerous studies have demonstrated the anticancer effects of various bioactive dietary compounds (DiMarco-Crook and Xiao 2015; Chimento et al. 2016; de la Parra et al. 2016), suggesting their potential use as chemopreventive agents. However, the molecular mechanisms linking nutrition and cancer are not fully elucidated. Nutrients can influence numerous cellular processes involved in cancer development and progression by regulation of gene expression through epigenetic mechanisms, such as DNA methylation, histone modifications and non-coding RNAs (Supic et al. 2013, 2016).

Among the nutrients, vitamin D attracts huge scientific interest due to its association with cancer risk and treatment (Deeb et al. 2007; Feldman et al. 2014). This review summarizes the recent findings on the molecular mechanism of vitamin D action, with focus on microRNA regulation and function in cancer.

Vitamin D synthesis, degradation and mechanism of action

Vitamin D is a fat-soluble steroid hormone with a wide spectrum of physiological effects throughout the body

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✉ Katarina Zeljic
katarina.zeljic@bio.bg.ac.rs; katjaze@yahoo.com

[1] Faculty of Biology, University of Belgrade, Studentski trg 3, Belgrade 11000, Serbia

[2] Faculty of Medicine, Military Medical Academy, University of Defence, Belgrade, Serbia

[3] Institute for Medical Research, Military Medical Academy, Belgrade, Serbia

(Dusso et al. 2005). Vitamin D is present in human skin in its precursor form (7-dehydrocholecalciferol) and converted to vitamin D_3 using the energy of ultraviolet radiation in sunlight. It can also be taken in the diet from food rich in cholecalciferol or ergosterol, vitamin D-fortified dairy products and supplements. These forms of vitamin D are then converted to the active form calcitriol [$1\alpha25(OH)_2D_3$] through two steps of hydroxylation at the 1-alpha and 25-C positions (Dusso et al. 2005). The first step of hydroxylation occurs in the liver, which is mediated by a 25-hydroxylase enzyme (such as CYP2R1, CYP27A1 and CYP2D25), and results in the synthesis of 25-hydroxyvitamin D_3 [$25(OH)D_3$], also known as calcidiol. Calcidiol is the main circulating form of vitamin D, which is transported through the bloodstream to the kidney for the second step of hydroxylation catalyzed by the 1α-hydroxylase (also known as CYP27B1).

In addition to 25- and 1α-hydroxylases, CYP24A1, a 24-hydroxylase enzyme also plays an important role in vitamin D metabolism. This enzyme is expressed in all cells that are responsive to calcitriol and protects the body from its excess (Feldman et al. 2014). CYP24A1 converts both $25(OH)D_3$ and $1\alpha25(OH)_2D_3$ into 24-hydroxylated products, which are molecules with reduced or no apparent biological activity. Besides CYP24A1, the synthesis of calcitriol is also tightly controlled by two hormones, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) (Feldman et al. 2014). PTH stimulates 1α-hydroxylase production and promotes calcitriol synthesis (Bikle 2014). On the contrary, FGF23 induces expression of 24-hydroxylase, but also directly suppresses activity and expression of the 1α-hydroxylase, thus preventing completion of the $1\alpha25(OH)_2D_3$ synthesis (Dusso et al. 2011; Bikle 2014). Increased calcium levels in blood inhibit PTH secretion and consequently suppress 1α-hydroxylase activity, while increased phosphate levels stimulate FGF23 expression (Bikle 2014).

The biological actions of calcitriol are mediated through the vitamin D receptor (VDR), which is a member of the steroid receptor family that acts as a nuclear receptor transcription factor (Fig. 1a). Calcitriol binds to the VDR, dimerizes with the retinoid X receptor (RXR) and forms a heterocomplex that interacts with the vitamin D response elements (VDRE) located in the promoter region of numerous target genes. This results in the recruitment of co-activators or co-repressors that modulate the transcriptional regulation of target genes. Apart from vitamin D-mediated genomic actions, vitamin D can also bind to plasma membrane caveolae-associated VDR, which activates multiple signaling pathways, such as phosphatidylinositol-3′-kinase (PI3K), phospholipase C and protein kinase C (PKC) (Haussler et al. 2011).

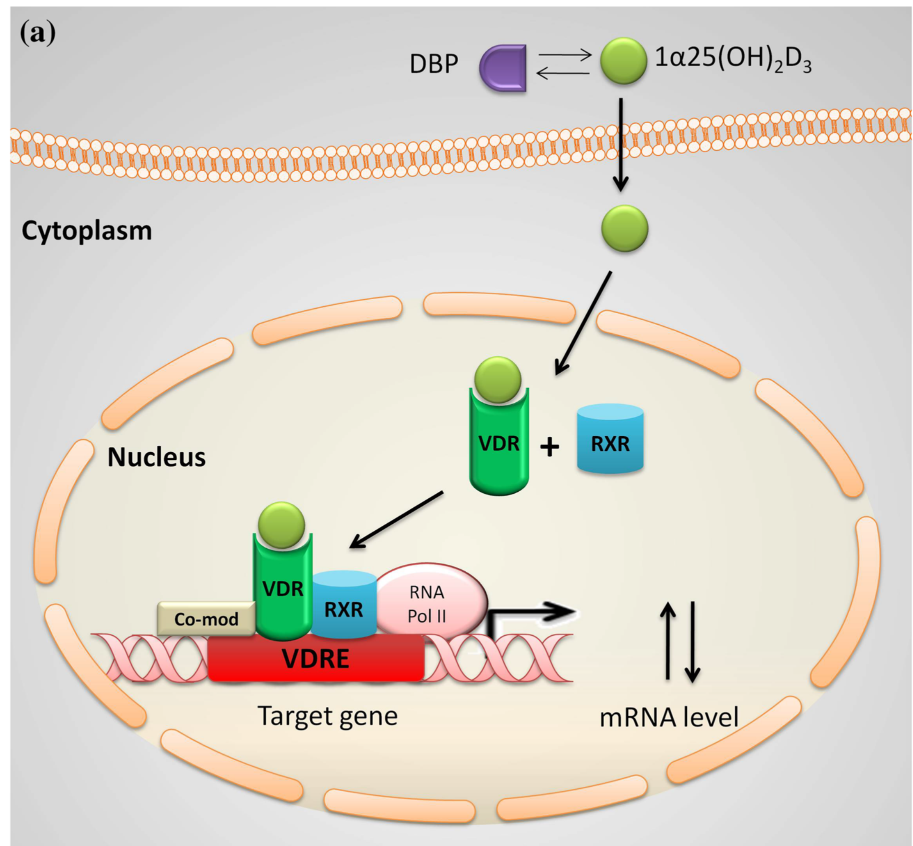
Role of vitamin D in cancer

The anticancer effects of vitamin D were first reported in vitro more than three decades ago (Abe et al. 1981; Colston et al. 1981). At the time it had been shown that growth of malignant melanoma cells was inhibited in the presence of vitamin D (Colston et al. 1981), and that vitamin D induced differentiation of myeloid leukemia cells to macrophages (Abe et al. 1981). Since then, an increasing number of studies have confirmed one of vitamin D's properties to be an anticancer effector in various cancer types (Deeb et al. 2007; Feldman et al. 2014). Also, multiple potentials of vitamin D and its synthetic analogs have been evaluated as an efficient treatment in cancer patients, with minimal risk of side effects, in numerous clinical studies (Feldman et al. 2014). However, knowing all components of the vitamin D anticancer molecular pathway would be of great importance for fully understanding and possible application of vitamin D to cancer prevention and treatment.

Anticancer effects of vitamin D include inducing differentiation and apoptosis, and inhibition of proliferation, angiogenesis, invasion and metastasis (Deeb et al. 2007; Feldman et al. 2014) (Fig. 1b). Specific signaling pathways are regulated by vitamin D in colon, breast and prostate cancers (Feldman et al. 2014). For instance, vitamin D inhibits β-catenin transcriptional activity through repression of the WTN-β catenin signaling pathway which is activated in most colorectal cancers (Larriba et al. 2011, 2013).

Anti-proliferative effects of vitamin D are mainly mediated by increased expression of cyclin-dependent kinase inhibitors p21 (WAF1/CIP1) and p27 (KIP1) leading to G0/G1 cell cycle arrest (Deeb et al. 2007). Inhibition of growth factors, i.e., insulin growth factor 1 (IGF1) and epidermal growth factor (EGF), and inducing the expression of growth factor inhibitors, such as transforming growth factor beta (TGFβ), lead to the inhibition of cancer cell proliferation (Vuolo et al. 2012). Vitamin D acts as an inhibitor of telomerase activity by reducing the expression of telomerase reverse transcriptase (TERT), which also induces apoptosis (Kyo and Inoue 2002; Jiang et al. 2004). Modulation of kinase pathways, such as ERK-MAPK and PI3K has been documented (Deeb et al. 2007). Proliferation of cancer stem-like cells is inhibited by vitamin D through cell cycle arrest (Peng et al. 2016).

Induction of apoptosis upon vitamin D treatment has been demonstrated in different cancer types, such as breast, colon, prostate, melanoma, and glioblastoma. (Hansen et al. 2001). Apoptosis is mainly triggered by suppression of anti-apoptotic genes, i.e., *BCL2* and inducing pro-apoptotic *BAX* and *BAK* (Lamprecht and Lipkin 2003). Caspase pathways are also triggered by vitamin D (Feldman et al. 2014).



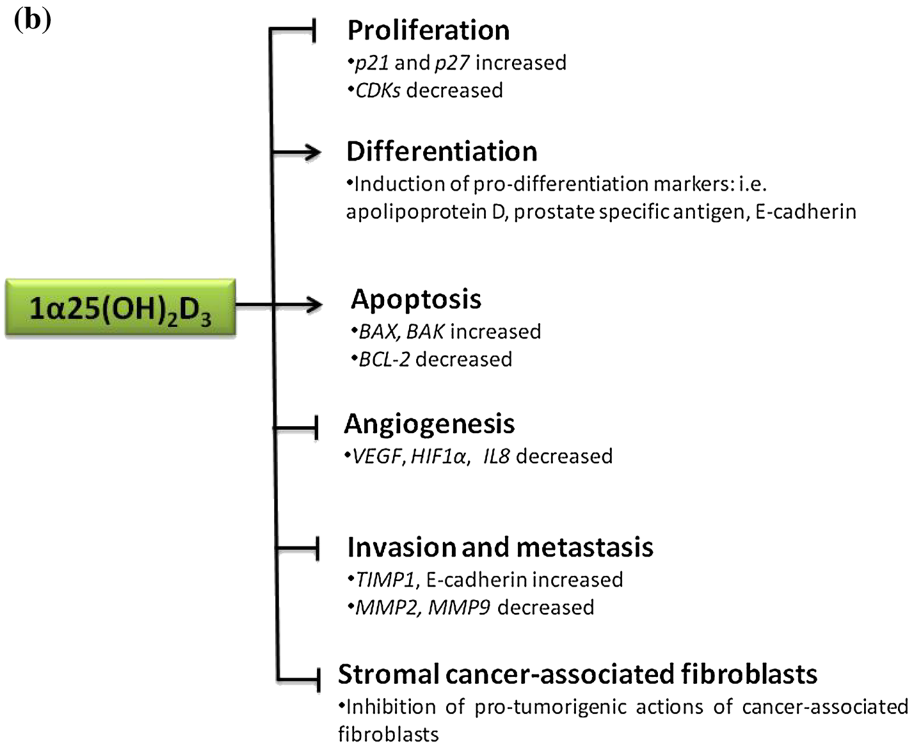


Fig. 1 Genomic action of metabolite active form of vitamin D (calcitriol) and its anticancer properties. **a** Vitamin D ($1\alpha25(OH)_2D_3$) is transported through the bloodstream by vitamin D-binding protein (DBP). Dissociated from the DBP, vitamin D binds to its receptor vitamin D receptor (VDR), which activates binding of the Retinoid X Receptor (RXR). A heterodimer consisting of vitamin D, VDR and RXR interacts with the vitamin D response elements (VDRE) located in the promoter region of the target gene which recruits co-modulators (*co-mod* co-activators and co-repressors). As a result, expression of the target gene will be induced or suppressed. **b** Vitamin D anticancer properties and examples of target genes. *p21 CDKN1A* (*WAF1*/*CIP1*)—cyclin-dependent kinase inhibitor 1 A; *p27 CDKN1B*—cyclin-dependent kinase inhibitor 1B; *CDKs* cyclin-dependent kinase, *VEGF* vascular endothelial growth factor, *HIF1α* hypoxia-inducible factor 1α *IL8* interleukin 8, *TIMP* tissue inhibitor of metalloproteinases 1, *MMP2, MMP9* matrix metalloproteinase

Stimulation of differentiation in response to vitamin D is demonstrated in various cancer types (Gocek and Studzinski 2009). One of the first examples was vitamin D-induced differentiation of leukemia cells into monocytes by increased expression of p21 (Liu et al. 1996). Vitamin D treatment-induced pro-differentiation markers, such as apolipoprotein D, prostate-specific antigen and E-cadherin (Palmer et al. 2001; Gocek and Studzinski 2009). Pro-differentiation mechanisms which include specific signaling pathways, such as WNT-β catenin, PI3K, NF-kβ are also regulated by vitamin D (Deeb et al. 2007; Gocek and Studzinski 2009).

Invasion and metastasis are mitigated by vitamin D through inhibition of cathepsins and matrix metalloproteinase (MMP), such as MMP2 and MMP9 (Bao et al. 2006b; Chen et al. 2015), increase of tissue inhibitors of metalloproteinase-1 (TIMP-1), and cathepsin inhibitors (Bao et al. 2006b) as well as E-cadherin expression (Lopes et al. 2012).

Vitamin D can inhibit angiogenesis by suppressing expression of vascular endothelial growth factor (VEGF) through hypoxia-inducible factor 1 alpha (HIF1α) inhibition (Mantell et al. 2000; Ben-Shoshan et al. 2007) and interleukin 8 (IL8) (Bao et al. 2006a). In some studies VEGF was found to increase upon vitamin D treatment (Fernandez-Garcia et al. 2005; Garcia-Quiroz et al. 2014), which suggests that vitamin D effects on angiogenesis might depend on tumor and cell type.

Recent evidences show that vitamin D inhibits pro-tumorigenic actions of stromal cancer-associated fibroblasts (CAFs) surrounding the tumour mass, probably through inhibition of NF-kB signaling (Shany et al. 2016). It has been reported that migration of CAFs, derived from colorectal cancer patients, has been inhibited due to vitamin D treatment (Ferrer-Mayorga et al. 2016). Furthermore, vitamin D modulates expression of numerous genes in CAFs, which was associated with longer survival of colorectal cancer patients (Ferrer-Mayorga et al. 2016). Also, vitamin D imposes switching CAFs pro-tumorigenic into more benign phenotype (Ferrer-Mayorga et al. 2016). These new findings expanded the translational importance of using vitamin D as an anticancer agent in treatment of not just tumour mass but also cancer-associated fibroblasts.

MicroRNA and regulation of vitamin D signaling

MicroRNA (miRNA) is a class of small non-coding RNA (ncRNA) with ~18 to 22 nucleotides, which has an important role in post-transcriptional regulation of gene expression and gene silencing. This class of ncRNAs is involved in regulation of numerous key cellular processes, including development, differentiation, cell proliferation, and apoptosis (Kim et al. 2009). Biogenesis of miRNA is a complex multistep, tightly controlled process (Fig. 2). Once synthesized, miRNAs regulate gene expression by directly binding to a complementary sequence in the 3′-untranslated region (3′-UTR) of mRNA, causing either mRNA degradation or translational repression (Kim et al. 2009).

Deregulation of miRNA expression has been reported in numerous diseases, including cancer (Iorio and Croce 2012). It is widely recognized that miRNAs act as an important trigger in cancer initiation and progression (Croce 2009; Iorio and Croce 2012). miRNAs have also been reported to regulate several genes involved in vitamin D pathway, such as *VDR* (Fig. 3a), *CYP24A1, CYP27B1* and *RXRα*. Four miRNAs, *miR-125b, miR-27b, miR-298* and *miR-346*, have been shown to target *VDR* (Mohri et al. 2009; Zhang et al. 2011; Chen et al.

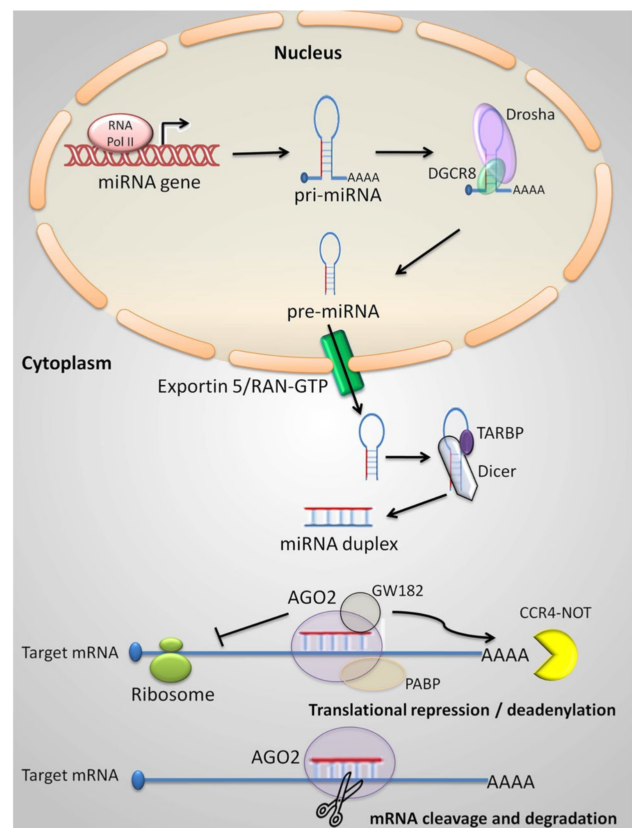


Fig. 2 miRNA biogenesis—canonical pathway. *Nucleus* RNA polymerase II starts the transcription of the miRNA gene. As a result, the primary transcript, pri-miRNA is synthesized, which will be processed by Drosha and Di George Critical Region 8 (DGCR8), which results in the formation of pre-miRNA. Pre-miRNA is transported via Exportin 5/RAN-GTP to the cytoplasm, where it is further processed by Dicer and TARBP (TAR RNA-binding protein). miRNA becomes part of the RISK complex (consisting of AGO2, GW128 and PABP), which in the case of incomplete pairing of miRNA with mRNA, leads to translational repression or deadenylation by CCR4-NOT. Perfect miRNA–mRNA pairing results in mRNA cleavage and degradation

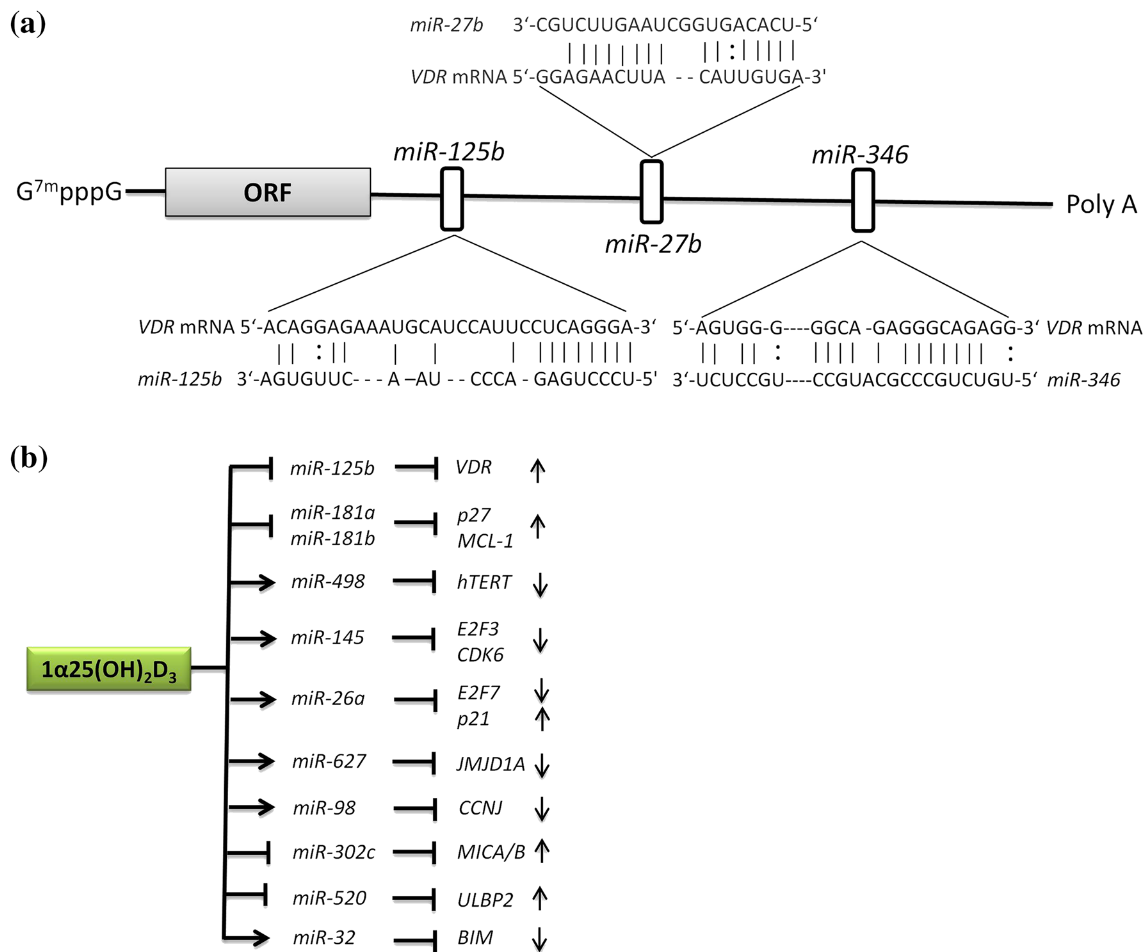


Fig. 3 **a** Schematic presentation of VDR mRNA and predicted target sequence of *miR-125b, miR-27b* and *miR-346*. **b** Examples of miRNAs that are regulated by vitamin D and target genes in different cancer types. ORF—open reading frame; *VDR*, vitamin D receptor; *p27, CDKN1B*—cyclin-dependent kinase inhibitor 1B; *MCL-1*, myeloid cell leukemia 1; *hTERT*, human telomerase reverse transcriptase; *E2F3*, E2F transcription factor 3; *CDK6*, cyclin-dependent kinase 6; *p21, CDKN1A* (*WAF1/CIP1*)—cyclin-dependent kinase inhibitor 1A; *E2F7*, E2F transcription factor 7; *JMJD1A*, Jumonji domain containing 1A; *MICA/B*, MHC class I polypeptide-related sequence A/B; *ULBP2*, UL16-binding protein 2

2014; Li et al. 2015). The first miRNA targeting *VDR* was identified by Mohri et al. (2009), who demonstrated that *miR-125b* directly regulates *VDR* gene expression in the MCF-7 breast cancer cell line and its over-expression can abolish the anti-proliferative effects of vitamin D. Furthermore, *miR-125b*-mediated suppression of *VDR* plays an important role in regulating hair follicle differentiation (Zhang et al. 2011). *miR-27b* was reported to be a regulator of *VDR* gene expression in melanoma, LS-180 colon cancer, PANC1 pancreatic cancer cell lines and human lung fibroblast MRC5 cells (Pan et al. 2009; Essa et al. 2012; Li et al. 2015). Li et al. (2015) verified by luciferase reporter assay that *miR-27b* directly targets *VDR* 3′UTR, which leads to decrease of VDR protein, but not mRNA levels. Pan et al. (2009) also demonstrated that the *miR-298* binding site within the 3′UTR of *VDR* is highly conserved in mice, rats and humans; they verified the direct interaction using a luciferase reporter assay. *miR-346* was found to suppress *VDR* expression during gut mucosal inflammation by direct targeting of *VDR* 3′UTR (Chen et al. 2014).

CYP24A1 has been shown to be regulated by *miR-125b* (Komagata et al. 2009) and a *miR-17~92* cluster (Borkowski et al. 2015). Functional analysis validated direct targeting of *CYP24A1* by *miR-125b* in KGN and MCF-7 cell lines (Komagata et al. 2009). *CYP27B1* expression was found to be directly regulated by *miR-21* in *Mycobacterium leprae*-infected monocytes, which was validated by the luciferase reporter assay (Liu et al. 2012).

It has been shown that *RXRα* is post-transcriptionally regulated by *miR-27a, miR-27b, miR-128-2* and *miR-574-3p* (Ji et al. 2009; Adlakha et al. 2013; Guerit et al. 2013). Rat *RXRα* was directly down-regulated by *miR-27a* and *miR-27b* in activation of hepatic stellate cells (Ji et al.

2009). Direct interaction between *miR-128-2* and *RXRα* has been confirmed in HEK293T cells by the luciferase reporter assay (Adlakha et al. 2013). *miR-574-3p*-mediated suppression of *RXRα* was found to be important in regulating mesenchymal stem cell differentiation to chondrocytes (Guerit et al. 2013).

Vitamin D modulates microRNA expression in cancer

Vitamin D can regulate the transcription of miRNA genes through VDR binding to its sequence motif located in the promoter of target miRNA genes, miRNA maturation through regulating genes involved in miRNA processing (such as Drosha, and Dicer) or miRNA stability (Giangreco and Nonn 2013).

Also, there are suggestions that vitamin D not only increases specific miRNAs, but up-regulates miRNAs expression on the global level, by VDR-dependent chromatin opening and increased pri-miRNA expression (Giangreco and Nonn 2013).

Examples of such regulation of miRNA expression by vitamin D on both transcriptional and post-transcriptional levels in different cancer types are given below (Fig. 3b; Supplementary Tables 1, 2, 3). Cell lines with tissue of origin are listed in the Supplement Table 4.

Ovarian cancer

It has been reported that the active metabolite form of vitamin D suppresses human telomerase reverse transcriptase (*hTERT*) expression and growth of ovarian human cancer cell lines OVCAR3 through *miR-498* induction in a vitamin D dose-dependent manner (Kasiappan et al. 2012). OVCAR3 cells were exposed to different calcitriol concentrations for 24 h, and showed dose-dependent induction of *miR-498* expression. At the lowest concentrations, *miR-498* was first induced, suggesting *miR-498* to be an early response gene to calcitriol treatment (Kasiappan et al. 2012). In the regulatory region of *miR-498* gene, a functional VDRE was identified, which was verified by ChiP assay. Vitamin D treatment induced VDR-RXR and co-activators binding to VDRE of *miR-498* gene. By luciferase reporter assay, *miR-498* direct targeting of *hTERT* 3′-UTR was confirmed. The ability of vitamin D to suppress growth of ovarian cancer and *hTERT* expression was prevented by *miR-498* depletion. Thus, anticancer effects of vitamin D in this ovarian cancer cell line were found to be mediated through transcriptional up-regulation of *miR-498* expression and consequently *hTERT* down-regulation (Kasiappan et al. 2012). In addition, *miR-498*-mediated *hTERT* down-expression is a key event mediating the anti-leptin activity of calcitriol in estrogen-sensitive tumours in women (Kasiappan et al. 2014).

Cervical cancer

In a recent study (Gonzalez-Duarte et al. 2015), vitamin D-sensitive cervical cancer cell lines (HeLa and SiHa) and vitamin D non-responsive C33-A cells were treated with 1 µM calcitriol for 24 and 48 h. Upon calcitriol treatment, the mRNA as well as protein level of *Dicer*, but not *Drosha*, was increased after 24 and 48 h of treatment in the case of SiHa cells, while increased expression was observed only after 48 h in HeLa cells. As C33-A cervical cancer cells do not express the vitamin D receptor, no change in either *Dicer* or *Drosha* mRNA and protein levels were observed (Gonzalez-Duarte et al. 2015). Also, expression analysis in SiHa cells revealed that 16 miRNAs were down-regulated after 24 h of treatment and 15 miRNAs down-regulated after 48h of calcitriol treatment compared with non-treated SiHa cells. Only *miR-3921* was down-regulated at both time points (Gonzalez-Duarte et al. 2015). Numerous miRNAs were up-regulated upon calcitriol treatment after 24 and 48 h, where *miR-22, miR-2963p, miR-29c, miR-342-5p, miR-4455, miR-4462* and *miR-4656* were induced at both time points. One of the induced miRNAs was *miR-498*, also found to be up-regulated in ovarian, breast and endometrial cancer cell lines (Kasiappan et al. 2012), while *miR-22* up-regulation was also confirmed in prostate, colon and bladder cancer cells (Wang et al. 2011; Alvarez-Diaz et al. 2012; Ma et al. 2015). This study showed vitamin D modulation of *Dicer* through the VDRE found in the *Dicer* promoter, which consequently modulated expression of miRNAs (Gonzalez-Duarte et al. 2015).

Breast cancer

Peng et al. (2010) demonstrated calcidiol protective effects against cellular stressors, such as serum starvation, hypoxia, H_2O_2-induced oxidative stress and apoptosis in the epithelial breast cancer cell line MCF-12F. Also, in 24-h low-serum-stressed MCF-12F cells, levels of multiple miRNAs, including *miR-182, miR-200b, miR-200c, miR-26b* and *let-7b* increased, while levels of *miR-18a, miR-106* and *miR-30c* decreased, compared with non-stressed cells. Treatment with calcidiol (250 nM) reversed or inhibited expression of stress-induced miRNAs, which additionally confirmed the protective effects of the main circulating vitamin D form, calcidiol, as well as the important role of miRNAs as mediators of vitamin D biological functions (Peng et al. 2010). Therefore, the possibility of using calcidiol as a natural chemopreventive agent in stress-induced carcinogenesis through maintaining normal miRNA expression level is suggested.

Dose-dependent vitamin D induction of *miR-498* at the transcriptional level was confirmed in breast (MCF-7) and endometrial (Ishikawa) cancer cell lines, implicating *miR-498* regulation in vitamin D's anticancer effects in many vitamin D-sensitive cancers (Kasiappan et al. 2012).

In MCF-7 and MDA-MB-231 breast cancer cell lines, vitamin D treatment resulted in the reduction of *miR-302c* and *miR-520* expression and increased susceptibility of cancer cells to cytotoxic effects of natural killer cells and up-regulated the NKG2D pathway ligands *MICA/B* and *ULBP2*, putative targets of *miR-302c* and *miR-520* (Min et al. 2013).

A recent study has shown that VDR negatively regulates expression level of *miR-199a/miR-214* cluster (*miR-199a-3p, miR-199a-5p, miR-214*) through modulation of dynamin-3 gene (*Dnm3os*) in breast cancer cell lines (MCF-7, T47D) as well as in murine VDR knock-out (VDRKO) and VDR wildtype (WT-145) mammary tumor cell lines (Alimirah et al. 2016). Vitamin D treatment of T47D cells (50nM, 24h) induced *VDR* and *p21* expression on protein and mRNA level (Alimirah et al. 2016). *miR-214* over-expression attenuated vitamin D signaling in both T47D and MCF-7 cell lines (Alimirah et al. 2016).

Prostate cancer

Several studies examined the association between vitamin D treatment in prostate cancer cell lines and miRNA expression. In LNCaP prostate cancer cells, numerous miRNAs were up-regulated (i.e., *miR-21, miR-22, miR-29a/b, miR-134*) while *miR-17/92* cluster members were down-regulated (*miR-17, miR-18a, miR-20a/b*) after treatment with vitamin D (100nM) and testosterone (5nM) (Wang et al. 2011), indicating additive and/or synergistic effects of vitamin D and testosterone treatment on the expression of miRNAs (Wang et al. 2011). A later mechanistic study demonstrated that *PPARA* (peroxisome proliferator-activated receptor alpha), the predicted target of the *miR-17/92* cluster, was up-regulated, which resulted in increased lipogenesis and altered energy metabolism to the production of neutral lipids (Wang et al. 2013).

In another study (Thorne et al. 2011), RWPE-1 non-malignant prostate epithelial cells, RWPE-2 and P69SV40T human prostate cancer cells were treated with 100 nM calcitriol. The cell cycle was arrested after 24 h of calcitriol treatment. Thorne et al. (2011) reported that VDR induced histone modifications of *p21* (*WAF1/CIP1*) gene's promoter, which was followed with increased *miR-106b* expression. *p21* was confirmed as the direct target of *miR-106b*. Vitamin D treatment induced cell cycle arrest due to increased *miR-106b* and concomitant decreased *p21* expression. It has been shown that the *miR-106b* gene is located in the intron of the *MCM7* gene. Vitamin D regulates the *MCM7* gene through VDRE and consequently up-regulates expression of *miR-106b*. Therefore, in prostate cancer cell lines, vitamin D exerts anticancer properties through induction of *MCM7, miR-106b*, and decrease of *p21*, which altogether leads to cell cycle arrest (Thorne et al. 2011).

In primary prostate cells (PrE), PrECa, RWPE-1 and RWPE-2 cell lines, *miR-100* and *miR-125b* were up-regulated after 50 nM vitamin D treatment for 24 h as opposed to non-treated cells. However, this effect was not confirmed in LNCaP, DU145 and PC3 cells (Giangreco et al. 2013). Giangreco et al. (2013) also demonstrated that miRNAs are required mediators of vitamin D-regulated expression of *E2F3* and *PLK1* genes. Vitamin D treatment of PrE and PrE-Ca cells increased *miR-100* and *miR-125b*, while decreasing *E2F3* and *PLK1* expression levels in a VDR-dependent manner. *miR-100* and *miR-125b* were, further, found to have anti-migratory, anti-proliferative and anti-colonigenic properties either in the presence or absence of vitamin D treatment, but depending on the cell type.

Expression of *miR-98* was shown by Ting et al. (2013) to be induced in a VDR-dependent manner, and anticancer properties were significantly promoted by vitamin D treatment in the prostate cancer cell line LNCaP via G2/M cell cycle arrest and cyclin J gene (*CCNJ*) down-regulation. They used the ChiP assay to demonstrate that vitamin D regulates *miR-98* expression directly, at the transcriptional level, by VDR binding to VDRE, identified in the *miR-98* gene promoter. Also, vitamin D indirectly up-regulated *miR-98* post-transcriptionally through suppression of microRNA processing proteins LIN28A and LIN28B. Anti-proliferative effects of vitamin D were decreased due to *miR-98* knockdown. Direct interaction between *miR-98* and *CCNJ* 3′UTR was demonstrated using a luciferase reporter assay. Overall, results of the study suggested *miR-98* to be a key mediator of vitamin D anti-proliferative effects in prostate cancer.

Bladder cancer

So far, only one study has investigated calcitriol regulation of miRNA expression in human bladder cancer cell lines of different tumorigenic and metastatic capacities, 253J (low tumorigenic and non-metastatic) and 253J-BV (highly tumorigenic and metastatic derivative line) (Ma et al. 2015). Both cell lines expressed endogenous VDR as well as CYP24A1 proteins, which were additionally induced after 48 h of treatment with calcitriol (10, 100 and 500 nM) in a dose-dependent manner (Ma et al. 2015). It was demonstrated that numerous miRNAs are differentially modulated upon calcitriol treatment (500 nM) in 253J and 253J-BV bladder cancer cell lines, showing induced expression after 24 and 48 h of treatment. In 253J cell line, *miR-17, let-7a* and *miR-1201* were induced at both time points upon

calcitriol treatment, while numerous miRNAs were found to be up-regulated in 253J-BV, including *miR-22, miR-96* and *miR-125*. Vitamin D was shown to differentially induce miRNAs depending on carcinogen properties of different bladder cancer cell lines. However, there are no mechanistic insights on how vitamin D regulates miRNA expression in bladder cancer cell lines.

Colorectal cancer

In vitro studies conducted on HT-29 and HCT-116 cell lines treated with calcitriol (100 nM) for 24 h demonstrated that *miR-627* was significantly up-regulated (Padi et al. 2013). In the same study, histone demethylase, *JMJD1A* (Jumonji domain containing 1A), was confirmed as the direct target of *miR-627*. Briefly, calcitriol treatment of colorectal cancer cell lines augmented its anticancer properties via *miR-627* up-regulation which consequently led to down-regulation of *JMJD1A*. By decreasing *JMJD1A*, methylation of H3K9 and H3K27 histones was increased, while being reduced in the case of H3K4 histone which suppressed expression of *JMJD1A* target genes, such as *GDF15* (Growth differentiation factor 15) (Padi et al. 2013). The anti-proliferative effects of vitamin D and *JMJD1A* decrease were blocked upon *miR*-627 inhibition. In addition, in the human colon cancer clinical specimens, lower *miR-627* expression was observed in colon cancer tissue compared with normal colon mucosa (Padi et al. 2013). As tumor stages and the presence of nodal metastases were not associated with *miR-627* expression, it was hypothesized that decreased expression of *miR-627* is a feature of the early stages of colorectal cancer formation. Overall, vitamin D anticancer epigenetic activities appear to be mediated through *miR-627* in colorectal cancer cells. The exact molecular mechanism of vitamin D up-regulation of *miR-627* still remains to be elucidated.

Experiments with human colon cancer cells (SW480-ADH and HCT-116) showed time-, dose- and VDR-dependent induction of *miR-22* by calcitriol treatment (Alvarez-Diaz et al. 2012). By inhibiting *miR-22*, anti-proliferative and anti-migratory effects of vitamin D were also inhibited. Furthermore, anti-miR-22 transfection abolished vitamin D down-regulation of target genes *OGN* (osteoglycin), *NELL2* (neural tissue-specific epidermal growth factor-like repeat domain-containing protein), *HNRPH1* (heterogeneous nuclear ribonucleoprotein H1), *RERE* (arginine glutamic acid dipeptide repeats) and *NFAT5* (nuclear factor of activated T cells 5). Reduced expression of *miR-22* in colon cancer tumors was observed, compared with normal tissue (Alvarez-Diaz et al. 2012). Also, an association between the expression of *miR-22* and *VDR* was confirmed (Alvarez-Diaz et al. 2012). Thus, tumor-suppressor *miR-22* was proposed as a mediator in the expression of vitamin D's anticancer properties. How vitamin D regulates *miR-22* expression remains unknown.

Gastric cancer

A recent study by Chang et al. (2015) reported that anticancer effects of vitamin D in gastric cancer cells (SGC-7901 and AGS), treated with 200 nM calcidiol for 48h, are mediated through induction of *miR-145* and consequent down-regulation of its targets *E2F3* and *CDK6*. Interaction between VDR and VDRE upstream of the *miR-145* gene was verified by ChiP assay, highlighting transcriptional regulation of *miR-145* expression by vitamin D. That *miR*-145 directly target *CDK6* and *E2F3* was confirmed by a luciferase reporter assay. Vitamin D anti-proliferative effects in gastric cancer cells were decreased after *miR-145* inhibition. *miR-145* inhibited cell proliferation through *E2F3* down-regulation and downstream cell cycle genes *CDK2* and *CCNA2*. In addition, it was demonstrated that *miR-145* blocks S/G2 transition of gastric cancer cells. Also, down-regulated *miR-145* expression was found in gastric cancer tissue compared with normal tissue and in gastric cell lines compared with normal cells, which indicate that *miR-145* functions as a tumor suppressor (Chang et al. 2015). Together, these results shed new light on miRNA-mediated anti-growth effects of vitamin D in gastric cancer.

Melanoma

According to the literature data, only two studies explored vitamin D miRNA modulation in melanoma. Expression levels of the *VDR* gene as well as several miRNAs were investigated in vitamin D-sensitive (MeWo, SK-Mel28, SM, SK-Mel5) and vitamin D-resistant (SK-Mel25, IGR, Meljuso) melanoma cell lines (Essa et al. 2010). In the vitamin D-sensitive melanoma cell lines MeWo and SK-Mel25, *miR-125b* expression level was inversely associated with the level of *VDR* mRNA, indicating the possible role of *miR-125b* in regulation of VDR expression and vitamin D resistance (Essa et al. 2010). The same study showed that vitamin D sensitivity could be restored using epigenetic drugs, such as histone deacetylase inhibitor and the DNA methyltransferase inhibitor 5-azacytidine (5-Aza) (Essa et al. 2010). *miR-27b* was less expressed in vitamin D-sensitive than -resistant melanoma cell lines (Essa et al. 2012). Combined treatment with vitamin D and 5-Aza significantly reduced the level of *miR-125b* and *miR-27b* while increasing the level of *VDR* mRNA. Essa et al. (2012) also reported that expression levels of *miR-125b* and *miR-27b* in normal human monocytes were not indicative for distinguishing malignant from benign melanocytes.

Lung cancer

In a study conducted by Guan et al. (2013) on lung cancer cell line A549, miRNA *let-7a-2* was up-regulated upon vitamin D treatment (10^{-8}, 10^{-6} mol/L) in a dose-dependent manner. Mechanistic insight demonstrated that the calcitriol–VDR complex up-regulates the expression of *let-7a-2* through interacting with VDRE located in the pre-let-7a-2 promoter, thereby mediating the increased *let-7a-2* expression after calcitriol induction and promoting anti-proliferative effects. Electrophoretic mobility shift and ChiP assays confirmed vitamin D transcriptional regulation of *let-7a-2* expression in vitro and in vivo.

Leukemia

Several studies on leukemia cell lines have dealt with vitamin D treatment and regulation of cellular processes via miRNA alteration. After exposure to low concentrations of calcitriol (0.1–100 nM), decreased levels of *miR-181a* and *miR-181b* were observed in promyeloblastic leukemia cells HL60 and promonocytic leukemia cells U937 (Wang et al. 2009). Down-regulation of *miR-181a* was associated with up-regulation of *p27* mRNA and protein expression, thus inducing G1 cell cycle arrest. Over-expression of *miR-181a* abolished vitamin D-induced *p27* up-regulation, expression of monocytic differentiation markers and stopped G1 cell cycle arrest (Wang et al. 2009). Decreased expression of *miR-181b* was reported in HL60 cells upon vitamin D treatment, accompanied by anti-apoptotic *MCL-1* up-regulation (Zimmerman et al. 2010). It has been reported that vitamin D-induced p53/63 (lyn kinase) activity might modulate *miR-181b* expression, but further mechanistic studies are warranted (Wang et al. 2000; Zimmerman et al. 2010). Combined treatment of HL60 and NB4 cells during monocyte differentiation with vitamin D (100 nM) and phorbol 12-myristate 13-acetate (PMA) (20nM) down-regulated (*miR-181a, miR-181b, miR-130a, miR-135b, miR-146a* and *miR-181d*) (Lutherborrow et al. 2011).

In the study of Duggal et al. (2012), HL60 and U937 cell lines were treated with vitamin D analog doxercalciferol (100 nM) and the rosemary plant-derived antioxidant carnosic acid (10 μM) separately or in combination for 48 h. The findings provided evidence for doxercalciferol-induced monocyte differentiation and cell cycle arrest, which was significantly enhanced with carnosic acid addition. After exposure to either doxercalciferol or carnosic acid or in combination, the *miR-181a* level decreased, followed by *p27* mRNA and protein up-regulation (Duggal et al. 2012). However, expression of *miR-181a* was reduced more strongly in the treatment combining both substances. These results have potential translational significance especially in overcoming the problem of hypercalcemia upon vitamin D treatment of leukemia patients within a clinical setting.

Vitamin D down-regulated the *miR-17-5p/20a/106a* cluster, *miR-125b* and *miR-155*, which was followed with up-regulation of AML1, VDR and CCAAT/enhancer-binding protein (C/EBPβ) (Iosue et al. 2013). In Ago2-depleted HL60 cells, vitamin D-dependent down-regulation of *miR-17-5p/20a/106a, miR-125b* and *miR-155* was impaired. This highlights the requirement of Ago2 for proper vitamin D-induced modulation of miRNAs during the differentiation process (Iosue et al. 2013).

In HL60, NB4 and U937 cell lines treated with vitamin D (100nM), expression of *miR-26a* was increased and followed by c-myc down-regulation (Salvatori et al. 2011). Salvatori et al. (2012) found that *miR-26a* directly targets *E2F7* transcriptional repressor which results in increased *p21* expression and thus G1/S cell cycle arrest. Thus, vitamin D regulation of proliferation and induced differentiation in myeloid leukemia cells is mediated through *miR-26a*.

The expression level of *miR-32* was increased upon calcitriol treatment in human myeloid leukemia cells, HL60 (1nM) and U937 (10nM) as well as in isolated monocytes from healthy individuals (Gocek et al. 2011). Consequently, pro-apoptotic BIM mRNA and protein levels were down-regulated, which suggested *BIM* as a putative *miR-32* target (Gocek et al. 2011). Vitamin D-induced *miR-32* up-regulation was abolished by silencing Drosha and Dicer. Over-expression of *miR-32* promoted vitamin D-induced differentiation of leukemia cells and resulted in decreased *BIM*, thus leading to increased cell survival. Other agents, but not vitamin D, which could inhibit *miR-32*, will be more effective in eradicating leukemia cells.

Recent findings have demonstrated increased susceptibility to natural killer cells (NK92) in human acute myeloid leukemia (Kasumi-1) and K-562 cell lines after treatment with calcitriol for 24h in a dose-dependent manner (Min et al. 2013). Upon vitamin D treatment, down-regulation of *miR-302c* and *miR-520c* was found in Kasumi-1 and K562 cell lines, depending on the dose of vitamin D applied, which indicates a role for *miR-302c* and *miR-520c* as molecular regulators of vitamin D-induced susceptibility to natural killer cells (Min et al. 2013). Functional studies confirmed that *miR-302c* and *miR-520c* serve as negative regulators of NKG2D ligand pathway genes *MICA, MICB* and *ULBP2* by directly interacting and reducing their mRNA and protein levels (Min et al. 2013).

Animal models

The great majority of studies which have investigated vitamin D modulation of miRNA expression have been conducted in maintained cancer cell culture systems. Regarding

miRNA modulation by vitamin D treatment in physiologically normal animal models, one study has been recently published. Namely, the influence of vitamin D treatment on miRNA expression levels was investigated in the *Danio rerio*-zebrafish animal model in vivo (Craig et al. 2014). Upon calcitriol treatment for 7 days after fertilization, 31 miRNAs precursors were differentially expressed (8 down-regulated and 23 up-regulated) in zebrafish (7-day-old post-fertilization larvae in vivo). Functional studies confirmed the role of *miR-125b* in regulating *CYP24A1* gene and protein expression levels in the zebrafish larvae model, which had previously been confirmed in humans (Komagata et al. 2009).

In the study of vitamin D effects on miRNA expression modulation in prostate cancer by Thorne et al. (2011), a mice model was used, namely wildtype C57 BL/6xFBV, treated for 12 and 24 h with calcitriol. They reported increased *miR-106b* expression in prostate tissue followed by p21 (WAF1/CIP1) repression upon vitamin D treatment.

In prostate cancer mice models (TRAMP mice and *wild type* PTEN mice), vitamin D treatment (25 ng/g of mice weight) increased levels of *miR-98* in the blood of both mice models. This highlights the potential use of *miR-98* as a biomarker in prostate cancer and development of a possible vitamin D-based therapy (Ting et al. 2013).

Findings in the ovarian cancer cell line OVCAR3 regarding induced *miR-498* expression levels as mediator upon calcitriol treatment were confirmed in in vivo nu/nu mice models inoculated with *miR-498* OV2008-transfected cells, and treated with vitamin D synthetic analog EB1089 (Kasiappan et al. 2012).

An in vitro study by (Padi et al. 2013) reported that calcitriol exerts anti-proliferative effects by inducing *miR-627* with subsequent down-regulation of *JMJD1A* in colorectal cancer cell lines. These findings were confirmed in the colorectal cancer HT-29 xenograft nude mice model treated with 0.4 µg of calcitriol. Upon calcitriol treatment tumor growth was suppressed in mice, and this was abolished by blocking *miR-627* activity by over-expressing the *JMJD1A* 3′UTR sponge. Additionally, in the mice model stably expressing a *miR-627* tumor xenograft, colon cancer growth was suppressed (Padi et al. 2013). Together, these findings highlight the important role of *miR-627* in promoting anticancer effects of vitamin D in in vitro as well as in vivo models.

Human cohort

The link between vitamin D level and miRNA expression profile has not been extensively studied so far, and studies conducted on a human cohort are limited. The main findings from the few investigations conducted in humans so far are presented here.

In 13 pregnant woman, mRNA and miRNA expression levels in peripheral blood were measured in groups with low (<25.5 ng/ml) and high (>31.7 ng/ml) serum levels of the main vitamin D circulating form calcidiol (Enquobahrie et al. 2011). In total, 305 genes (299 up- and 6 down-regulated) were found differentially expressed between the two groups, mainly genes which are known to have roles in the functioning and development of numerous physiological systems. Ten microRNAs (*miR-589, miR-601, miR-573, miR-138, miR-320d, miR-196a, miR-92b, miR-423-3p, miR-484, miR-93, miR-574-5p*) were down-regulated and *miR-574-5p* was up-regulated in subjects with low calcidiol levels in early pregnancy compared with participants with high calcidiol concentrations. A large number of identified miRNAs target genes were found to be differentially expressed. The study demonstrated that low levels of early pregnancy calcidiol are associated with differences in mRNA and miRNA expression, which could lead to the development of various pathophysiological processes and increased risk for pregnancy complications. At the same time, intrauterine vitamin D deficiency could have subsequent consequences later in childhood and adulthood. However, the study group was small, consisting of only 13 subjects, limiting the generalization of their results, among other concerns in the study (Enquobahrie et al. 2011).

One of the human studies investigated the expression of plasma miRNA in 40 subjects prior to and after 12 months of vitamin D supplementation in high doses (19 subjects given 20,000 IU/week and 21 subjects given 40,000 IU/week), and compared them with a placebo group (37 subjects) (Jorde et al. 2012). Prior to vitamin D supplementation, subject serum levels of calcidiol were positively correlated with plasma *miR-532-3p* expression levels. After 12 months of supplementation, expression levels of *miR-221* were significantly different between subjects and placebo group (Jorde et al. 2012). Although the study used a relatively large group of subjects (in total 77), their results should be taken with caution as findings from the previously conducted pilot study were not reproduced and authors reported an inconsistent association between vitamin D and microRNA levels in plasma (Jorde et al. 2012).

Calcidiol and calcitriol serum and tissue levels were measured in a group of 66 prostate cancer patients treated with 400, 10,000 or 40,000 IU/day for 3–8 weeks prior to prostatectomy (Giangreco et al. 2013). Expression levels of *miR-100, miR-125b, miR-103, miR-331-3p, miR-146a, miR-155, miR-197, miR-106b, miR-141, miR-301a, let-7a* and *let-7b* were down-regulated in prostate cancer tissue compared with normal epithelium. An association between decreased *miR-100* and *miR-125b* expression and *E2F3* increase in prostate tumor was also found. However, after vitamin D treatment, expression levels of *miR-100* and *miR-125b* increased in a vitamin D dose-dependent

manner. Expression levels of *miR-100* and *miR-125b* were positively associated with prostate calcitriol levels. Positive associations were also found between serum calcitriol and calcidiol with *miR-100* and *miR-125b* levels (Giangreco et al. 2013). Overall, this study demonstrated the possibility of using vitamin D supplementation in prostate cancer patients.

Vitamin D levels were measured in a group of 97 acute myeloid leukemia patients in a study by Lee et al. (2014). Here, deficient and insufficient calcidiol levels were associated with worse relapse-free survival (Lee et al. 2014). Although the authors reported that 13 miRNAs were up-regulated and 4 miRNAs were down-regulated in patients with low calcidiol levels (<32 ng/ml), after multiple testing, none of the miRNAs was associated with the level of the main circulating form of vitamin D.

A recently published study by Beckett et al. (2015) found an association between levels of the microRNA *let-7a/8* circulating in serum with vitamin D intake which was dependent on *VDR* gene allele for single nucleotide polymorphisms BsmI (rs1544410) and ApaI (rs7975232). The study involved 200 elderly participants who were surveyed for vitamin D food and supplemental habits. The study demonstrated the importance of considering genotypic variants in vitamin D-related gene-VDR in studies focusing on miRNA expression and vitamin D serum levels. Overall, the findings illustrated the interplay between vitamin D epigenetic modulations and genome variations and highlighted the importance of evaluating human genome variations which could be responsible for differences in responses to vitamin D treatment (Beckett et al. 2015).

Studies with human cohorts are inevitably more complicated compared with in vitro models, particularly bearing in mind crosstalk between different dietary components which could in vitamin D's bioactive form also modulate miRNA expression profiles. In addition, inter-individual genome variability in miRNA and vitamin D-related genes should be considered when interpreting results, as the presence of single nucleotide polymorphisms, for instance, could result in different responses to vitamin D or treatments with other dietary components (Shah et al. 2012).

Conclusion and future perspectives

A growing body of evidence convincingly demonstrates vitamin D as an important cancer chemopreventive and therapeutic agent (Lamprecht and Lipkin 2003; Deeb et al. 2007). Thus, better understanding of the vitamin D molecular pathways is required in different experimental models. So far, little was known about miRNA molecular mediation of the functional effects of vitamin D. From the information presented in this review, it is obvious that vitamin D could have anticancer effects through alteration of miRNA expression in various types of malignancies, such as ovarian, cervical, breast, prostate, bladder, colorectal, gastric, leukemia, melanoma, and lung cancer. Also, miRNAs regulated by vitamin D are specific for certain cancer types. Owing to the limited number of studies which have investigated the role of vitamin D on miRNA expression modulation in cancer, mainly conducted in cell culture systems and animal models, results are inconclusive in terms of a complete elucidation of connections. Further studies are also required with human cohorts.

Numerous studies have reported mainly modulated expression of specific miRNAs upon vitamin D treatment in different cancer types via canonical VDRE regulation. However, there are suggestions that vitamin D can up-regulate the expression of pri-miRNAs on a global level by VDR-dependent chromatin opening (Giangreco and Nonn 2013). Additionally, it is assumed that miRNA expression could be modulated by vitamin D via non-genomic VDR-dependent activation by possible alteration of the miRNA processing machinery or changes in miRNA stability (Giangreco and Nonn 2013). As far as we know, there are still no experimental confirmations of vitamin D modulation of miRNA via non-genomic pathway, thus this assumption remains to be elucidated in the future.

Taken altogether, studies cited in this review have provided new mechanistic insights into vitamin D anticancer effects through miRNA modulation. All findings have potential translational significance. Further studies investigating the role of vitamin D and modulation by dietary components in general of miRNA in cancer and other pathologies are expected in the future.

One of the problems arising in the potential application of vitamin D as a chemoprotective agent is defining the dose which will have optimal biological effects, while not being accompanied by toxic and other side effects. Also, inconsistent results obtained in different types of cancer cell lines and in vivo models, including humans should be examined further. Thus, using vitamin D as a therapeutic agent with our current state of knowledge is still a matter of considerable controversy. At the same time, in in vitro studies it is easy to control the inclusion of dietary components and, therefore, to determine the precise mechanisms by which miRNA expression is altered. However, extrapolating results from in vitro studies to humans could be problematic, as it is unlikely that only one dietary component is having an effect, with apparent associations being the result of synergistic or antagonistic effects of dietary components.

Elucidating the molecular mechanisms of vitamin D modulation of miRNA will contribute to a better understanding of the potential use of vitamin D for therapeutic and preventive purposes in cancer management. Studies of vitamin D modulatory effects on miRNAs expression are

expected to gain more attention in the future. Also, apart from vitamin D, numerous bioactive dietary components are currently under investigation as potential modulators of miRNA molecular signatures in different cancer types. Knowing the details of molecular functions of vitamin D and other dietary components will help in developing the concept of personalized nutrition, where miRNAs could serve as biomarkers and molecular targets which could be modulated by nutritional interventions in health and disease.

The evidence for miRNA regulation by vitamin D treatment discussed in this review gives rising hope for opening a new area of translational biomedical science for the development of novel vitamin D- and miRNA-based therapeutics and improved treatment for an increasing number of cancer patients worldwide.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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