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RESEARCH ARTICLE

FUNCTIONAL TESTING OF VITAMIN D RESPONSE ELEMENTS IN THE LCE2 GENE CLUSTER WITH RELEVANCE TO PSORIASIS.

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Abstract

Psoriasis is a chronic inflammatory skin disease characterized by abnormal keratinocyte proliferation and differentiation. A genetic risk factor for psoriasis is a deletion of LCE3B and LCE3C genes encoding structural proteins in terminally differentiated keratinocytes, suggesting that compromised skin barrier function has a role in psoriasis susceptibility. Because analogs of 1,25-dihydroxyvitamin D₃ (1,25D) are used in psoriasis treatment, we hypothesized that 1,25D acts via the vitamin D receptor (VDR) to upregulate expression of LCE2B genes, potentially mitigating the absence of LCE3B/LCE3C gene products.

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Introduction:-

Psoriasis is a chronic inflammatory skin disease characterized by abnormal keratinocyte proliferation and differentiation [1]. Psoriasis affects 1.5–2% of the population in western countries [2]. In 1985 a case report was published by (Morimoto S, Kumahara Y) that led us to the conclusion that raising blood 1,25-dihydroxyvitamin D₃ (1,25D) can dramatically improve psoriasis symptoms in some patients [3]. And from that point 1,25D analogs were developed, including calcipotriene [4] treatment, which are now routinely used as topical agents in psoriasis.

In that point we know that the bioactions of 1,25D are mediated by vitamin D receptor (VDR) expressed in many tissues, including skin [5]. However, the specific genes that are regulated by 1,25D to improve psoriasis symptoms have yet to be defined. Genome wide searches for psoriasis susceptibility loci have implicated as many as 36 chromosomal regions [6], including 12 named linkages (PSORS1-12). One of these, the PSORS4 locus, is associated with an assembly of skin differentiation genes on human chromosome 1 [7]. A reported PSORS4 risk allele consists of a deletion encompassing two genes (Fig. 1) encoding late cornified envelope 3B and 3C (LCE3B and LCE3C) proteins [8].

This deletion is found in 63–72 % of patients with psoriasis according to ethnicity [9]. LCE3B and LCE3C belong to a cluster of five LCE3 genes that are expressed late in keratinocyte differentiation, and the protein products of these genes are crosslinked to facilitate formation of the cornified envelope [10]. Transcripts of LCE3A, -3C, -3D and -3E are upregulated in psoriasis [11]. Tape stripping to induce superficial skin injury also upregulates LCE3A/3C/3D/3E expression [12]. In contrast, other LCE genes, LCE2B (approximately 71 kb from the PSORS4 deletion), are expressed at moderate levels in normal skin, but are downregulated in psoriasis or superficial injury [12]. Previous work in our lab showed that the late cornified envelope-2B (LCE2B) gene is upregulated by 1,25D. this may mitigate the absence of LCE3B/LCE3C gene products.

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We hypothesis At least one VDRE in the LCE2 gene cluster binds VDR/RXR and stimulates transcription of the nearby LCE2B gene

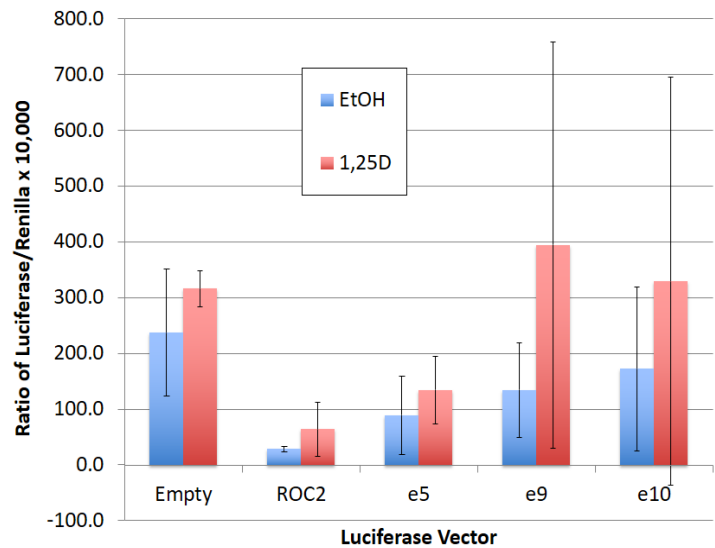
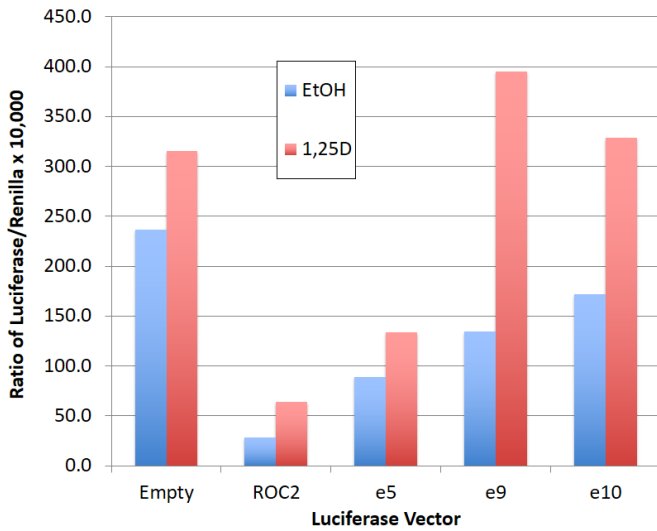
In this study we will examine three candidate of vitamin D response element (VDRE) sequences, LCE2.e5 and LCE2.e9 and LCE2.e10, located in the LCE2 gene cluster.

Materials & Methods:-

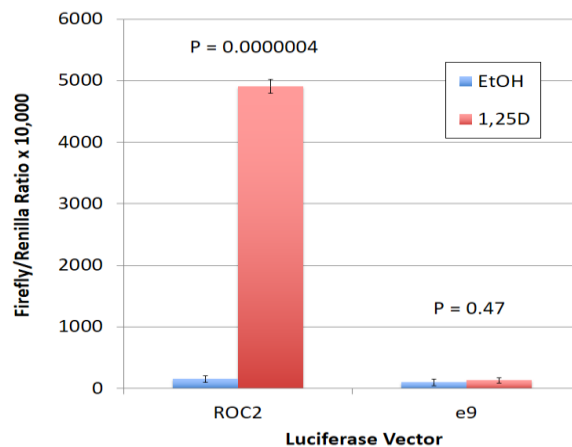
To test this, we used a luciferase plasmid to see if any of these VDREs can regulate luciferase in a 1,25D-dependent manner. And here the steps:

1. plasmid preparation (pSG5-hVDR): E-coli containing the desired plasmids were streaked onto LB-agar plates containing ampicillin and tetracycline for isolation of single colony.
2. Then Single colony were inoculated into TB broth containing ampicillin / tetracycline for overnight growth. After that, Bacteria were collected by centrifugation, and lysed. Plasmid DNA was purified using a midiprep kit (Invitrogen).
3. transfection into HEK293 with Renilla plasmid (this plasmid tells us if the transfection worked): HEK-293 cells were transfected with five LUC vectors: empty Luc, ROC2-Luc, LCE2.e5-Luc, LCE2.e9-Luc and LCE2.e10-Luc.
4. treatment with ethanol or 1,25D: Transfected cells were treated for 24 hours with 10^{-8} M 1,25D.
5. Cell lysis and dual luciferase assay: Each well was lysed and assayed for both firefly and RENILLA luciferase (Dual Luciferase Assay Kit, Promega). LARII solution contains substrate for firefly luciferase, Stop &GLO contains quencher for firefly luciferase and substrate for Renilla luciferase.

Results:-



Repeat with ROC2 and e9



Preliminary data in HEK293 cells (Exp I, Exp II) suggested that all three VDREs appear might be responding to 1,25D but these results were not statistically significant due to high variability. The LCE2.e9 element was chosen for a second experiment (Exp III) together with the ROC2 control. The second experiment showed high consistency but unfortunately the e9 element, in contrast to the ROC2 element, showed very low activity.

Conclusion:-

LCE2B (e5, e9, e10) is not significant due to high variability in our assay in our cell lines and using VDR ligands. We recommend Future Experiments to Test other LCE2 genes (LCE2A, 2C and 2D) to see if they are also upregulated by 1,25D. and to Repeat our results in HEK293 and other cell lines including keratinocytes.

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