The Interaction of β-catenin, Vitamin D, Resveratrol, and Two Common VDR Polymorphic Variants in Colorectal Carcinogenesis

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Abstract

Vitamin D and resveratrol have been widely studied in recent years, especially their apparent abilities to impact a host of physiologic processes. Resveratrol is purported to have anti-aging, anti-inflammatory, and anticancer properties, among others. Vitamin D has been reported to possess similar properties, as well as detoxification, anti-oxidation, and cardiovascular benefits, in additon to its classical role in bone mineral homeostasis. The actions of vitamin D are mediated by the vitamin D receptor (VDR), and the VDR gene contains several polymorphisms, one of which results in two phenotypically distinct isoforms, designated M1 and M4. VDR M4 is thought to be physiologically more active than M1 in vitamin D-dependent transcriptional activity and in binding to partner proteins such as TFIIB and RXR. Another protein known to interact with VDR is β-catenin, a member of the Wnt/β-catenin signaling pathway that can drive colorectal carcinogenesis. The goal of this study was to investigate the ability of vitamin D and resveratrol to regulate the Wnt/β-catenin system via stimulation of β-catenin-VDR interaction and subsequent inhibition of β-catenin-mediated transcription. Our data support and extend existing literature by demonstrating that both 1,25D and resveratrol enhance the VDR-β-catenin interaction. It is also evident that 1,25D-stimulated VDR is capable of inhibiting β-catenin transcriptional activity. Additionally, as seen in VDR transactivation assays, the M1 and M4 forms are functionally variable in their potential to reduce β-catenin-mediated transcription. VDR M4 is again physiologically more active than M1 in this context, and our mutagenesis results indicate that the key difference is likely the glutamic acid residue at postion 2 in VDR M1. Both SNPs are responsive to 1,25D stimulation, and their differences appear to narrow in the presence of this hormone. These data support the notion that VDR influences pathways important for colorectal carcinoma (CRC) development. Supplementation with vitamin D and resveratrol may therefore overcome the disadvantage of carrying a "weaker" VDR SNP, and may prove to be an important chemopreventive measure, in conjunction with other lifestyle changes, to deter the development of CRC.

Background

Vitamin D and VDR

Vitamin D_3 is acquired either directly from dietary sources or produced via a nonenzymatic, UV light-dependent reaction in the skin, converting 7-dehydrocholesterol to vitamin D_3. Vitamin D_3 is then hydroxylated in the liver to form 25-hydroxyvitamin D_3 (25D) the major circulating and clinically measurable form. This precursor is then converted to 1,25-dihydroxyvitamin D_3 (1,25D), the physiologically active hormone. The classic functions of 1,25D are in the regulation of calcium and phosphate homeostasis, raising blood levels of both ions through intestinal absorption, renal reabsorption, and bone resorption. Though the majority of 1,25D is produced in the kidneys, locally produced 1,25D in epithelial and other tissues has been implicated in triggering xenobiotic detoxification, vascular protection, as well as anti-inflammatory and anticancer effects in epithelial cells. The effects of 1,25D are mediated by the vitamin D receptor (VDR) protein, a nuclear receptor that regulates the expression of 1,25D/VDR-target genes. Two important polymorphic variants (Fok1, F/f) result in phenotypically different isoforms, VDR M1 (from the f gene variant) and M4 (from the F variant). M1 is 427 amino acids (AAs) long, while a single nucleotide polymorphism in the initial start codon yields a 424 AA protein, called M4, which starts 3 AAs downstream of the M1 start codon at a second, in-frame ATG. The alleles encoding these unique isoforms occur at approximately 40% for VDR M1/f and 60% for M4/F in the human population. Previous studies suggest functional differences in the two variants with respect to vitamin D-stimulated transcription as well as to their interaction with partner proteins, such as TFIIB and β-catenin.

Resveratrol

Resveratrol, a polyphenol found in raspberries, grapes, and other vegetables, is a phytoalexin generated by plants under stress. In humans, this compound is purported to have anti-aging, anti-inflammatory, and anticancer properties. Some studies have supported the notion that resveratrol prevents the translocation of β-catenin into the nucleus, while others have found that resveratrol has no effect on β-catenin accumulation or cellular distribution. Resveratrol has been shown to reduce β-catenin target gene expression, including cyclin D1 and conductin, in colorectal carcinoma (CRC) cells. Other studies have linked resveratrol with cancer stem cell inhibition and apoptosis in human CRC cells. In addition to these *in vitro* studies, epidemiological data have linked consumption of foods high in resveratrol with reduced risk of CRC.

Wnt/β-catenin signaling system

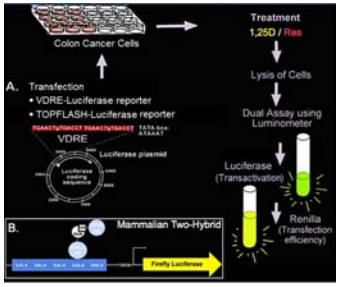
The Wnt/β-catenin signaling system is integral to the cell cycle, regulating embryonic development, as well as maintenance of stem cell populations and homeostasis of adult tissues. β-catenin is a member of the Wnt/β-catenin signaling pathway that can drive colorectal carcinogenesis. Prior research has established β-catenin as VDR-interacting protein (VIP).

Hypothesis

We postulate that the VDR M4 polymorphism is more effective in attenuating β-catenin activity in the Wnt/β-catenin signaling pathway compared to M1, and the inhibitory effect on β-catenin action of both variants is enhanced by 1,25D and resveratrol.

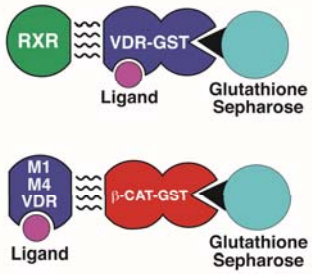
Methods

VDR-VDRE Transcription, Mammalian-Two-Hybrid, and TOPFLASH β-catenin Transcription Assays



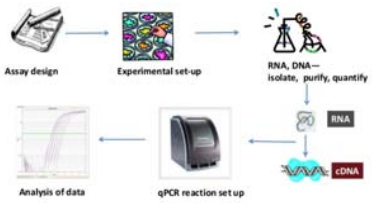
- To evaluate the transcriptional activities of VDR M1 vs M4 in the presence of 1,25D and resveratrol, a VDRE-based transcription assay was employed.
- The intracellular interaction of β-catenin and VDR was probed via a mammalian-two-hybrid assay.
- The effects of VDR SNPs, 1,25D, and resveratrol on β-catenin transcription was explored with a TOPFLASH β-catenin transcription assay utilizing a luciferase plasmid with a β-catenin/Lef1/TCF binding site (not shown).

Glutathione-S-Transferase (GST) Pull-down Assay



- An *in vitro* GST fusion protein assay system was also employed to test the variable interactions of the VDR SNPs with β-catenin (β-CAT) in the presence or absence of 1,25D.

Quantitative Real-Time PCR



Results

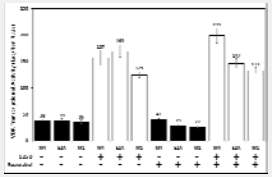


Figure 1. VDRE-based transcription assay in HCT-116 cells comparing the transcriptional activities of VDR M4, a VDR E2A mutant, and VDR M1 in the presence of 10^{-8} M 1,25-dihydroxyvitamin D (1,25D), $2.5x10^{-5}$ resveratrol, or both compounds.

- 1,25D stimulates the transcriptional activity of VDR M1, M2, and E2A.
- With the addition of 1,25D both VDR M4 and VDR E2A demonstrate increased activity over VDR M1.
- With the addition of resveratrol alone, VDR M4 is more transcriptionally active than VDR E2A and M1 while VDR E2A and VDR M1 were statistically equivalent.

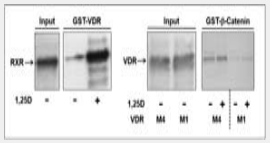


Figure 3. *In vitro* GST fusion protein assay probing the interaction of β-catenin with VDR M1 and M4 in the absence or presence of 1,25D.

- VDR M1 and VDR M4 bind to β-catenin, but M4 shows stronger binding in this *in vitro* assay.
- The positive control interaction between VDR-RXR is shown at left.

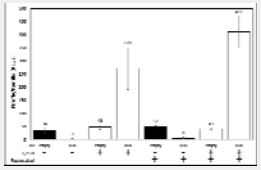


Figure 4. Mammalian two hybrid assay in HCT-116 cells evaluating the interaction between VDR and β-catenin in the presence of 10^{-8} M 1,25D, $2.5x10^{-5}$ resveratrol, or both compounds.

- VDR-β-catenin interaction is increased in the presence of 1,25D and resveratrol individually.
- The simultaneous presence of 1,25D and resveratrol enhances this interaction greater than either compound alone.

← Quantitative real-time PCR experiments in colon cancer cells were used to test the ability of either VDR M1 or M4 to sequester and inhibit β-catenin activity by measuring the expression of endogenous β-catenin target genes.

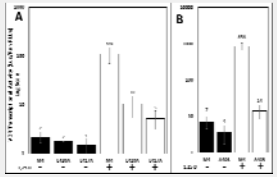


Figure 2. VDRE-based transcription assay in HCT-116 cell line assessing the different transcriptional activities between VDR M4 and mutant VDRs E420A, L417A, and Δ403, in the absence or presence of 10^{-8} M 1,25D.

- With the addition of 1,25D, the mutant VDRs E420A and L417A both display significantly decreased transcriptional activity (note log scale) when compared to the wild type VDR M4.
- The addition of 1,25D activates VDR M4 significantly more than the VDR truncation mutant Δ403 which lacks the AF-2 transactivation domain.

Table 1. Quantitation of VDR SNPs M1 and M4 Binding to β-Catenin.

VDR M4 [+1,25D]	**VDR M1** [+1,25D]
VDR M4 [-1,25D]	**VDR M1** [-1,25D]
1.68 (0.12)	**2.05** (0.21)
VDR M4 [-1,25D]	**VDR M4** [+1,25D]
VDR M1 [-1,25D]	**VDR M1** [+1,25D]
3.31 (0.34)	**2.62** (0.25)

Binding was assessed via GST-β-catenin "pull-down" assays as described in Figure 3, followed by quantitation via densitometric scanning of radiographs. Values represent the mean fold effect (-/+ SD) from 10 independent experiments as the one shown in Figure 3.

- Both VDRs M4 and M1 bind to β-catenin more efficiently in the presence of 1,25D.
- In the presence or absence of 1,25D, VDR M4 binds β-catenin with higher affinity than VDR M1.

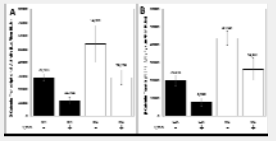


Figure 5. β-catenin transcriptional assay (TOP-FLASH) assessing the differences between VDRs M4, E2A, and M1 in their ability to inhibit β-catenin-mediated transcriptional activation in the absence or presence of 1,25D.

- VDR M4 and E2A are more effective than VDR M1 at attenuating β-catenin transcription activation with or without the presence of 1,25D.
- The addition of 1,25D further enhances this attenuation by all VDR proteins, with VDR M4 and E2A remaining more active than VDR M1.

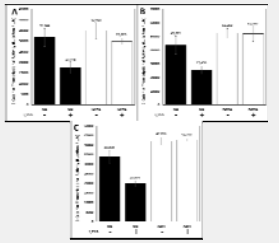


Figure 6. β-catenin transcriptional assay (TOP-FLASH) assessing the differences between VDRs M4, L417A, E420A, and Δ403 in their ability to inhibit β-catenin-mediated transcriptional activation in the absence or presence of 1,25D.

- In the absence of 1,25D, VDR L417A and E420A are not statistically significantly different from VDR M4 in their abilities to attenuate β-catenin transcription.
- With the addition of 1,25D, VDR M4 becomes more active than L417A, E420A, and Δ403 at reducing β-catenin transcriptional activity.

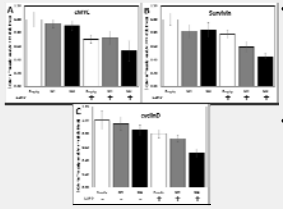


Figure 7. Analysis of VDR-mediated attenuation of β-catenin transcriptional activation using quantitative real-time polymerase chain reaction (qPCR), probing β-catenin target genes cMYC, survivin, and cyclinD1.

- VDR M4 attenuates transcriptional activation by β-catenin of its nuclear target genes in their natural chromatin context more effectively than VDR M1.
- 1,25D further enhances this inhibitory effect, with VDR M4 continuing to be more effective than VDR M1.

Conclusions

- Our data extend prior reports that VDR directly binds to β-catenin and both vitamin D and resveratrol appear to enhance this interaction.
- VDR inhibits β-catenin-mediated transcriptional activity and this inhibition is enhanced by the presence of 1,25D.
- VDR polymorphisms M1 and M4 are functionally variable, both in their ability to activate vitamin D-dependent genes and in their potential to reduce β-catenin-mediated transcription.
- VDR M4 remains physiologically more active than M1 likely due to the glutamic acid residue at position 2 of VDR M1 (see results with E2A).
- Both VDR M1 and M4 are responsive to 1,25D stimulation, and their differences appear to narrow in the presence of this hormone.
- VDR influences the Wnt/β-catenin pathway, which is important for CRC development, and supplementation with vitamin D and resveratrol may exert chemopreventative pressure, in conjunction with other lifestyle changes, to deter the development of CRC.

Future Directions

- An important next step is identifying how the VDR-β-catenin interaction affects CRC development, *in vivo*, and whether a meaningful difference between the two VDR polymorphisms exists in the context of CRC.
- Clinical trials will be necessary to evaluate if *in vivo* supplementation with vitamin D and resveratrol promote significant chemoprotection against CRC development.
- Future studies should explore the possible effects of resveratrol on β-catenin activity, as well as on transcriptional regulation of β-catenin target genes.
- Finally, it will be useful to define more completely the effects of 1,25D and resveratrol on the interaction between VDR and β-catenin, and to probe the potential involvement of additional modulators, such as SIRT1, in the VDR-β-catenin signaling axis.

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