The Vitamin D Receptor and RXR Heteropartner Cooperate via Synergistic Activation to Control Target Gene Expression

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Abstract

Vitamin D is a fat-soluble vitamin that is essential for maintaining cellular growth and development. The biologically active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25D), binds to the vitamin D receptor (VDR), a nuclear protein that mediates 1,25D actions. 1,25D binding to VDR triggers heterodimerization with the retinoic X receptor (RXR) and the VDR-RXR heterocomplex then binds to vitamin D response elements (VDREs) to modulate target gene expression. In the present study, we tested specific ligands (agonists) for their potential role in activating VDR-RXR by employing luciferase reporter plasmids that contain distinct VDREs in human embryonic kidney cells (HEK-293). When cells were transfected with 1,25D alone, or with either RXR-specific or VDR-specific ligands such as bexarotene (RXR) or novel VDR analogs alone, showed modest transactivation of the luciferase reporter gene. However, VDR-RXR-mediated activity was significantly increased in the presence of both 1,25D and RXR ligands. Similar results were obtained using an everted repeat VDRE (PER6). These results suggest that RXR alone either (1) binds and partially activates VDR or (2) that when RXR binds to ligand, the liganded Rex can partially activate VDR. Thus, when both ligands are present, the significantly elevated heterodimer activity implicates a synergistic effect via a dual ligand interaction process. VDR-RXR is thought to be a nonpermissive heterodimer, which can be activated only by the "primary" (VDR) ligand, but our novel results suggest that VDR-RXR can heterodimerize via RXR behavior and be activated by either RXR or VDR ligands. In summary, this study illuminates a coordinated interaction between VDR and RXR in transcriptional regulation of vitamin D-target genes.

Background

Vitamin D is a fat-soluble vitamin that is obtained through the skin when exposed to ultraviolet rays, diet, or supplements. Once it is recreated, vitamin D travels to the kidneys and is converted into the biologically active form, 1,25-dihydroxyvitamin D₃ (1,25D), which enters the bloodstream. Bexarotene (RXR) is a synthetic retinoid analog of the endogenous lipid 9-cis-retinoic acid (9-cis RA), which is a biologically active form of vitamin A. Bexarotene binds to the RXR receptor, a nuclear receptor that functions as a transcription factor for cell development. The active 1,25D ligand can bind to the vitamin D receptor (VDR), a nuclear receptor that mediates 1,25D actions, and heterodimerizes with RXR that is bound to its ligand. The ligand-heterodimer complex (VDR-RXR) then binds to vitamin D response elements (VDRE) to modulate target gene expression. Two types of heterodimers exist: A nonpermissive heterodimer is shown to be only activated by the primary ligand or synergistically activated by both ligands whereas a permissive heterodimer is capable of being activated in the presence of either ligands and also synergistically activated by both ligands. Currently it is not known if the VDR-RXR complex is influenced in its activity when both ligands are present, and that is the scope of the present study.

Methodology

The VDRE-based dual luciferase assay measures regulated gene expression at the transcriptional level via luciferase. Human embryonic kidney cells (HEK-293) are plated and transfected with plasmids that code for two commonly used reporter luciferase proteins, derived from firefly (Photinus pyralis) and sea pansy (Renilla reniformis). Transfection efficiency is measured via luciferase of the luciferase protein, which represents the level of VDR or RXR activity. Transfection efficiency is measured via luciferase of the luciferase protein, which functions as a normalizing control. A significant finding of this study is that the VDR-RXR heterodimer is more active compared to treating 1,25D and RXR alone, indicating a notable synergism.

These findings suggest:
- In the presence of both 1,25D and RXR ligands, the VDR-RXR heterodimer is more active compared to treating 1,25D and RXR alone, indicating a notable synergism.
- VDR-RXR may possess permissive heterodimer behavior, as Bex alone either binds and partially activates VDR or RXR, which then partially activates the unliganded VDR.
- When working with endogenous receptors, cells treated with 10^M Bex showed higher transactivation-mediated activity than Bex alone.
- VDR-RXR respond differently and PER6 may be stronger compared to XDR3 with respect to 1,25D and Bex.

Conclusion

We will continue to examine how 1,25D and Bexarotene interacts with the vitamin D pathway by:
- Continuing to test vitamin D impact using various VDREs (MOP2, ROC)
- Investigating how novel in vitro assays are measured in a functional assay between the VDR-RXR heterodimer.

Future Directions

Is VDR-RXR Cooperation Similar on a Different VDRE (PER6)?

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