

# Vitamin D resistant genes – promising therapeutic targets of chronic diseases

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## REVIEW

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## Vitamin D resistant genes – promising therapeutic targets of chronic diseases

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Vitamin D is an essential vitamin indispensable for calcium and phosphate metabolism, and its deficiency has been implicated in several extra-skeletal pathologies, including cancer and chronic kidney disease. Synthesized endogenously in the layers of the skin by the action of UV-B radiation, the vitamin maintains the integrity of the bones, teeth, and muscles and is involved in cell proliferation, differentiation, and immunity. The deficiency of Vit-D is increasing at an alarming rate, with nearly 32% of children and adults being either deficient or having insufficient levels. This has been attributed to Vit-D resistant genes that cause a reduction in circulatory Vit-D levels through a set of signaling pathways. CYP24A1, SMRT, and SNAIL are three genes responsible for Vit-D resistance as their activity either lowers the circulatory levels of Vit-D or reduces its availability in target tissues. The hydroxylase CYP24A1 inactivates analogs and pro-hormonal and/or hormonal forms of calcitriol. Elevation of the expression of CYP24A1 is the major cause of exacerbation of several diseases. CYP24A1 is rate-limiting, and its induction has been correlated with increased prognosis of diseases, while loss of function mutations cause hypersensitivity to Vit-D. The silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and its corepressor are involved in the transcriptional repression of VDR-target genes. SNAIL1 (SNAIL), SNAIL2 (Slug), and SNAIL3 (Smuc) are involved in transcriptional repression and binding to histone deacetylases and methyltransferases in addition to recruiting polycomb repressive complexes to the target gene promoters. An inverse relationship between the levels of calcitriol and the epithelial-to-mesenchymal transition is reported. Studies have demonstrated a strong association between Vit-D deficiency and chronic diseases, including cardiovascular diseases, diabetes, cancers, autoimmune diseases, infectious diseases, etc. Vit-D resistant genes associated with the aforementioned chronic diseases could serve as potential therapeutic targets. This review focuses on the basic structures and mechanisms of the repression of Vit-D regulated genes and highlights the role of Vit-D resistant genes in chronic diseases.

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## 1. Introduction

Vitamin D (Vit-D) is the precursor of parathyroid hormone, a regulator of several metabolic processes and physiological responses.<sup>1</sup> In addition to regulating bone metabolism, Vit-D has also been implicated in the prevention and treatment of many extra-skeletal diseases.<sup>2</sup> Several epidemiological, pre-clinical, and cellular studies vouch for its roles in different types of cancers and chronic kidney disease.<sup>3</sup> The prohor-

mone, Vit-D, is converted to the biologically active metabolite, calcitriol, in the liver and kidneys. Vit-D receptor (VDR) binds calcitriol and triggers a signal cascade that regulates the expression of diverse genes. Vit-D can also be activated and metabolized by CYP11A1.<sup>1</sup>

The major functions of this fat-soluble vitamin include the regulation of calcium and phosphate levels for the maintenance of integrity in bones, teeth, and muscles, proliferation of cells, differentiation, immune functions and genome stability.<sup>4,5</sup> In the target organs, Vit D is believed to work through a nuclear-mediated receptor-based mechanism involving calcium and phosphorus transfer proteins. Vit-D is endogenously synthesized in the layers of the skin by the action of UV-B radiation, while dietary sources like fish, fortified food, and supplements serve as additional sources of Vit-D.<sup>6,7</sup> The absorption efficiency of Vit-D in tissues is reported to

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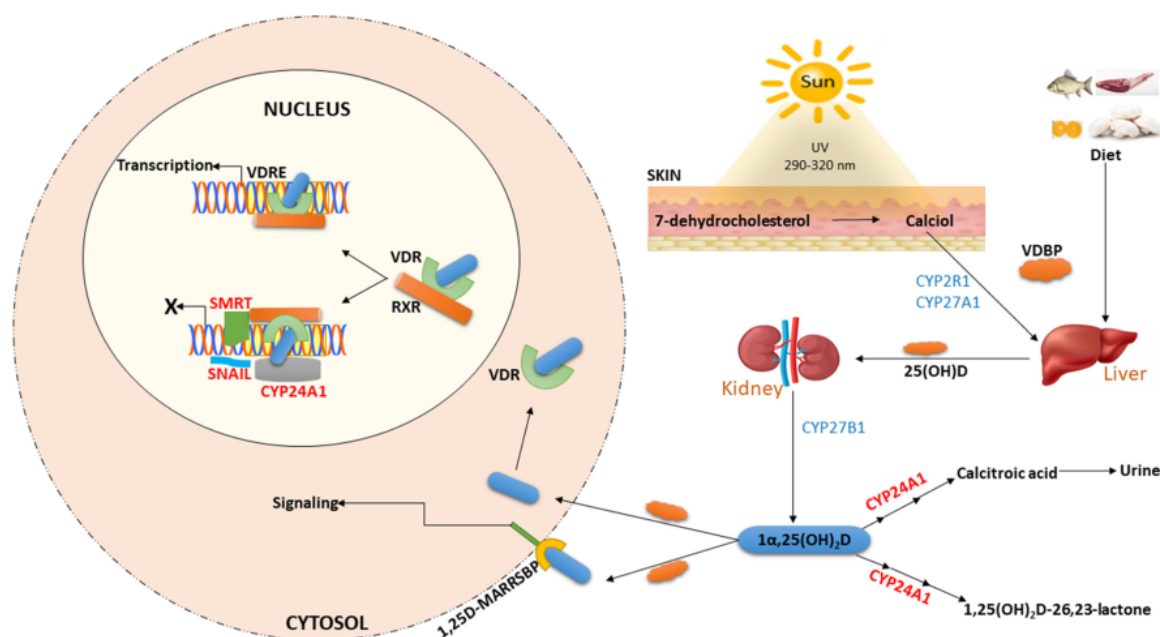
vary between 55% and 99%. Though Vit-D is readily available from different sources, Vit-D deficiency/insufficiency is prevalent even in tropical regions.<sup>8,9</sup> Vit-D in circulating blood is categorized as (i) Vit-D deficiency when the level of 25-hydroxyvitamin-D3 (25(OH)D3) is less than or equal to 20 ng mL<sup>-1</sup>, (ii) Vit-D insufficiency when the level of (25(OH)D3) is between 21 and 29 ng mL<sup>-1</sup>, and a value anywhere above 30 ng mL<sup>-1</sup> is considered sufficient.<sup>10</sup>

Adults in most developing and developed countries face 25(OH)D3 deficiency, and more than 50% of the world's population is at risk of 25(OH)D3 deficiency.<sup>11</sup> As per the reports of the Centre for Disease Control and Prevention (CDC), the level of 25(OH)D3 is deficient in almost 32% of children and adults.<sup>12</sup> Deficiency and/or insufficiency perturbs calcium homeostasis, causing bone demineralization,<sup>13</sup> softening, rickets,<sup>14</sup> fatigue,<sup>15</sup> osteoporosis<sup>16</sup> and depression.<sup>17</sup> 76% of India's population is Vit-D deficient as per a recent study that included 4624 subjects across 81 cities.<sup>18</sup> Significant risk of cancer, cardiovascular disease, diabetes, and autoimmune disorders are associated with the deficiency or insufficiency of Vit-D.<sup>19,20</sup> Recent observations among the people with low Vit-D levels highlight the role of Vit-D resistant genes.<sup>21</sup> Vit-D resistant genes cause reduction in circulatory Vit-D levels through a set of signalling pathways resulting in Vit D deficiency or insufficiency.

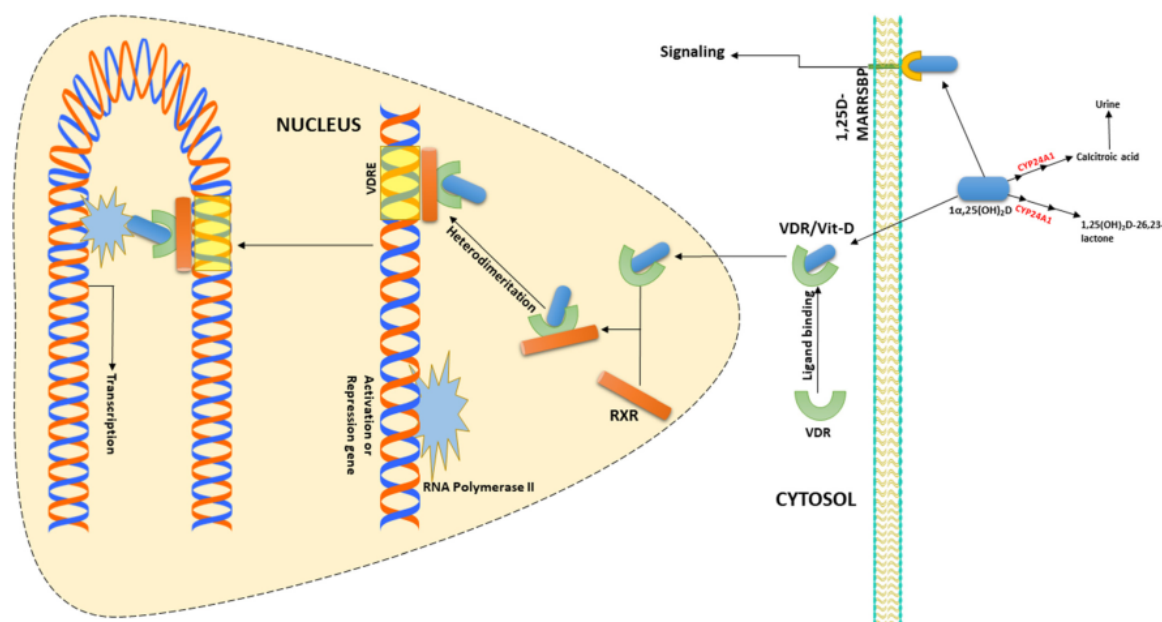
## 2. Synthesis and metabolism of vitamin-D

Endogenous Vit-D3 or exogenous Vit-D2 binds Vit-D-binding protein (VDBP) and reaches the liver, where Vit-D-25-hydroxylases, CYP2R1 and 25(OH)D3 1 $\alpha$ -hydroxylase (CYP27B1) hydroxylate Vit-D3 to calcidiol (25(OH)D3).<sup>22</sup> This most abundant form of Vit-D in circulation reaches the proximal tubule where it is hydroxylated by CYP27A1 to 1 $\alpha$ ,25-dihydroxy Vit-D (1 $\alpha$ ,25(OH)<sub>2</sub>D3), also referred to as calcitriol. Calcitriol reaches the intestine, kidneys and bone, where it regulates the absorption/re-absorption and mobilization of calcium and phosphate.<sup>23–25</sup> The levels of calcitriol in turn are tightly regulated, by hydroxylation of both at C-24 and C-23 side chains catalysed by 25(OH)D3-24-hydroxylase (CYP24A1)<sup>22</sup> (Fig. 1).

The nuclear Vit-D receptor (VDR) binds calcitriol and regulates both genomic and non-genomic targets.<sup>26</sup> Calcitriol bound VDR is phosphorylated and forms a dimer with the retinoid-X receptor (RXR), and this complex translocates to the nucleus.<sup>27,28</sup> It binds to the promoter of target genes and recruits coactivators or corepressors to regulate expression (Fig. 2). On the other hand, in a non-genomic pathway calcitriol binds to the 1,25(OH)<sub>2</sub>D3-membrane-associated rapid response steroid-binding protein (1,25D-MARRSBP), a mem-



**Fig. 1** Vitamin D metabolism. Vitamin-D3 is either taken from the diet or is synthesized in layers of skin upon exposure to sunlight (290–315 nm). The precursor form cholecalciferol or 7-dehydrocholesterol (7-DHC) is further metabolised to the active form and is released into circulation. Hydroxylation of cholecalciferol into 25-hydroxyvitamin-D3 [25(OH)D3] is catalysed in the liver by Vit-D 25-hydroxylase (25-OHase) followed by 25(OH)D3-1 $\alpha$ -hydroxylase enzyme (CYP27B1 or 1 $\alpha$ -OHase) converting it to 1 $\alpha$ ,25-dihydroxy Vitamin-D3 [1 $\alpha$ ,25(OH)<sub>2</sub>D3] in the kidneys. 1 $\alpha$ ,25(OH)<sub>2</sub>D3 binds to the vitamin-D RXR complex, translocated to the nucleus where it binds to the vitamin-D Response Element (VDRE) activating the downstream targets and responses. CYP24A1 catabolizes 1 $\alpha$ ,25(OH)<sub>2</sub>D3 and 25(OH)D3 into 24-hydroxylated metabolites and inactivates VDR ligands. VDR interacts with CYP24A1 and SMRT both of which repress VDR target genes, osteocalcin and 24-hydroxylase D3.



**Fig. 2** Formation and activation of the Vit-D/VDR-RXR complex. The vitamin-D nutritional status index is the 25(OH)D3 level in serum in place of the active form, 1,25(OH)2D3 and this might be because of the extra-renal fusion of 1,25(OH)2D. Furthermore it binds with the Vit-D receptor-retinoid X receptor (VDR-RXR) heterodimer on the vitamin-D response element (VDRE) () which is located in the promoter of vitamin-D responsive genes.

brane-bound VDR known as ERp57a.<sup>29</sup> This interaction alters intracellular signalling, (*e.g.*, calcium and MAPK pathway) through enhancing or disturbing the protein-protein interaction between signaling molecules and causes phenotypic variations.<sup>30,31</sup>

### 3. Vitamin-D resistance and vitamin-D resistant genes

One of the most pronounced effects of the hormonally active 1, 25 dihydroxy Vit-D3 is the increased synthesis of CYP24A1, which catabolizes 1, 25 dihydroxy Vit-D3.<sup>22</sup> Therefore, Vit-D3 regulates its own metabolism, protecting from hypercalcemia. The level of circulatory Vit-D is reduced when the catabolism of Vit-D precedes the anabolism.<sup>32</sup> SMRT and SNAIL, induce Vit-D resistance by arresting VDR and lowering the uptake of Vit-D by target tissues. CYP24A1 is an inducible gene which is expressed in target tissues, including kidneys, intestine and bone. SMRT is mainly expressed in the fat tissues and expressed throughout the body. SNAIL is a transcription factor which is expressed in throughout the body but predominantly in the gall bladder.

#### 3.1. Structure of CYP24A1

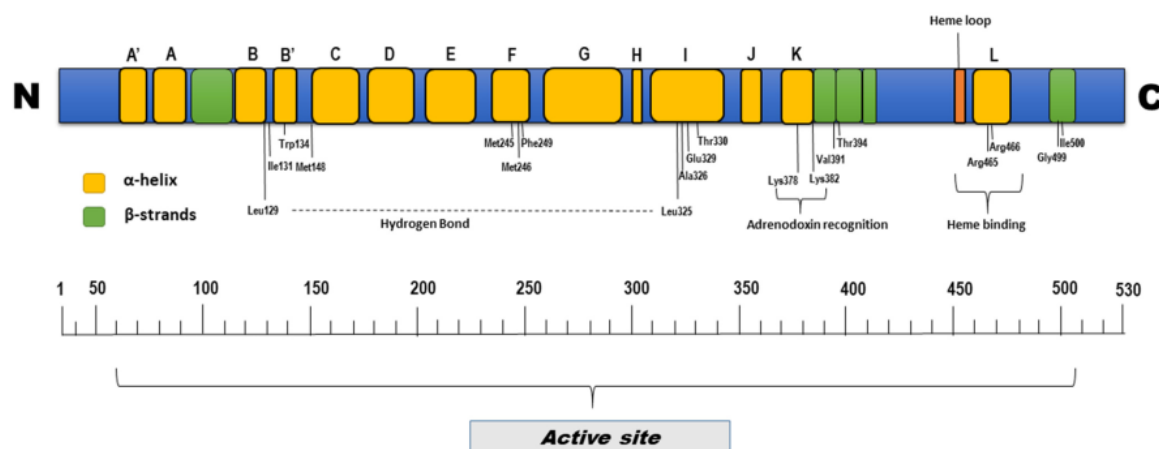
Crystallographic analysis of CYP24A1 revealed the presence of the P450 fold, displaying an alpha beta motif, twelve alpha helices (A-L), four  $\beta$ -sheets ( $\beta$ 1- $\beta$ 4) and five short helices.<sup>33-35</sup>

The substrate-binding cavity is formed from  $\beta$ 1 and  $\beta$ 4 sheets and helices E, F, G, I, and K that surround the heme and the B-C loop. Furthermore, 13 out of 19 residues, namely Ile-131, Trp-134, Met-148, Met-245, Met-246, Phe-249, Ala-326, Glu-329, Thr-330, Val-391, Thr-394, Gly-499, and Ile-500 are involved in substrate binding and catalytic reactions of which only 6 residues, namely, Ile-131, Trp-134, Met-148, Met-246, Ala-326, and Gly-499 have roles in substrate binding.<sup>34-37</sup> The Ala-326 residue in I helix determines the depth to which the substrate penetrates the binding pocket.<sup>34</sup> The K, K', and L helix residues next to a loop rich in lysine residues, provide an interface for redox protein binding<sup>34,38</sup> (Fig. 3).

#### 3.2. CYP24A1 in vitamin D resistance

The gene that encodes CYP24A1 is an oncogene, and it contributes to aggressive tumours by revoking the local anticancer effects of calcitriol.<sup>39</sup> The extrarenal production of CYP24A1 in cancer cells, catabolizes 1, 25 dihydroxy Vit-D3.<sup>39</sup> CYP24A1 is rate limiting and its elevated expression has always been correlated with increased prognosis of diseases.<sup>40</sup> Loss of function mutations in CYP24A1 cause hypersensitivity to Vit-D3, and the reintroduction of CYP24A1 clone restores normocalcemia.<sup>41</sup> Four different SNPs, namely, rs4809957, rs6068816, rs6091822 and rs8124792, in the CYP24A1 gene have been identified to be associated with colon cancer and all reduce the activity/affinity of CYP24A1 to its substrate, increasing/maintaining the much-required elevated levels of Vit-D3 in the cancer milieu.<sup>42</sup>





**Fig. 3** Structure of CYP24A1. Crystallographic analysis of CYP24A1, the presence of P450 fold, displaying alpha beta motif, twelve alpha helices (A–L) four  $\beta$ -sheets ( $\beta$ 1– $\beta$ 4) and five short helices. The substrate-binding cavity is formed from  $\beta$ 1 and  $\beta$ 4 sheets and helices E, F, G, I, and K that surround the heme and the B–C loop. Further 13 out of 19 residues, namely Ile-131, Trp-134, Met-148, Met-245, Met-246, Phe-249, Ala-326, Glu-329, Thr-330, Val-391, Thr-394, Gly-499, and Ile-500 involved in substrate binding and catalytic reactions.

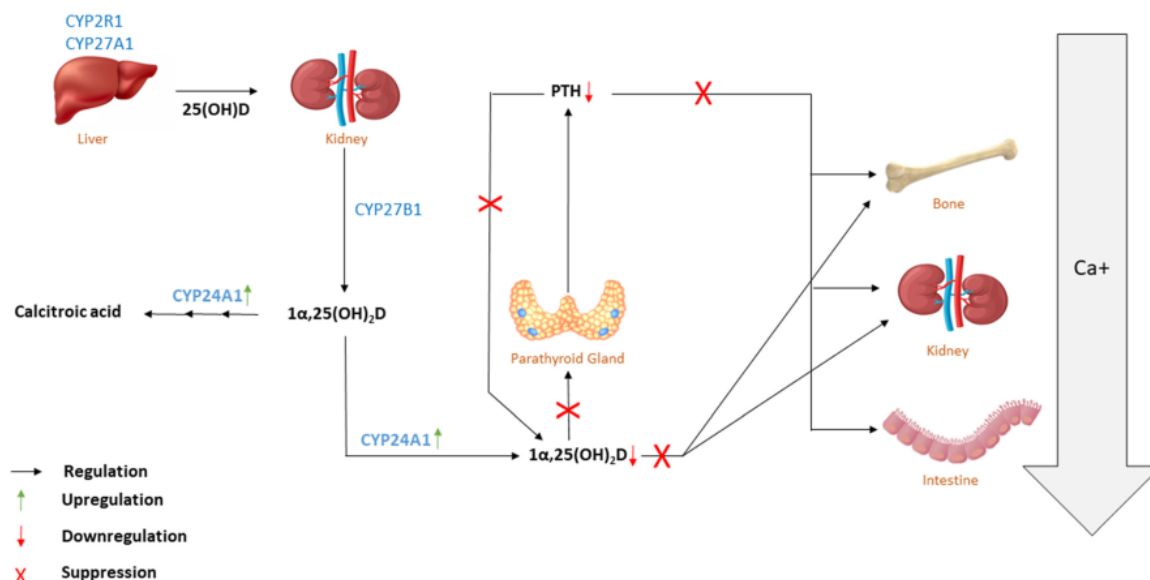
CYP24A1 inactivates analogues and prohormonal and/or hormonal forms of calcitriol.<sup>43</sup> Elevation of the expression of CYP24A1 that results in Vit-D deficiency or insufficiency is the major cause of chronic kidney disease. Elevated CYP24A1 leads to a dysfunctional Vit-D metabolism and declining Vit-D levels.<sup>44</sup> Data obtained from a uremic rat model and from humans have indicated the relationship between a dysfunctional Vit-D metabolism and changes in CYP24A1 expression by elevated levels of phosphate and FGF-23 expression.<sup>45</sup> In CKD patients, increase in the CYP24A1 expression resulted in decreased Vit-D status in serum phosphate and FGF-23 levels disturb calcium and phosphate homeostasis leading to renal osteodystrophy and contributing to the other renal complications.<sup>44</sup>

Calcitriol-VDR-RXR is a prominent target of CYP24A1,<sup>1,2</sup> the complex induces CYP24A1 either by chromatin remodelling or by driving the transcription of the CYP24A1 gene.<sup>46–48</sup> Thus, the expression of CYP24A1 is partly mediated by calcitriol, which controls the level of calcidiol and calcitriol in kidneys. Both the C23 or C24 side-chain carbons of 25(OH)D<sub>3</sub> or 1,25(OH)<sub>2</sub>D<sub>3</sub> are hydroxylated by the enzyme. The former results in lactone formation while the later results in side-chain cleavage and oxidation to a carboxylic acid.<sup>22</sup> CYP24A1 has been demonstrated as a bifunctional enzyme capable of the 24-hydroxylation of 1,25(OH)<sub>2</sub>D, calcitriolic acid, and 23-hydroxylation, culminating in 1 $\alpha$ ,25-(OH)<sub>2</sub>D<sub>3</sub>-26,23-lactone.<sup>49–56</sup> Though the biological activity of the C23-hydroxylation metabolites is unclear, reports state that the terminal product, 1,25(OH)<sub>2</sub>D-26, 23-lactone,<sup>57</sup> could act as a VDR antagonist.<sup>55,56</sup> Furthermore, 1,25(OH)<sub>2</sub>D-inducible 24-hydroxylation and calcitriolic acid production are observed in several kidneys, bones, intestines, skin, and breast cells.<sup>58–60</sup> In the C24-hydroxylation pathway, carbon 23 of 24-oxo-1 $\alpha$ ,25(OH)<sub>2</sub>D is hydroxylated to generate 24-oxo-1  $\alpha$ ,

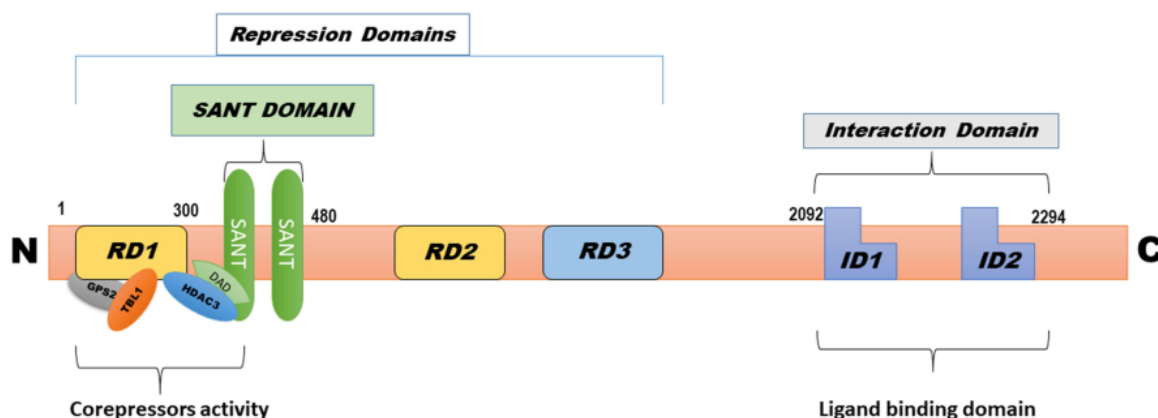
23,25(OH)<sub>3</sub>D. This is cleaved between C23 and C24 to produce 24, 25, 26, 27-tetranor-1 $\alpha$ ,23(OH)<sub>2</sub>D which is converted to calcitriolic acid, the excretory product of 1,25(OH)<sub>2</sub>D<sub>3</sub> in bile. The degradation of 1,25(OH)<sub>2</sub>D<sub>3</sub> occurs either systemically or locally within the cells of target tissues due to upregulation of CYP24A1. As a result this suppresses the action of Parathyroid gland which in turn decreases the level of PTH. Since PTH has contradicting effects on regulation of catabolic and anabolic enzymes, a decreased ratio 25(OH)D<sub>3</sub> to 1,25(OH)<sub>2</sub>D<sub>3</sub> is perceived as a biomarker for vitamin D resistance which results in low bone, kidney and intestinal calcium absorption (Fig. 4)<sup>1,57–63</sup>

### 3.3. Structure of SMRT

The polypeptide chains of the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) are disordered, hence the SMRT protein is referred to as intrinsically disordered protein (IDP).<sup>64,65</sup> IDPs are known to play important roles in signaling cascades mediating the protein-protein interaction. SMRT interacts with several transcription factors, nuclear receptors, HDACs, and a wide range of other proteins.<sup>66</sup> The corepressor motifs of both SMRT and the nuclear receptor corepressor (NCoR) possess domains ID1 and ID2 that interact with the nuclear receptor.<sup>67</sup> Binding of coactivators or corepressors bring about a conformational change in helix-12 resulting in the formation of suitable coactivator binding sites and enhances ligand binding.<sup>68</sup> A SANT (*i.e.*, Swi3, Ada2, N-CoR, and TFIIB)-like fold was predicted by Aasland *et al.* based on the secondary structure of SMRT.<sup>69</sup> This SANT-like fold has been later found to have two domains, of which one recruits and activates HDAC3, mediated by inositol-3-phosphate signalling and the other binds to the tail region of histone, as an HDAC3 substrate<sup>70,71</sup> (Fig. 5). In addition, SMRT expresses different isoforms, some containing ID3, and regulates the interaction between SMRT and nuclear



**Fig. 4** The action of PTH and CYP24A1 in  $\text{Ca}^{2+}$  metabolism. The degradation of  $1,25(\text{OH})_2\text{D}_3$  occurs either systemically or locally within the cells of target tissues due to upregulation of CYP24A1. As a result this suppresses the action of Parathyroid gland which in turn decreases the level of PTH. Since PTH has contradicting effects on regulation of catabolic and anabolic enzymes, a decreased ratio  $25(\text{OH})\text{D}_3$  to  $1,25(\text{OH})_2\text{D}_3$  is perceived as a biomarker for vitamin D resistance which results in low bone, kidney and intestinal calcium absorption.



**Fig. 5** Structure of SMRT. Structural and Functional domains of the SMRT protein, the corepressor motifs of both proteins possess domains ID1 and ID2 that interact with nuclear receptor. A SANT (*i.e.*, Swi3, Ada2, N-Cor, and TFIIB)-like fold is present on the secondary structure of SMRT. This SANT-like fold has been later found to have two domains, out of which one (DAD) recruits and activates HDAC3, mediated by inositol phosphate signalling and the other binds to the tail region of histone, as an HDAC3 substrate. HDAC3, TBL1 are core interacting proteins associated with highly conserved amino-terminal repression domain 1 (RD1) and influence chromatin remodelling functions of SMRT.

receptors.<sup>72</sup> A peptide of the SMRT ID2 domain competitively binds the hydrophobic pocket and blocks the binding of the corepressor, which forms three  $\alpha$ -helical turns in the corepressor, resulting in the displacement of the helix-12 from its active site of LBD.<sup>73,74</sup> The N-termini of ID1 and ligand binding domain bring a conformational change in the

repressor.<sup>75,76</sup> HDAC3, transducing  $\beta$  like protein1, core interacting proteins associated with corepressors, binds the highly conserved amino-terminal repression domain 1 and influences the chromatin remodelling functions of SMRT. Three proteins, HDAC3, TBL1/TBLR1, and GPS2, form the core complex with interactions from the amino acids 167–480 of SMRT.<sup>77</sup>

Proteins GPS2 and TBL1/TBLR1 recruited at the amino-terminal portion of RD1 interact directly and form a complex.<sup>78,79</sup> The DAD-specific motif comprises of first sixteen residues in the DAD domain (residues 412 and 480).<sup>80</sup> A TRiC chaperone assembles the HDAC3/DAD complex that phosphorylates and activates HDAC3.<sup>81–83</sup>

### 3.4. Functions of SMRT in regulating vitamin D resistance

The VDR complexed with RXR binds to the VDRE at the promoter and recruits SMRT with the help of ID1 of SMRT. The residues Arg4, Val2, Leu1, Ala2, His4, Ile5, Glu7, Val8, Ile9, and Tyr13 of SMRT-ID1 interact with VDR. These residues are also crucial for mediating VDR-target gene repression *in vivo*. RNAi studies of the unliganded VDR have shown the involvement of SMRT in the transcriptional repression of VDR-target genes, osteocalcin and CYP24A1 in HEK 293. Residues within and outside the extended helix motif of SMRT-ID2 are required for VDR-specific interaction. SMRT mutations that result in incompatible VDR interactions cannot repress VDR-target genes. These results are a clear indication of the involvement of the SMRT corepressor and specific residues of SMRT-ID2 generally required for optimal NR binding in VDR-mediated repression.<sup>84</sup>

### 3.5. Structure of SNAIL

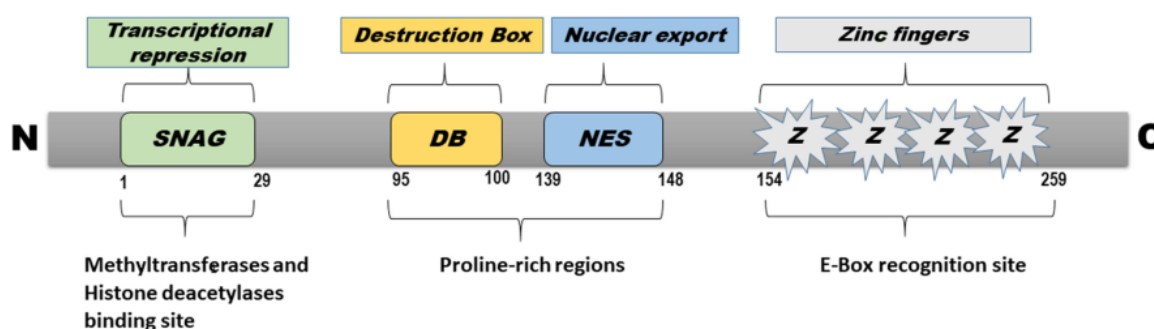
SNAIL1 (SNAIL), SNAIL2 (Slug), and SNAIL3 (Smuc) are zinc-finger transcription factors that have a significant role in gene regulation.<sup>85</sup> Around 4 to 6 C2H2-type zinc fingers in the carboxy-terminal region of SNAIL, are involved in the sequence-specific interaction with a DNA promoter containing a E-box sequence. SNAG (SNAIL/Gfi), the evolutionarily conserved domain at the amino terminus of all vertebrate SNAIL family, is involved in epigenetic regulation and transcriptional repression.<sup>86</sup> The C-terminal region of these proteins is highly conserved and consists of four zinc fingers which facilitate DNA protein interactions.

The conserved SNAG domain at the N-terminal of vertebrate SNAIL family members recruits the polycomb repressive complex (PRC) to the target gene promoters.<sup>85,87</sup> H3/H4 deacetylation, H3K4 demethylation and H3K9 and H3K27 hypermethylation are induced by the complex followed by recruitment of DNA methyltransferases at the target gene promoter.<sup>88</sup> These changes result in the silencing of SNAIL target genes. A protein destruction box (DB) domain and a nuclear export signal (NES) domain are located within the central proline-rich regions and mediate ubiquitination and proteolytic degradation<sup>89</sup> (Fig. 6).

### 3.6. Functions of SNAIL in regulating vitamin D resistance

A cascade of signaling mediators such as ILK, PI3-K, MAPKs, GSK-3 $\beta$  and NF $\kappa$ B control the expression of SNAIL at transcriptional and post-transcriptional levels.<sup>90</sup> SNAIL expression is induced by signals from receptor tyrosine kinases involving the activation of FGF, EGF, or inhibition of GSK-3 $\beta$ .<sup>91</sup> SNAIL is transcriptionally activated in hepatocytes, epithelial cells, and mesothelial cells by the indirect action of TGF- $\beta$ /Smad, facilitating epithelial mesenchymal transition. In notch signaling, SNAIL is expressed either by direct regulation through notch activation or SNAIL is stabilized by elevated expression of HIF-1 $\alpha$ , which recruits lysyl oxidase (LOX). The LOX-like 2 (LOXL2) attenuates GSK-3 $\beta$ -dependent SNAIL degradation through the oxidation of K98 and/or K137 lysine residues in SNAIL. Wnt signalling also suppresses the degradation of SNAIL by repressing the activity of GSK-3 $\beta$ .<sup>92–95</sup>

NF- $\kappa$ B binds between 194 and 78 bp of the SNAIL promoter and activates transcription.<sup>96</sup> GSK-3 $\beta$  targets SNAIL for ubiquitin-mediated proteasomal degradation.<sup>97</sup> Furthermore, the ubiquitination of SNAIL by TNF- $\alpha$ /NF- $\kappa$ B is inhibited by CSN2 and hence interferes with GSK-3 $\beta$  or  $\beta$ -Trep interaction. Thus, SNAIL is maintained in a non-phosphorylated and non-ubiquitinated state.<sup>98</sup> Cellular localization also influences the expression of SNAIL. For example, p21-activated kinase 1



**Fig. 6** Structure of SNAIL. SNAIL is Zinc-finger transcription factors with 4 to 6C2H2-type Zinc fingers in the carboxy-terminal region, involved in sequence-specific interactions with a DNA promoter containing a E-box sequence. SNAG (SNAIL/Gfi), the evolutionarily conserved domain at the amino terminus of SNAIL, promotes transcriptional repression. The C-terminal region of these proteins is highly conserved and consists of four zinc fingers which facilitate DNA Protein interactions. An evolutionarily conserved SNAG domain towards the N-terminal region is responsible for transcriptional repression and binding to histone deacetylases and methyltransferases. A protein destruction box (DB) domain and a nuclear export signal (NES) domain are located within the central proline-rich regions, which mediate ubiquitination and proteolytic degradation.



(PAK1) phosphorylates SNAIL at the S246 residue followed by localization to the nucleus<sup>99</sup>

Wnt, TGF $\beta$  and other growth factors are involved in signaling pathways of epithelial – mesenchymal transition associated with metastasis, and, collectively, these activate the master regulators of the transition including Snail1 (Snail), Snail2 (Slug). These not only repress epithelial markers, claudin, E-cadherin, mucin-1, occludin, PTEN, RKIP and others but also activate mesenchymal markers such as matrix metalloproteases, N-cadherin and vimentin. The most important and most studied genes in cancer metastasis is E-cadherin (CDH1), induced by 1 $\alpha$ , 25-dihydroxy Vit-D<sub>3</sub>, in cells expressing VDR.<sup>100,101</sup> During late colon cancer progression, VDR expression is downregulated, which is one of the major causes for therapy failure with Vit-D analogs. SNAIL binds to the VDR and downregulates VDR in colon cancer cells.<sup>102</sup> Moreover, a line of evidence suggests the inverse relationship between SNAIL and VDR, resulting in decreased levels of circulatory Vit-D. Snail and Slug expression has been positively correlated with E-cadherin expression, invasiveness, dedifferentiation and aggressiveness in breast, gastric, and colon tumours, hepatocellular carcinoma (HCC), and synovial sarcoma.<sup>103–108</sup> Snail1 is reported to be critical for the metastasis of lymph nodes in human breast cancer cell line MDA-MB231.<sup>109</sup> *In vitro* and *in vivo* studies indicate the inhibition of growth and the cell migration of lung cancer.<sup>110</sup>

An inverse relationship between levels of calcitriol and epithelial–mesenchymal transition is reported. VDR, E-cadherin, Snail and Slug which were at the baseline in human colorectal cancer and were enhanced in the epithelial phenotype with the decreased expression of Snail and Slug, increased E-cadherin, reduced vimentin and decreased migration in response to calcitriol. Based on sensitization experiments,<sup>111</sup> the therapeutic effects of radiation in colon cancer cells have been enhanced by calcitriol and increased Slug expression is reported to mitigate this effect and hence may be considered as a biomarker for calcitriol therapy.

#### 4. Role of vitamin D resistant genes in chronic diseases

Studies have demonstrated a strong association between Vit-D deficiency and chronic diseases, including cardiovascular diseases, diabetes, cancers, autoimmune diseases, infectious diseases, and others.<sup>112</sup> Identification of Vit-D resistant genes associated with the aforementioned chronic diseases could serve as potential therapeutic targets. The following sections describe the studies that have recognized the role of Vit-D resistant genes in chronic diseases (Table 1).

##### 4.1. Cancer

Vit-D influences cancer development and progression in several ways and is designated to be anticancerous.<sup>113</sup> However, evidence suggest that in several types of cancers there is dysregulation in the metabolism and functions of Vit-

D resulting in resistance to its antitumorigenic effects.<sup>2</sup> Research into understanding the perturbations to the metabolism and its altered functions in cancer pathophysiology will aid in the development of new strategies for successful cancer therapy based on Vit-D.

Active Vit-D has several antitumor effects, including pro-differentiative, antiproliferative and proapoptotic functions in a number of tissues *via* its binding to VDR.<sup>113</sup> The local concentrations of active Vit-D are determined by 24-hydroxylase (CYP24A1) and 1-hydroxylase (CYP27B1).<sup>114</sup> Modulation of CYP24A1 and VDR expression in human tumors may compromise VDR signalling.<sup>115</sup> RT PCR data reveal the upregulation of CYP24A1 mRNA in colon,<sup>116</sup> ovary and lung tumors<sup>115</sup> and its down regulation in breast tumor,<sup>117</sup> while VDR mRNA was down-regulated in colon, breast and lung tumors, but up-regulated in ovarian tumors. 1,25(OH)2D<sub>3</sub> treatment had no effect on VDR expression after 48 h though it stimulated CYP24A1 expression in SW620, MCF-7 and HT-29 cell lines.<sup>115</sup>

The Vit-D resistant gene CYP24 has been reported as a putative oncogene in breast cancer, and has been identified at a higher level in colon cancer. Studies suggest a strong association of Vit-D resistance at advanced stages of cancer, resulting in resistance to Vit-D supplements. Studies also report the overexpression of CYP24A1 in the cervical, ovarian, basal cell and squamous cell carcinomas.<sup>118</sup> Matusiak and Benya have identified the translocation of the CYP24 protein to the cytoplasm at aberrant crypt foci in metastatic colon cancer.<sup>119</sup> Another study supported this evidence where ketoconazole arrested prostate cancer proliferation by inhibiting CYP24A1.<sup>120</sup>

The expression of CYP24A1 is induced by the negative feedback mechanism of calcitriol-VDR activation. Current studies on cancer progression focus on four major mechanisms of CYP24A1 regulation, which include (i) excessive amplification of the gene on chromosome 20 at the q13.2–3 locus.<sup>121</sup> In this connection, studies have demonstrated the detection of excessive CYP24A1 amplification in malignant stages of colon cancer<sup>122</sup> (ii) miR-125b that inhibits the CYP24 mRNA was low in breast cancer,<sup>123</sup> (iii) overexpression of upstream kinases: CYP24A1 overexpression in prostate cancers is induced by casein kinase 2 signaling<sup>124,125</sup> and (iv) epigenetic regulation of the CYP24A1 promoter region: inhibition of both DNMT and histone deacetylase increases the expression of CYP24A1.<sup>126,127</sup> The low levels of CYP24A1 in the normal colon tissue could be due to promoter hypermethylation and that hypomethylation results in the CYP24A1 overexpression in tumor tissue, which indicates that epigenetics plays an important role in the control of CYP24A1 expression in cancer.<sup>128</sup>

SMRT levels are elevated in prostate cancer cell lines<sup>129</sup> and in PC-3 and DU-145 cells than in normal prostate epithelial cells and most primary prostate cancer cell cultures.<sup>130</sup> Furthermore, a higher expression of SMRT is reported in a CWR22Rv1 sub-cell line with decreased Vit-D sensitivity.<sup>131</sup> A non-synonymous SNP of SMRT has been reported to be associated with breast cancer.<sup>132</sup> The SMRT gene was also amplified



**Table 1** Role of vitamin-D resistant genes in various chronic diseases

Disease	Gene	Study model	Outcome	Ref.
Breast cancer	CYP24A1	Suppression of CYP24A1 in the breast cancer cell lines MCF7 and MDA-MB-231 RNA was isolated from breast tumor biopsies Normal breast mammary lesions, breast carcinomas <i>in situ</i> and invasive tumours	CYP24 has been reported as a putative oncogene in breast cancer. Upregulation of CYP24A1 In breast cancer, CYP24A1 expression is upregulated and the Vit-D metabolism and signaling pathways are deregulated, promoting tumours	156 115 157
	SMRT	Five SNPs were genotyped in 1218 familial BRCA1/2-mutation negative breast cancer cases and 1509 controls (rs1804645, rs6094752, rs2230782, rs2076546, and rs2229840)	Involvement of SNP SRC-3, in breast cancer development	132
Colon cancer	CYP24A1	RNA isolated from Colon tumor biopsies Tissue specimens of cancerous lesions and histologically normal mucosa outside the tumor border	Upregulation of CYP24A1 calcitriol's anti-proliferative effect is counteracted by the overexpression of CYP24A1.	115 116
	SNAIL	Hman SW480-ADH colon cancer cells that ectopically express mouse hemagglutinin-tagged SNAIL protein (SNAIL-HA)	In human colon tumors CYP24A1 mRNA is highly expressed SNAIL overexpression significantly decreased VDR mRNA and protein expression, and inhibited the induction of E-cadherin by 1,25(OH) <sub>2</sub> D <sub>3</sub> .	158 159
Ovarian cancer	CYP24A1	RNA was isolated from ovarian tumor	Upregulation of CYP24A1 in ovary tumor	115
	SNAIL	Snail knockdown in mouse ovarian cancer cells OV2944-HM-1 (HM-1)	Snail knockdown reduces the expression of CXCR2 ligands (CXCL1 and CXCL2), and chemokines that attract MDSCs to the tumor <i>via</i> CXCR2	136
Lung adenocarcinoma	CYP24A1	Lung tumor samples were collected for determining CYP24A1 expression	CYP24A1 upregulation is associated with poorer survival in lung AC	160
Prostate cancer	CYP24A1	Human prostate cancer cell lines (LNCaP, PC3, and DU145) were obtained and treated with calcitriol	CYP24A1 upregulation is associated with highly advanced stage of prostate cancer	40
	SMRT	Sublines of CWR22rv1, CWR22R-1, and CWR22R-2, were screened with differential sensitivity to Vit-D	A reduction in VDR-mediated transcription was observed in CWR22R-2 cells when SMRT was expressed.	131
	CYP24A1	VDR null mice were fed a high-calcium and high-phosphate rescue diet containing 20% lactose and Vit-D	Evaluation of CYP24A1 expression	146
Atherosclerotic lesion	CYP24A1	High glucose-induced human renal podocytes	The progression of atherosclerotic lesions in the aorta of transgenic rats that exhibit constitutive CYP24A1 expression	149
Coronary artery calcification		Genotyping analysis	CYP24A1 gene is associated with coronary artery calcium (CAC) quantity in 3 independent populations	150
Asthma	CYP24A1	Genotyping analysis	SNPs or haplotypes of CYP24A1 have been found to be associated with asthma in humans ( $P = 0.0299$ )	154
Multiple sclerosis	CYP24A1	Brain samples were derived from 134 adult individuals H1ESC and HepG2 cells (DNase I hypersensitivity site identification)	In UKBEC, rs2248359 had the greatest impact on CYP24A1 expression in frontal cortex and temporal cortex despite the gene being widely expressed in human brain and MS.	155

in ductal carcinoma *in situ* (DCIS), and in early breast lesion.<sup>133</sup> In contrast, deletion of the gene was more common in advanced cases of mixed DCIS and invasive carcinomas. SMRT gene truncation mutations are also detected in the Cancer-Genome-Atlas breast cancer analyses.<sup>134</sup>

SNAIL seemed to be overexpressed in many cases of cancer. Moody *et al.* reported SNAIL to be facilitating epithelial-mesenchymal transition (EMT) in mammary tumor and promoting tumor recurrence *in vivo*<sup>135</sup> (Fig. 7), and expression patterns of SNAIL correlated with poor prognosis of breast cancer. Furthermore, the level of SNAIL was elevated in ovarian cancer<sup>136</sup> and colon cancer.<sup>102</sup> Microarray data revealed the increased expression of SNAIL in metastatic prostate cancer compared to the non-metastatic state.<sup>137</sup> SNAIL1 was not expressed, but its isoform SNAIL2 is expressed at low levels in

normal tissues. In contrast, both of them seemed to be at an elevated level in cancerous tissues.

#### 4.2. Kidney diseases

Patients with chronic kidney disease (CKD) are diagnosed with insufficient or deficient Vit-D levels. Also, patients with hyperparathyroidism and hyperplasia in the parathyroid gland are reported to have imbalanced bone and mineral metabolism.<sup>138,139</sup> As kidney function declines, 1,25(OH)<sub>2</sub>D<sub>3</sub> levels also decline, resulting in reduced calcium levels and a rise in phosphate levels, which leads to an increase in PTH and FGF23.<sup>140–142</sup> Reduced renal mass in patients with CKD leads to a decreased level of 1,25-(OH)<sub>2</sub>D, 25(OH)D<sub>3</sub>, along with low CYP27B1 activity.<sup>143,144</sup> According to a recent study high PTH disrupts not only CYP27B1 expression but also

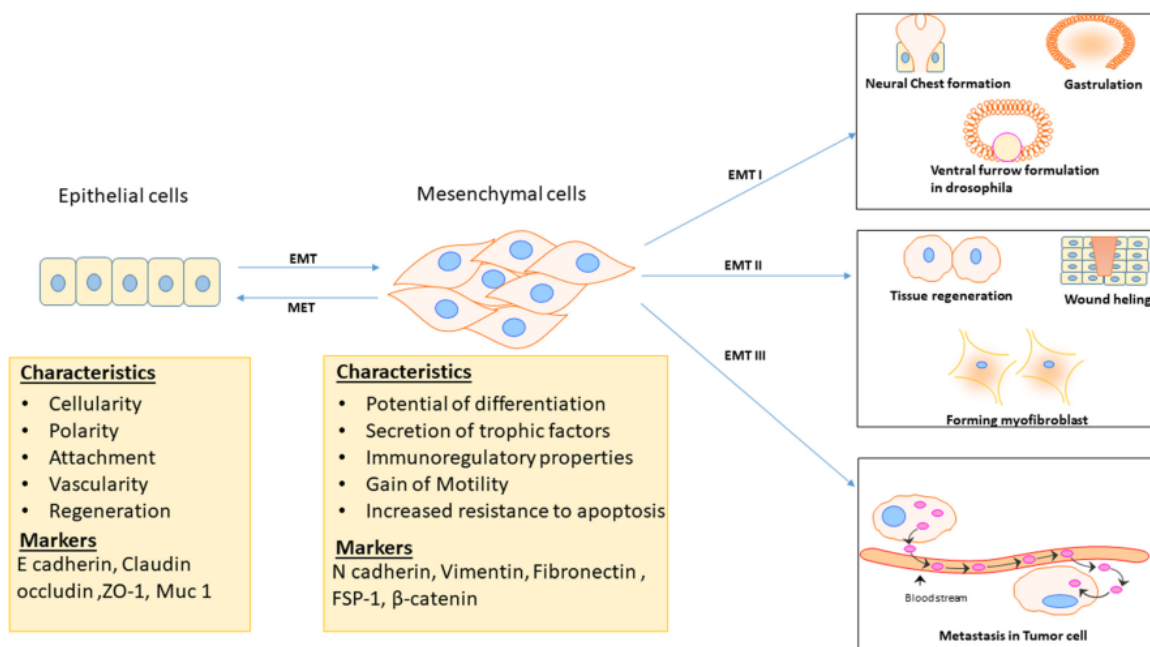


Fig. 7 The role of EMT in malignancy. The role of Epithelial-to-mesenchymal transition (EMT) and Mesenchymal-to-epithelial transition (MET) in malignancy, cells transition from an epithelial phenotype to a mesenchymal phenotype. Activation of different types of EMT program results in the various functions.

affects CYP24A1 expression.<sup>138,139</sup> It is also evident that CYP27B1 mRNA in some CKD cases remains unchanged.<sup>145</sup> Studies have reported decreased levels of 25(OH)D3 and 1,25-(OH)2D3 and increased levels of CYP24A1 mRNA and protein in the uremic kidney with no significant changes in CYP27B1 levels.<sup>45</sup> On the contrary studies on animals induced with Vit-D deficiency have been revealed low CYP24A1 mRNA expression.<sup>146</sup> The overexpression of CYP24A1 deprives kidney tissue of Vit-D and it triggers inflammation and fibrosis in turn, aggravating kidney disease.<sup>147</sup>

#### 4.3. Cardiovascular diseases

The mechanism of action of Vit-D has been largely elucidated from clinical studies. Despite the abundance of circumstantial evidence, it remains controversial whether Vit-D is involved in cardiovascular health.<sup>148</sup> A study has evidenced that high-fat and high-cholesterol feeding greatly accelerates the progression of atherosclerotic lesions in the aorta of transgenic rats that exhibit constitutive CYP24A1 expression.<sup>149</sup> Another study has shown the association of the CYP24A1 gene with coronary artery calcium levels in 3 independent human populations.<sup>150</sup>

#### 4.4. Asthma

*In vitro*, *in vivo*, and clinical studies support the link between Vit-D supplementation and onset of allergies, suggesting that calcitriol suppresses dendritic cell maturation and sub-

sequently Th1 cell development.<sup>151–153</sup> Another study reported SNPs or haplotypes of CYP24A1 to be associated with asthma in humans, associated with 1,25-(OH)<sub>2</sub>D, 25-(OH)D, and Ig-E levels, which cause severity in asthma.<sup>154</sup>

#### 4.5. Brain related diseases

Inflammation, neurotransmitter synthesis, and calcium balance are affected by Vit-D. In foetuses and adults, the neurons and the glial cells express CYP27B1, particularly in substantia-nigra, supraoptic, and hypothalamus paraventricular tissues. A study reports the Vit-D receptor to be highly expressed in the brain, including the hypothalamus, pons, basal ganglia, hippocampus, and developing brain tissues. The relationship between 25(OH)D deficiency and depression, Alzheimer's disease, epilepsy, neurocognitive decline, and dementia has been studied from three human brains (post-mortem) to identify the potential risk allele. The identified allele C of SNP rs2248359, a known multiple sclerosis (MS) disease risk gene, is strongly correlated with increased CYP24A1 expression. According to a range of experiments performed in H1 human embryonic stem cell line (H1ESC) and HepG2 cells the SNP was located within the promoter region. Additionally, the rs2248359 variant in UK Brain Expression Consortium (UKBEC), had a greater impact on the expression of CYP24A1 in the frontal and temporal cortex, despite the gene being widely expressed throughout the brain.<sup>155</sup>

## 5. Prospects

Vit-D resistance characterised by decreased level of Vit-D in the circulation and insufficiency in the target organs is on the rise. Vit-D resistant genes namely CYP24A1, SMRT and SNAIL are associated with the pathogenesis of chronic diseases. Research related to the use of inhibitors of Vit D resistant genes is on the rise. For example, the use of sulfone and sulfoximine derivatives of 1,25(OH)<sub>2</sub>D<sub>3</sub>, CTA018/MT2832 (16,23-diene-25sulfone analog) antagonist of CYP24A1 and agonist of VDR and the use of ketoconazole and liarozole for stabilizing CYP24A1 or the use of NPI-0052 and DETANONOate for inhibiting the expression of SNAIL are proving to be promising. In addition, the use of phytochemicals genistein, gambogic acid, and EGCG for inhibiting CYP24A1, SNAIL is on the rise. There is a lot of scope for research and translation related to the natural compounds that can modulate the expression and function of these crucial genes dictating the circulatory levels of Vit D. Advancement of research in this area will open up a new arena in the field of therapeutic modulators of Vit-D resistant genes.

## 6. Conclusion

This review has summarised the role of Vit-D resistant genes, their structures and functions in the progression of chronic diseases. With the knowledge of the prevalence of vitamin D deficiency in the population and understanding the roles of vitamin D resistant genes CYP24A1, SNAIL, and SMRT in dictating the deficiency/insufficiency and bioavailability of vitamin D, these genes could be considered potential therapeutic targets. Furthermore, identifying natural compounds that modulate the functions of these enzymes could prove to be a noble strategy to improve the levels of vitamin D, thus overcoming the adverse effects of vitamin D deficiency in health and disease.

## Conflicts of interest

The authors declare no conflicts of interest.

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