

Vitamin D, Resveratrol, and Control of LCE3 Genes with Implications for Psoriasis

Shane F. Batie¹, Mark R. Haussler¹, Peter W. Jurutka^{1,2}, G. Kerr Whitfield¹

¹Department of Basic Medical Sciences, University of Arizona College of Medicine – Phoenix, AZ

²School of Mathematical and Natural Sciences, Arizona State University, Phoenix, AZ

Abstract

Psoriasis (Pxs) is a chronic inflammatory skin disease with abnormal keratinocyte proliferation and differentiation. One genetic risk factor for psoriasis (denoted PSORS4) is a deletion of LCE3B and LCE3C genes encoding structural proteins in terminally differentiated keratinocytes. Analogs of the hormonal form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25D) are routinely used to treat Pxs, a skin disease that affects over 7 million patients in the US. However, this therapy, even when combined with an anti-inflammatory (e.g., betamethasone), is ineffective in some patients, particularly those with a severe disease phenotype, underscoring the need for better agents. Further, the mechanism of action of vitamin D analogs is not understood, although their ability to reduce proliferation and promote differentiation of psoriatic keratinocytes is both valued in therapy and is complementary to anti-inflammatory agents. Given that 1,25D acts via the vitamin D receptor (VDR) to regulate gene expression, this project is focused on elucidating expression alterations in psoriasis-relevant genes mediated by the 1,25D-liganded VDR in human keratinocytes. Whereas VDR activity is increased when bound to 1,25D, less is known about the ability of other nutritionally-derived lipids to act on VDR. The current study is designed to: 1) evaluate resveratrol, an antioxidant found in the skin of red grapes, as an effector of VDR signaling and potent activator of LCE gene transcription in human keratinocytes, and 2) determine whether resveratrol acts synergistically with 1,25D to regulate the expression of LCE3 genes, with the potential to boost skin repair and ameliorate the symptoms of psoriasis.

Introduction

- Psoriasis has a large genetic component, with risk loci named PSORS1-12.
- One genetic risk factor, PSORS4, is the deletion of two late cornified envelope (LCE) genes, LCE3B and LCE3C (Figure 2), in a cluster of LCE3 genes with a role in skin repair (1).
- 1,25-dihydroxyvitamin D₃ (1,25D) is routinely used to treat mild/moderate psoriasis. Although the exact mechanism(s) by which this process occurs is unknown, our recent results suggest that upregulation of LCE3 genes could be part of this therapeutic action.

Methods

- Total RNA from culture was prepared employing primary human HEK neonatal keratinocytes using an Arium kit (BioRad) yielding a 50 µL volume containing 2-10 µg RNA from 10⁷ cells.
- 1st strand cDNA synthesis was performed using an iScript kit (BioRad) and 2 µg RNA in a 20 µL reaction.
- Real time PCR was performed in an ABI 7500 Fast machine using the Roche Fast Start Universal SYBR Green Master Mix, custom primers for each mRNA to be tested, and 2 µL of 1st strand cDNA in a 10 µL reaction.
- A VDRE sequence was identified ~29 kb upstream of the LCE3A gene. Two copies of this sequence with four bases on either side were synthesized with an additional four base overhang for cloning into the HindIII/BglII sites of the pLUC-MCS reporter plasmid to create pLUC-LCE3.
- HEK-293 cells were transfected in 24-well plates at 60,000 cells/well. Each well received 2.0 µL Express-In Reagent, 250 ng of either empty pLUC-MCS plasmid or pLUC-LCE3, 25 ng of pSG5-hVDR, 20 ng of pRL-null and 1 µL of 100X sodium pyruvate.
- After transfection, wells were treated with ligands or ethanol control for 20 hours. Whole cell lysates were harvested and analyzed for Firefly luciferase and Renilla luciferase activity using a Dual Luciferase assay kit and a Sirius Luminometer according to the manufacturers' protocols.

HYPOTHESIS: It has been shown that 1,25D (2) and alternative candidate VDR ligands/modulators (2,3) upregulate the other members of the LCE3 gene cluster to compensate for the loss of LCE3B and 3C in susceptible individuals. We hypothesize that resveratrol synergizes with 1,25D to activate the LCE3 gene cluster beyond the traditional 1,25D therapy, as it does in other biosystems.

Background

MODEL OF VITAMIN D ACTION

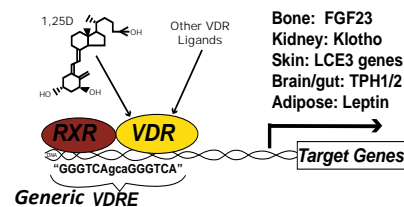


Figure 1: Model of 1,25D/vitamin D receptor (VDR) action. Upon binding the 1,25D or other ligand, VDR forms a heterodimer with the retinoid X receptor (RXR) and binds to a vitamin D responsive element (VDRE), whereupon it attracts coactivators to stimulate transcription of target genes in various tissues.

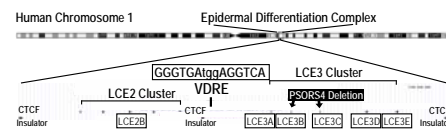


Figure 2: The PSORS4 deletion removes the LCE3B and LCE3C genes, leaving the LCE3A, 3D and 3E genes intact. Both the LCE3 and the adjacent LCE2 gene clusters are bracketed by binding sites for the CTGF factor, which may serve as regulatory insulators to allow for separate transcriptional control of each cluster. A candidate VDRE for control of the LCE3 gene cluster is shown; this VDRE is transcriptionally active in a heterologous reporter construct (next figure), suggesting it could exert transcriptional regulation of the LCE3 cluster.

Results: Experiment 1

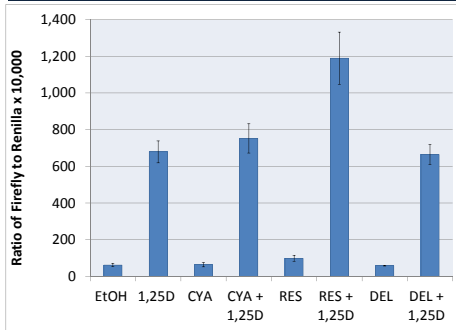


Figure 3: Luciferase assay measuring transcriptional activation from the LCE3 VDRE in transfected HEK293 cells. Cyanidin (CYA), resveratrol (RES), and delphinidin (DEL) showed modest, if any activation over the ethanol control vehicle. When 1,25D was combined with CYA or DEL, there was no apparent increase in activation over 1,25D alone. However, when cells were treated with RES and 1,25D in combination, an apparent synergistic relationship was observed in transcriptional activation of the artificial LCE3 VDRE luciferase gene construct.

Results: Experiment 2

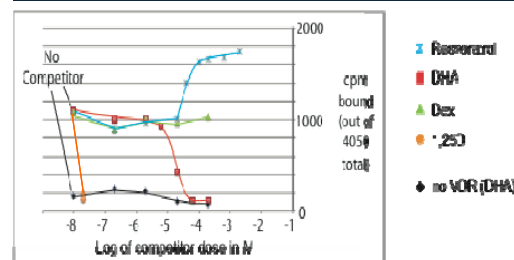


Figure 4: Ability of candidate ligands to compete for binding to VDR with approximately 0.4 nM [³H]-1,25D in VDR-containing cell lysates. Dexamethasone (Dex, green) is a negative control with no appreciable binding to VDR. Radioinert 1,25D (orange circles) is the high affinity VDR ligand (nanomolar range) that shows the full range of competition. Docosahexaenoic acid (DHA, red squares) is a known low affinity ligand (micromolar range). Resveratrol (blue) does not compete with vitamin D, but rather shows an increase in binding affinity of radioactive 1,25D. The black line with diamonds shows a DHA competition curve using lysates from COS-7 cells that were not transfected with the VDR expression plasmid. These results are a compilation of two similar assays performed independently.

Results: Experiment 3

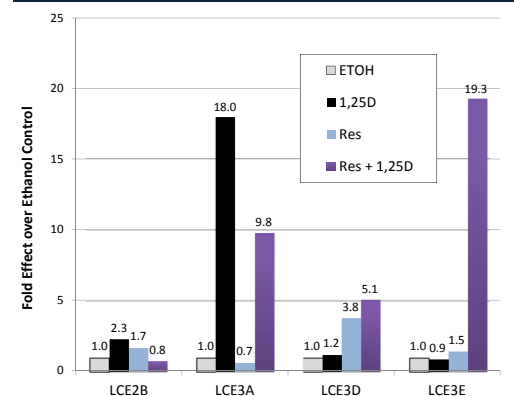


Figure 5: Real time PCR of total RNA from cells treated with ethanol, 1,25D, resveratrol, or a combination of resveratrol and 1,25D. As anticipated, the positive control gene CYP24A1 demonstrated the highest fold effect of 1,25D over the ethanol control (1154-fold, not shown), in keeping with the exquisite sensitivity of this gene to regulation by 1,25D. Omitting the CYP24A1 results in this plot allows us to examine the genes of interest on a scale which allows for comparison of their regulation by the tested ligands. Resveratrol alone showed a modest activation of LCE2B, LCE3D, and LCE3E genes. When cells were treated with the combination of resveratrol and 1,25D, however, a synergistic fold effect over the ethanol and 1,25D controls was observed with the LCE3B and LCE3E genes. The LCE3A gene was regulated in a different pattern, although inconsistencies in the results with the 1,25D (alone) treatment leave open the possibility that the pattern of regulation of LCE3A may in fact be similar to that of LCE3D and LCE3E. Consistent with this conclusion is the fold-induction of LCE3A (9.8-fold) by the combination of resveratrol and 1,25D, which falls between the 5.1- and 19.3-fold induction of LCE3D and LCE3E, respectively, with the dual treatment. In contrast, the LCE2B gene behaved in a very different manner, with no activation by the combination of resveratrol and 1,25D, but modest induction by either resveratrol or 1,25D alone, suggesting that the LCE2 gene cluster (separated from the LCE3 cluster by insulator elements) may be regulated quite differently by these two compounds.

Discussion and Conclusions

The data collected in this study confirm and extend the original hypothesis that resveratrol can synergize with 1,25D to activate the LCE3 gene cluster beyond the traditional 1,25D therapy. Although other compounds tested in our laboratory, such as cyanidin, delphinidin, and curcumin, seem to act by binding in the VDR ligand binding pocket (in competition with 1,25D), there is recent evidence from the laboratory of Dr. Peter Jurutka (4) that resveratrol acts via completely different mechanisms. Resveratrol is now thought to function by activating SIRT1 which then may, in turn, potentiate VDR action on transcription by a mechanism such as deacetylation. Indeed, resveratrol enhances rather than competes for 1,25D binding to VDR (Fig. 4). This scenario would explain why 1,25D and resveratrol can synergize in the activation of VDR since they do so by separate and independent means.

Although overall positive, our study does have limitations. The keratinocytes tested in our system were shown to be heterozygous for the PSORS4 deletion. Ideally, these experiments would be performed in a homozygous deletion lineage. This strategy would yield a clearer picture of the potential for resveratrol and 1,25D to upregulate the surrounding LCE3 genes. Obtaining a homozygous deletion lineage has proven difficult, as the company we purchase the cells from does not test for the genotype, but future work could address this limitation.

Vitamin D has long been used as a treatment for psoriasis, but the molecular mechanisms by which this treatment is successful have been in question. In a specific subset of patients with psoriasis, namely those with the PSORS4 deletion, vitamin D therapy may potentially be augmented with compounds shown to improve 1,25D-induced gene expression, such as the polyphenol red wine constituent, resveratrol.

Key References

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