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Vitamin D and alternative splicing of RNA

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Abstract

The active form of vitamin D (1 α ,25-dihydroxyvitamin D, 1,25(OH)₂D) exerts its genomic effects via binding to a nuclear high-affinity vitamin D receptor (VDR). Recent deep sequencing analysis of VDR binding locations across the complete genome has significantly expanded our understanding of the actions of vitamin D and VDR on gene transcription. However, these studies have also promoted appreciation of the extra-transcriptional impact of vitamin D on gene expression. It is now clear that vitamin D interacts with the epigenome via effects on DNA methylation, histone acetylation, and microRNA generation to maintain normal biological functions. There is also increasing evidence that vitamin D can influence pre-mRNA constitutive splicing and alternative splicing, although the mechanism for this remains unclear. Pre-mRNA splicing has long been thought to be a post-transcription RNA processing event, but current data indicate that this occurs co-transcriptionally. Several steroid hormones have been recognized to coordinately control gene transcription and pre-mRNA splicing through the recruitment of nuclear receptor co-regulators that can both control gene transcription and splicing. The current review will discuss this concept with specific reference to vitamin D, and the potential role of heterogeneous nuclear ribonucleoprotein C (hnRNPC), a nuclear factor with an established function in RNA splicing. hnRNPC, has been shown to be involved in the VDR transcriptional complex as a vitamin D-response element-binding protein (VDRE-BP), and may act as a coupling factor linking VDR-directed gene transcription with RNA splicing. In this way hnRNPC may provide an additional mechanism for the fine-tuning of vitamin D-regulated target gene expression.

Keywords

Vitamin D; RNA; Transcription; Splicing; Heterogenous nuclear ribonucleoprotein C

1. Introduction

Alternative splicing provides an efficient mechanism by which the genome of any given organism can greatly expand its protein repertoire. The aim of this review is to describe our current understanding of the link between alternative splicing and vitamin D. The review will initially focus on the canonical transcriptional actions of vitamin D, but will then explore the interaction between vitamin D and a key component of the machinery associated with the maintenance of splicing fidelity, heterogeneous nuclear ribonucleoprotein C (hnRNP). In this way, the review will highlight a potentially important new mechanism for further expansion of the genomic actions of vitamin D.

1.1. Genomic actions of the vitamin D receptor

1 α ,25-dihydroxyvitamin D [1,25(OH) $_2$ D], the biologically active metabolite of vitamin D, performs the majority of its biological functions by regulating gene transcription through a nuclear high-affinity vitamin D receptor (VDR), a member of the superfamily of nuclear receptors that bind steroid hormones and other lipophilic ligands [1,2]. Upon ligand (1,25(OH) $_2$ D) binding, VDR heterodimerization occurs with the nuclear retinoid X receptor (RXR) [3]. The resulting VDR–RXR complex can then bind to specific DNA sequences, termed vitamin D-response elements (VDREs) within proximal or distal promoter regions of target genes [4,5]. After binding to a VDRE, a variety of VDR-interacting nuclear proteins (co-regulators) are recruited to the pre-initiation transcriptional complex, which act to enhance or suppress the rate of gene transcription by the liganded VDR [6,7]. Other factors that add to the diversity of VDR-mediated regulation of gene expression include the presence of distal enhancer elements [8], as well as the potential for ligand-independent actions of VDR [9].

As the only high-affinity receptor for 1,25(OH) $_2$ D, the VDR mediates genomic responses to 1,25(OH) $_2$ D [10]. Recent developments in deep sequencing technologies for unbiased analysis of VDR binding loci have expanded our understanding of the genome-wide actions of 1,25(OH) $_2$ D-VDR. Chromatin immunoprecipitation (ChIP) followed by DNA sequencing of the immunoprecipitated products, ChIP-sequencing (ChIP-Seq), using a variety of cell types has shown that there are between 1000 and 13,000 VDR-specific genomic binding sites [11,12]. The majority of these VDR binding sites appear to be distal to the target gene transcriptional start site, being located in either intergenic regions or within introns. This has had a dramatic effect on our understanding of transcriptional regulation by the 1,25(OH) $_2$ D-VDR complex. Most VDR-binding DNA loci have yet to be validated by specific quantitative PCR analysis of ChIP products, but, recent work by Pike and co-workers has confirmed that 1,25(OH) $_2$ D regulation of the genes for 24-hydroxylase (*CYP24A1*) and RANK ligand (*RANKL*), involves both proximal promoter VDREs and a complex set of downstream or upstream distal VDREs [13–15].

Genomic binding of VDR is also highly cell-specific. Tuoresmaki et al. recently conducted a re-analysis of publically available VDR ChIP-seq datasets for six different cell types. These datasets revealed a total of 23,409 non-overlapping genomic VDR binding sites, with a majority of these VDR loci (17,700) being unique to each of the analyzed cell types, and only 43 binding sites were shared by all six cell types, emphasizing the cell-specificity of

VDREs [16]. However, the underlying mechanisms that define cell-specific patterns of VDR binding remain unclear, and may involve alternative accessory factors for VDR. Intriguingly more than 60% of genomic VDR binding sites do not contain a canonical hexameric VDRE sequence direct-repeat (DR) 3 with two viable six-base half-elements separated by three base pairs with consensus sequence RGGTCAnnnRGTTCA, ($r = A$ or G), suggesting that VDR may use alternative mechanisms to interact with genomic DNA [16]. For example, liganded VDR may partner with currently undefined partner proteins or interface with other DNA-binding transcription factors, such as pioneer factors.

These alternative binding mechanisms may explain some of the cell-specific actions of VDR as well as its repressive functions on gene transcription [11]. However, there is now strong evidence supporting the contribution of other mechanisms that diversify the effects of vitamin D on the transcriptome and proteome. For example, epigenetic modifications are known to play a key role in the maintenance of VDR-directed gene expression and dysregulation of these mechanisms can lead to pathological conditions [17,18]. The impact of epigenetics on VDR signaling has been well defined for chromatin remodeling via DNA methylation and histone acetylation, with VDR co-activators and co-repressors interfacing with chromatin modifiers and remodelers [6,19,20]. In addition, recent studies have also implicated microRNAs (miRNAs) in mediating the fine-tuning of vitamin D-mediated responses [21–23]. The liganded VDR complex can either suppress or induce miRNAs by either direct transcriptional regulation of autonomous miRNA genes or indirect regulation of miRNAs via host gene promoter sequences [24]. Conversely, miRNAs may act to regulate $1,25(\text{OH})_2\text{D}$ synthesis, catabolism, or signaling to form dynamic feedback mechanisms (comprehensive review, see [24]).

The current manuscript will review another mechanism with the potential to influence vitamin D regulated gene expression – namely RNA splicing, and alternative splicing. In particular, this review will focus on the potential role of hnRNPC, a key nuclear factor in post-transcriptional RNA-processing, as a mediator of vitamin D receptor-directed transcription and RNA splicing.

2. Vitamin D and RNA splicing

2.1. Pre-mRNA splicing and alternative splicing overview

In humans and other complex metazoans, the vast majority of protein-coding genes contain several exons separated by introns that will not appear in the mature mRNA. Removal of introns and the ligation of exons that contain the protein-coding open reading frame and the 5' and 3' untranslated regions (UTRs) is accomplished by pre-mRNA splicing, a process which is facilitated by a complex of small nuclear RNAs (snRNAs), splicing factors, and numerous RNA-binding proteins that collectively form the spliceosome [25]. Nuclear pre-mRNA splicing entails two consecutive trans-esterification reactions. First, the 2'-hydroxyl of an adenosine of the branch point sequence in the intron carries out a nucleophilic attack on the phosphodiester bond at the 5' splicing site (SS). This results in cleavage at this site and ligation of the 5' end of the intron to the branch adenosine, forming a free 5' exon and a lariat structure. Subsequently, the phosphodiester bond at the 3' SS is attacked by the 3'-

hydroxyl of the 5' exon, leading to the ligation of the 5' and 3' exons and release of the lariat intron.

During splicing, the spliceosome dynamically, and in a stepwise fashion, assembles and disassembles across the pre-mRNA to direct the correct recognition and pairing of the splice sites [26]. Whilst some exons are constitutively spliced, in that they are included in every mRNA produced from a given pre-mRNA, many others are alternatively spliced to generate variable forms of mRNA from a single pre-mRNA transcript [27]. The capacity for alternative splicing in eukaryotes has greatly enhanced transcriptome and proteome diversity leading to a higher order of organismal complexity but without the need for expansion of the genome. Importantly, alternative splicing can also be regulated differently according to cell type, developmental stage, or signal-dependent patterns [25,28].

2.2. Alternative RNA splicing and the vitamin D system

Previous studies linking vitamin D and alternative splicing have focused on the metabolism of vitamin D (Table 1). Published reports have described splice variant mRNA transcripts for the vitamin D-activating enzyme 25-hydroxyvitamin D-1 α -hydroxylase (*CYP27B1*) [29–35]. Data from these studies have underlined a potential role for *CYP27B1* splice variants in regulating localized synthesis of 1,25(OH)₂D [32], notably in the context of tissue-specific regulation of this active form of vitamin D [31]. Other studies have suggested that dysregulation of *CYP27B1* splice variant expression may contribute to the development of neoplasias via effects on cell-specific synthesis of 1,25(OH)₂D [29,30,36]. The underlying mechanisms for this have yet to be defined, as reported splice variants of *CYP27B1* appear to be expressed in very low abundance, and do not appear to encode functional proteins [32]. Nevertheless, knockdown of some of these *CYP27B1* splice variants in human kidney cells using RNA-interference resulted in enhanced conversion of 25-hydroxyvitamin D (25OHD) to 1,25(OH)₂D [32], suggesting a functional role for RNA splicing with respect to vitamin D metabolism. In this context the most informative gene for vitamin D and alternative splicing appears to be the vitamin D-catabolic enzyme 24-hydroxylase (*CYP24A1*), which is potently induced in cells following exposure to 1,25(OH)₂D [37,38].

Alternative splicing of *CYP24A1* was first reported in the chick HD-11 macrophage cell line [39]. In this instance, exons 1 and 2 of the *cyp24a1* gene are spliced out and replaced by a pseudo exon composing part of intron 2 [39]. The resulting *CYP24A1*-splice variant (*CYP24A1-SV*) generates an in-frame shift in mRNA, using an alternative start codon that leads to a protein lacking the mitochondrial-targeting sequence for *CYP24A1* which is therefore metabolically inactive. Despite this, *CYP24A1-SV* appears to play a pivotal role in vitamin D metabolism by acting as a decoy for substrate 25OHD and attenuating endogenous conversion of 25OHD to 1,25(OH)₂D by *CYP27B1* [39]. Thus, *CYP24A1* may be particularly important in cells, such as macrophages, with significant intracrine *CYP27B1* activity. Notably *CYP24A1-SV* has been detected in cancer cells, in particular colorectal and prostate cancer cells [40,41]. The precise impact of the *CYP24A1* splice variant in this setting has yet to be fully defined. However, in common with its proposed role in macrophages, this may involve decoy actions on local vitamin D metabolism by these cells. In this way, expression of the variant protein form of *CYP24A1* may play a crucial role in

the efficacy of local 25OHD metabolism, with overabundance of *CYP24A1*-SV acting to diminish local concentrations of 1,25(OH)₂D at the cell-specific level.

Other studies have shown that vitamin D itself may influence *CYP24A* splicing. Muindi et al. reported that 1,25(OH)₂D can modulate *CYP24A1* pre-mRNA splicing in prostate cancer cells in a dose- and time-dependent fashion, although the mechanism for this is unclear [40]. Another recent study showed that 1,25(OH)₂D can also regulate *CYP24A1* splicing in colon cancer cells [42]. In multiple colon cell lines, RNA splicing patterns regulated by 1,25(OH)₂D were shown to be associated with cellular sensitivity to 1,25(OH)₂D, with more significant induction of *CYP24A1* splicing being observed in cells that were more sensitive to 1,25(OH)₂D treatment. Regulation of RNA splicing by vitamin D also appears to be gene selective, with vitamin D target genes other than *CYP24A1* showing no significant variations in splicing following treatment with 1,25(OH)₂D [42]. The underlying mechanism for 1,25(OH)₂D-mediated variations in *CYP24A1* RNA splicing has yet to be clearly defined, and may involve multiple signaling pathway, such as PKA activation and c-Jun terminal protein kinase inhibition [42]. Moreover, there is evidence that the role of 1,25(OH)₂D in pre-mRNA splicing is neither confined to cancer cells nor to the *CYP24A1* gene. Collectively these studies highlight the potential importance of RNA splicing as an alternative facet of vitamin D-mediated regulation of gene expression, and this will be discussed in greater detail in the remainder of the review.

2.3. Nuclear receptor co-transcriptional splicing

It is now clear that transcriptional regulation is physically and functionally integrated with pre-mRNA splicing [43–45]. Such co-transcriptional splicing has been demonstrated in a number of different organisms and has been shown to play a role in coordinating both constitutive and alternative splicing [46]. It has been reported that by recruiting receptor co-regulators that can both control gene transcription and splicing, steroid hormones (in this case progesterone and estrogen) may coordinately control gene transcription and splicing decisions leading to alternatively spliced transcripts [47]. This mechanism is controlled in a steroid nuclear receptor (NR) and promoter-dependent manner [47]. In particular a subset of hormonally recruited NR co-regulators including CoAA [48], CAPER α and CAPER β [49], SKIP [50], and co-factor of BRCA 1 (COBRA1) [51], have been identified as coupling factors involved in coordinating steroid hormone-activated gene transcription and transcript splicing. This double-duty for the NR and co-regulators, in response to a steroid hormone signal, may act to ensure that the appropriate gene product is generated in the appropriate cell type [52].

In a similar fashion, the VDR co-regulator NcoA62/SKIP can promote RXR-VDR dependent gene transcriptional activation or repression in a cell-specific manner [53]. However, it has also been identified as a non-snRNP component of spliceosome complexes, supporting a potential role for NCoA62/SKIP in pre-mRNA splicing [54,55]. Zhang et al. demonstrated that 1,25(OH)₂D is able to influence target gene splicing through recruitment of NcoA62/SKIP. In this case the co-activator function of NcoA62/SKIP in the VDR-activated transcriptional process was demonstrated in HeLa cells by showing that NcoA62/SKIP interacts with VDR, and is associated with recruitment of VDR to *CYP24A1* VDREs

following treatment with $1,25(\text{OH})_2\text{D}$ [50]. However, using glutathione S-transferase (GST)-pull down analyses and HeLa cell nuclear extracts, NcoA62/SKIP was also shown to interact with components of the splicing machinery, such as the U5 small nuclear ribonucleoprotein (snRNP) [50]. Moreover, disruption of native NCoA62/SKIP through expression of a dominant-negative NCoA62/SKIP blocked conventional splicing of $1,25(\text{OH})_2\text{D}$ -induced RNA transcripts from a VDRE-driven reporter minigene cassette. This resulted in a $1,25(\text{OH})_2\text{D}$ -dependent accumulation of unspliced transcripts, suggesting that NcoA62/SKIP is required for correct splicing in VDR-activated gene expression. The mechanism by which NcoA62/SKIP affects RNA splicing has yet to be clarified, but these data suggest that NcoA62/SKIP, a common VDR co-factor, is involved in coupling VDR mediated transcription to RNA splicing [50].

There is currently no direct evidence showing that vitamin D affects nascent mRNA alternative splicing. However, a recent proteomic study identified a number of proteins functioning as splicing factors that were induced by $1,25(\text{OH})_2\text{D}$ in colon cancer cells [56]. As detailed above, VDR-interacting proteins with traditional roles as VDR transcriptional co-activators or co-repressors, may also be involved in the regulation of alternative splicing [57]. The remainder of this review will focus on an alternative view of this concept, in which a nuclear factor with an established function in RNA splicing, hnRNPC, has also been shown to be involved in the VDR transcriptional complex as a VDRE-binding protein [58].

3. HnRNPC1/C2, RNA splicing, and VDR-mediated transcription

The protein complex known as hnRNPC is a member of a subfamily of hnRNPs that act as RNA binding proteins by complexing with heterogeneous nuclear RNA. Transcript variants of the hnRNPC gene encode two major isoforms (C1 and C2) that form an hnRNPC1/C2 heterotetramer consisting of 3 hnRNPC1 and 1 hnRNPC2 subunits [59,60]. Although hnRNPC1/C2 was identified over 30 years ago as a core component of 40S hnRNP particles, which form on all nascent RNA transcripts [61], conflicting reports still exist in regard to the sequence specificity and mode of hnRNPC1/C2 binding to RNA [62–65]. A recent study using in vivo crosslinking and immunoprecipitation followed by deep sequencing confirmed that hnRNPC1/C2 is predominantly positioned on pre-mRNAs via sequence-specific binding to neighboring uridine tracts of its RNA recognition motif (RRM) domains, with a defined spacing of 165 and 300 nucleotides [66]. In this study it was hypothesized that hnRNPC1/C2 act as an ‘RNA nucleosome’ that incorporates long regions of nuclear pre-mRNA [66]. This is consistent with the several previously reported functions of hnRNPC1/C2 in post-transcriptional RNA processing including RNA packaging [67,68], constitutive and alternative splicing [69–72] and RNA export [73].

3.1. HnRNPC1/C2 and RNA splicing

An essential role for hnRNPC1/C2 in pre-mRNA splicing was identified over 20 years ago [69,70]. However, more recent studies have described a versatile function for hnRNPC1/C2 in alternative splicing. Knockdown of hnRNPC1/C2 by small interfering RNAs (siRNAs) promoted exon skipping or the use of more internal 3'-splice sites, suggesting that hnRNPC1/C2 acts as a splicing enhancer by enforcing exon inclusion in a cell type-dependent manner [71]. Another study described an opposite role for hnRNPC1/C2 in

regulating alternative pre-mRNA splicing to promote exon exclusion [74]. In this latter case cooperative interaction of hnRNPC1/C2 and human antigen R (HuR), an RNA binding protein which has been previously identified as an alternative splicing regulator in mammalian cells [74], promoted Fas cell surface death receptor (*Fas*) gene exon 6 skipping through binding to the exon splicing silencer (ESS) URE6. This, in turn, inhibited molecular events leading to exon definition, and subsequent exon skipping [72].

More recent approaches utilizing newly developed techniques such as individual-nucleotide resolution UV cross-linking and immunoprecipitation (iCLIP)[75], have been able to determine more precise locations of hnRNPC1/C2 binding to nascent transcripts and provide insights into the mechanism of hnRNPC1/C2 regulated alternative splicing [66,76]. Using iCLIP followed by deep sequencing, Konig et al. characterized a precise transcriptome-wide binding pattern of hnRNPC1/C2 at single-nucleotide resolution. These studies determined that hnRNPC1/C2 can promote either exon exclusion or inclusion, depending on the exact binding location of hnRNPC1/C2 on the nascent mRNA. Alternative exons were skipped by direct binding of the exon to the RNPC1/C2 tetramer, while binding of the hnRNPC1/C2 tetramer to the preceding intron enhanced the inclusion of alternative exons [66]. Another recent study by the same team found that hnRNPC1/C2 directly competes with the core splicing factor U2 auxiliary factor 65 (U2AF65), with this mechanism acting to prevent a process known as Alu exonization [76]. Sequences of DNA known as Alu elements are amongst the most transposable of DNA elements in the human genome. However, Alu elements can introduce cryptic splice sites which, if used, lead to Alu exonization. There have been increasing numbers of reports of Alu exonization-associated disorders and the mechanisms that prevent spurious exonization of Alu elements are currently unknown [77]. In this setting hnRNPC1/C2 has been identified as a potential mediator of this protection against spurious exonization [76].

3.2. HnRNPC1/C2 and vitamin D resistance

Despite their classical interaction with chromatin-associated single-stranded RNA, several studies have shown that hnRNPC1/C2 is a pluripotent protein complex with the potential for alternative modes of action [58,78–80]. In particular, previous studies from our group have demonstrated a role for hnRNPC1/C2 as an additional component of the VDR transcription complex, in which hnRNPC1/C2 functions as a trans-regulatory factor via interaction with double-stranded DNA cis-elements[79,81,82].

A role for hnRNPC1/C2 in vitamin D-mediated signaling was first reported following an outbreak of rachitic bone disease in the New World Primate (NWP) emperor tamarin (Saguinas imperator) colony at the Los Angeles Zoo [83]. Analysis of epidermal cells from these animal showed that the NWPs were protected against potential adverse effects of sustained exposure to high circulating levels of 1,25(OH)₂D by elevated cellular expression of a protein that binds to VDR target gene promoter VDREs [79,81]. Overabundance of this so-called “VDRE-binding protein” (VDRE-BP) appears to attenuate vitamin D receptor-mediated gene expression by blocking the binding of 1,25(OH)₂D-liganded VDR to target gene promoter VDREs. This occurs via direct binding of the VDRE-BP to the VDRE DNA half-sites, and has been proposed as the underlying cause of 1,25(OH)₂D-insensitivity in

NWPs [81,84]. Subsequent studies showed that a VDRE-BP was identical to hnRNP1/C2 that was over-expressed in a human subject with a form of hereditary vitamin D resistant rickets (HVDRR) [79,82,85]. In this case, ChIP analyses confirmed the ability of hnRNP1/C2 to compete in trans with VDR for occupation of the VDRE in human cells. In normal 1,25(OH)₂D responsive human cells, the hnRNP1/C2 occupying the CYP24A1 VDRE at baseline was then displaced from the promoter VDRE by the 1,25(OH)₂D liganded VDR–RXR complex [79]. This reciprocal relationship between VDR and hnRNP1/C2 occurs in a time-dependent cyclical fashion following exposure to 1,25(OH)₂D, suggesting that hnRNP1/C2 may be a key determinant of the temporal patterns of VDRE occupancy [79]. In hnRNP1/C2 over-expressing fibroblasts and B cells from the HVDRR patient, the reciprocal association of VDR and hnRNP1/C2 with the VDRE was distorted relative to control vitamin D-responsive cells [79]. In a similar fashion, overexpression of hnRNP1/C2 in vitro has been shown to disrupt the relationship between VDR and hnRNP1/2, leading to suppression of 1,25(OH)₂D-induced gene expression [79]. These data suggest that vitamin D resistance in the case of the HVDRR patient was similar to that previously described for NWPs [58,79].

3.3. hnRNP1/C2 – a link between VDR-mediated transcription and alternative splicing?

Although there is evidence for effects of 1,25(OH)₂D on pre-mRNA splicing, the mechanism for this is unclear. Because of its capacity to interact with nascent pre-mRNA as well as double-strand DNA, we hypothesize that hnRNP1/C2 may play a key role in linking 1,25(OH)₂D-induced transcription with RNA splicing, thereby providing an additional mechanism for fine-tuning of gene expression (see Fig. 1). This hypothesis is supported by the increasing evidence for functional integration between transcription and splicing machinery in mammalian cells [86]. This dual role for hnRNP1/C2 may help to ensure more precise regulation of gene expression in response to vitamin D, in a manner that is more efficient for cellular energy. It is well recognized that the biosynthetic and metabolic energy cost of RNA splicing and the surveillance required to eliminate imprecisely spliced mRNAs is very high, as is the potential harm from defects in these processes [25].

A key question to be answered in future studies is whether the hnRNP1/C2 displaced from VDRE by liganded VDR is involved in facilitating RNA splicing. If this is the case, then another question arises as to how hnRNP1/C2 migrates from gene promoter regions to a specific RNA splice site to direct pre-mRNA splicing or alternative splicing. Recent studies have shown that a ‘mediator complex’ may function to “hand over” a splicing regulator, heterogeneous nuclear ribonucleoprotein L (hnRNPL), from gene promoters to the elongating RNA polymerase II complex, thereby mediating specific splice site selection during co-transcription splicing [87]. Moreover, as outlined above, VDR co-activators or co-repressors may also function as splicing factors and, as such, these may also act as coupling factors linking transcription and RNA splicing responses to vitamin D. Further analysis of vitamin D signaling, hnRNP expression and alternative splicing of RNA will broaden our perspective on how vitamin D is able to influence gene expression during normal physiology. However, alternative splicing may also be a contributor to human disease, and it in future studies it will also be important to assess the impact of vitamin D status on hnRNP function and normal RNA splicing.

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Fig. 1.

DNA and RNA binding functions of hnRNP1/C2 and the action of 1,25(OH)₂D. Schematic representation of the ability of hnRNP1/C2 to act as: (1) a vitamin D-response element-binding protein (VDRE-BP) in the absence of liganded (1,25(OH)₂D-bound) vitamin D receptor (VDR). In the presence of VDR-1,25(OH)₂D, hnRNP1/C2 is displaced from the VDRE; (2) a component of the RNA spliceosome, facilitating either exon exclusion or exon-inclusion. As yet it is unclear whether these two facets of hnRNP1/C2 function are linked.

Table 1

Overview of splice variants of the CYP24A1 and CYP27B1 genes.

Gene	Transcript variant		Organism/cell	Protein coding	Enzymatic activity	Biological meaning	Reference
CYP24A1	CYP24A1-wild type	Full length	All VDR-expressing cells	Yes	Yes	23- and 24-hydroxylation of 25(OH)D/1,25(OH) ₂ D	[38]
–	CYP24A1-SV	Deletion exons 1 and 2, insertion part of intron 2	Chick macrophage cell line, Human/kidney, placenta, skin, and macrophages	Yes	No	Suppress synthesis of 1,25(OH) ₂ D as a cytosolic decoy for CYP24A1/CYP27B1 substrates	[39–41]
	CYP24A1-SV2	Deletion exons 1 and 2	Human/colon cancer cells	Unknown	No	Unknown	
	CYP24A1-SV3	Deletion exon 10	Human/colon cancer cells	Unknown	No	Unknown	
–							
CYP27B1	CYP27B1-wild type	Full length	Human/renal or extra-renal cells	Yes	Yes	1 α -Hydroxylation of 25(OH)D	[38]
	SV ₁	Contains part of intron 2, exons 6–9	Human/kidney cell lines	Noncoding	–	Predicted function: coordinate localized synthesis of 1,25(OH) ₂ D by limiting the availability of the wild type 1 α -hydroxylase enzyme activity	[32]
	SV ₂	Contains part of intron 2, part of exon 6, exons 7–9	Human/kidney cell lines	Noncoding	–		
	SV ₃	Contains part of intron 2, exon 3, part of intron 3, part of exon 8 and exon 9	Human/kidney cell lines	Noncoding	–		
	SV ₄	Contains part of intron 2, exon 3, intron 3, part of exon 4 and exon 9	Human/kidney cell lines	Noncoding	–		
	SV ₅	Contains part of intron 2, exons 3–4 and exon 9	Human/kidney cell lines	Noncoding	–		
	SV ₆	Contains intron 2, exons 3–5, intron 5 and part of exon 6	Human/myelomonocytic cell lines	Noncoding	–		
	SV ₇	Contains part of intron 2, exons 3–5, part of exon 6 and part of exon 7	Human/myelomonocytic cell lines	Noncoding	–		
	SV ₈	Contains part of intron 2, part of exon 4, exon 5, intron 5, exon 6 and part of exon 7	Human/myelomonocytic cell lines	Noncoding	–		
	SV ₉	Contains part of intron 2,	Human/myelomonocytic cell	Noncoding	–		

Gene	Transcript variant	Organism/cell	Protein coding	Enzymatic activity	Biological meaning	Reference
	part of exon 4, exons 5–6 and part of exon 7	lines				
	SV ₁₀	Contains part of intron 2, part of intron 5, exon 6, and part of exon 7	Human/myelomonocytic cell lines	Noncoding	–	
	Hyd-V1	Insertion part of intron 2, deletion exons 4 and 5	Human/GBM cell lines	Yes	No	Predicted function: reduce 1 α -hydroxylase enzyme activity; contribute to the development of neoplasias via effects on cell-specific synthesis of 1,25(OH) ₂ D; potential as a diagnostic marker for cancer [29–31,33,36]
	Hyd-V2	Deletion exons 4 and 5	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V3	Deletion of part of exon 8	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V4	Insertion part of intron 2	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V5	Insertion part of intron 5	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V6	Insertion part of intron 1	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V7	Insertion part of intron 1, deletion exons 4 and 5	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V8	Insertion introns 1 and 5	Human/GBM, melanoma, cervix carcinoma and kidney cell lines	Yes	No	
	Hyd-V9	Insertion introns 2 and 6	Human/GBM cell lines	Yes	No	
	Hyd-V10	Insertion introns 1 and 2	Human/GBM cell lines	Yes	No	
	Hyd-V11	Insertion introns 1 and 3	Human/GBM cell lines	Yes	No	
	Hyd-V12	Insertion introns 1, 3 and 5	Human/GBM cell lines	Yes	No	
	Hyd-V13	Insertion introns 1, 2 and 5	Human/GBM cell lines	Yes	No	
	Hyd-V14	Insertion introns 1, 3 and 6, 7	Human/GBM cell lines	Yes	No	
	Hyd-V15	Insertion intron s1, 2 and 3, 5	Human/GBM cell lines	Yes	No	
	Hyd-V16	Insertion introns 2 and 5,	Human/GBM cell lines	Yes	No	

Gene	Transcript variant	Organism/cell	Protein coding	Enzymatic activity	Biological meaning	Reference
–	deletion exons 6–9					
	Insertion intron 1	Human endometrial cancer cells/breast cancer cells	Yes	No	Unknown	[34,35]
	Deletions exons 3–5	Human endometrial cancer cells/breast cancer cells	Yes	No		

GBM: glioblastoma multiforme.

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