IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 2 Ver. V (Feb. 2016), PP 48-53
www.iosrjournals.org

Functional testing of a Vitamin D Response Element near the Human LCE2B Gene

Rabea H Habib[1], Jui-Cheng Hsieh[2], G. Kerr Whitfield[2]

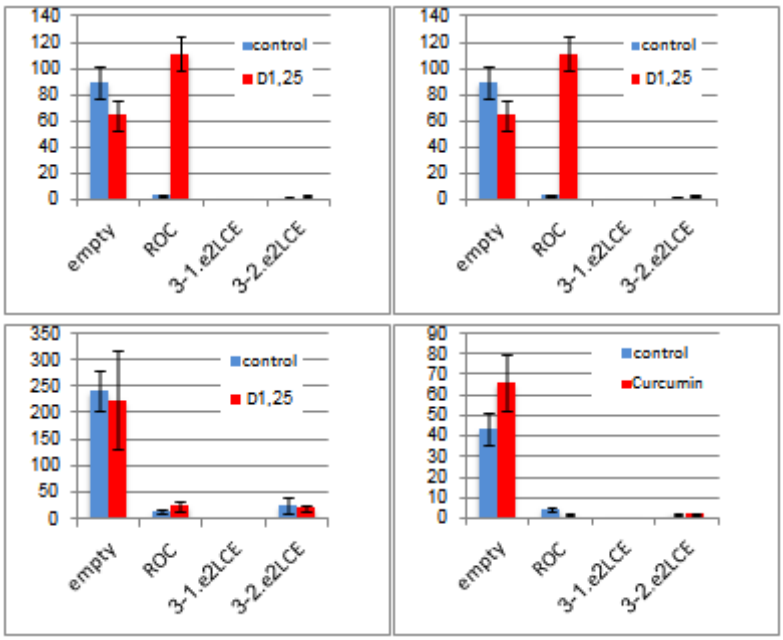
Abstract:

Introduction

- The hormonally active form of vitamin D (1,25D) can improve symptoms of psoriasis.
- Previous work in our lab showed that the LCE2B gene (one of 18 similar LCE genes) is upregulated by 1,25D. This may help repair skin after psoriasis injury.
- Two candidate vitamin D response element (VDRE) sequences, designated LCE2.e1 and LCE2.e3, located near the LCE2B gene were previously shown to bind VDR and RXR.

Methods

1. plasmid preparation.
2. Transfection into HEK293 and COS 7 along with Renilla plasmid (this plasmid tells us if the transfection worked).
3. Treatment with ethanol, 1,25D, or Curcumin.
4. Cell lysis and luciferase assays



Discussion

- In Exp I, we found that LCE2e.3-1 and LCE2e.3-2 both had no significant increase when 1,25D is added to the HEK293 cell line.
- In Exp II, we repeated the first experiment and had the same results.
- In Exp III, we tried different cell line, COS-7, to measure the activity of LCE2e.3-1 and LCE2e.3-2 when 1,25D is added, and we had the same results.
- In Exp IV, we chose different ligand, Curcumin, and had the same result.
- From the previous work, we can hypothesize that the LCE2e.3-1 and LCE2e.3-2 are 1,25D independent (This is yet to be proved by further experiments).

Conclusion

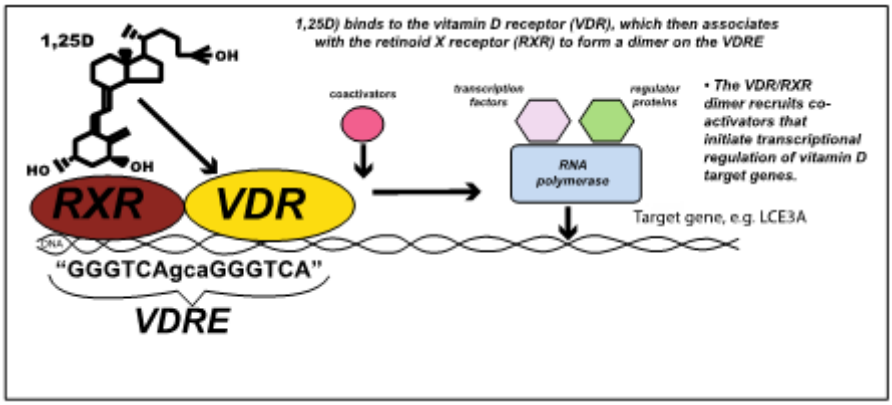
- LCE2B.e3 is not active in our assay in two different cell lines and using two different VDR ligands (1,25D and curcumin)

- unliganded VDR/RXR might be repressive
- the effect of 1,25D may differ depending on the cell line

I. Introduction

- The hormonally active form of vitamin D (1,25dihydroxyvitamin D or 1,25D) can improve symptoms of psoriasis.
- Previous work in our lab showed that the LCE2B gene (one of 18 similar LCE genes) is upregulated by 1,25D. This may help repair skin after psoriasis injury.
- Two candidate vitamin D response element (VDRE) sequences, designated LCE2.e1 and LCE2.e3, located near the LCE2B gene were previously shown to bind VDR and RXR.

Background



This element is found in two locations near the LCE2B gene.

Hypothesis

The VDRE near the LCE2B gene binds VDR/RXR and stimulates transcription of the nearby LCE2B gene.

To test this, we will use a luciferase plasmid to see if this VDRE can regulate luciferase in a 1,25D-dependent manner

Previous work

VDREs cloned into Firefly Luciferase reporter vector

II. Methods

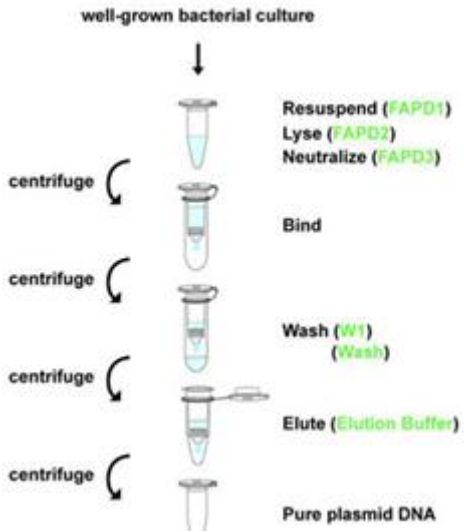
1. Plasmid preparation
2. Transfection into HEK293 and COS 7 along with Renilla plasmid (this plasmid tells us if the transfection worked)
3. Treatment with ethanol, 1,25D, or Curcumin.
4. Cell lysis and luciferase assay

1. Plasmid preparation

E-coli containing the desired plasmids were streaked onto LB-agar plates containing ampicillin and tetracycline for isolation of single colonies.

- Single colonies were inoculated into TB broth containing ampicillin / tetracycline for overnight growth.

- Bacteria were collected by centrifugation, and lysed. Plasmid DNA was purified using a miniprep kit (Fermentas, Gene-Jet).



2. Transfection

- HEK-293 or COS 7 cells were transfected with LCE2.e3-1 and LCE2.e3-2 reporter plasmids using ExpressInlipofection reagent.

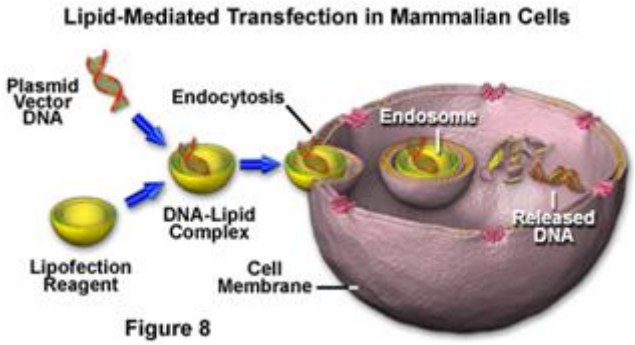
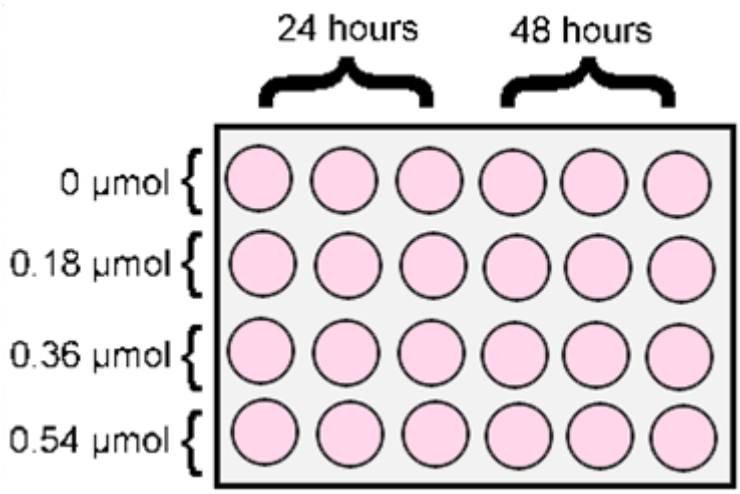


Figure 8

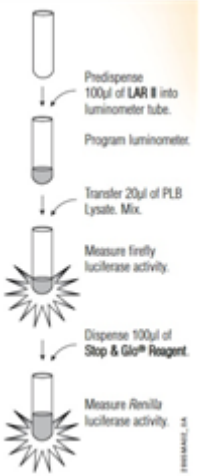
3. Treatment

Transfected cells were treated for 24 hours with 10 nanomolar 1,25D or 10 micromolar Curcumin.



4. Cell lysis and luciferase assay

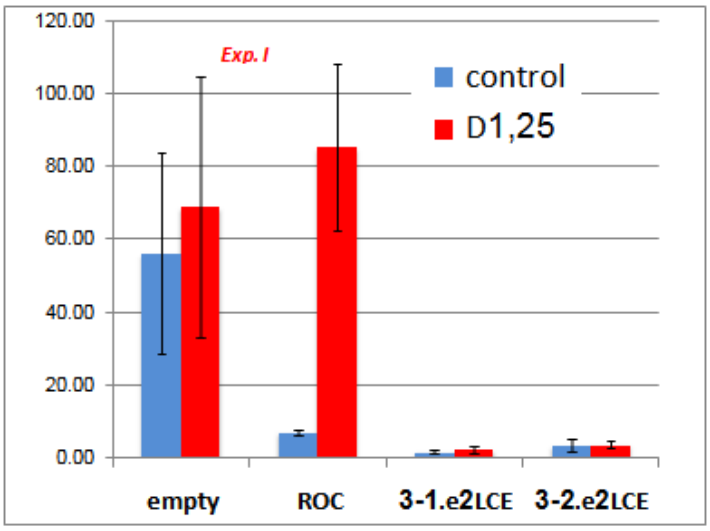
Each well was lysed and assayed for both firefly and
RENILLA luciferase (Dual Luciferase Assay Kit, Promega).

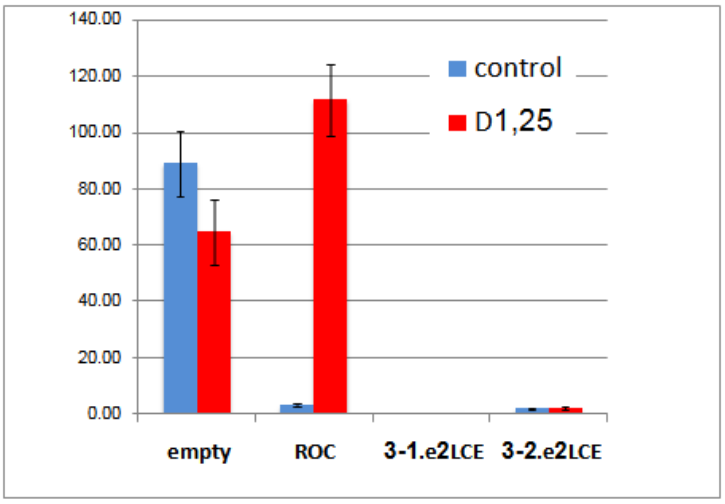


We used ¼ of the standard amounts in our assay.

III. Results

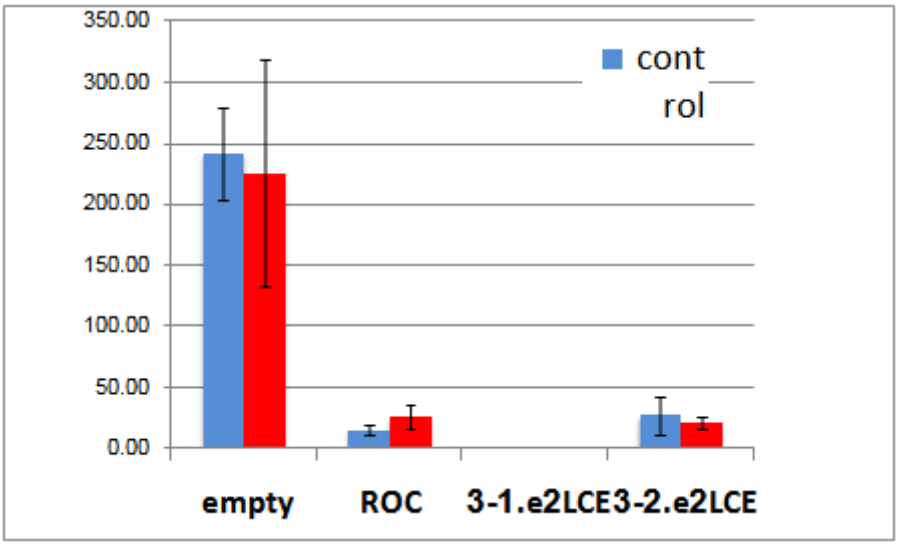
Results HEK293 with 1.25D





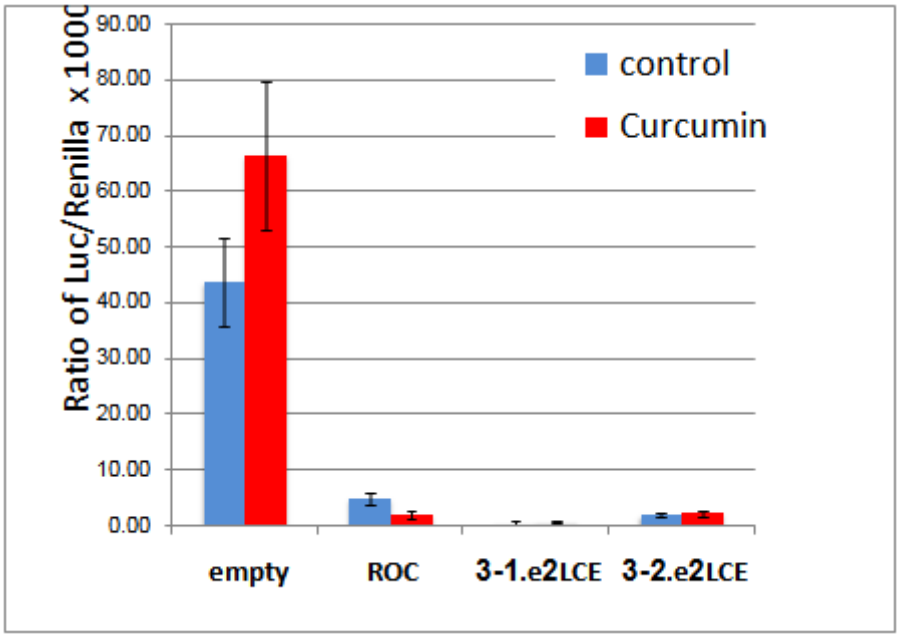
Two independent Experiments, each in triplicate.

Results COS 7 with 1,25D



One Experiment in triplicate.

Results HEK 293 with Curcumin



Unliganded VDR/RXR

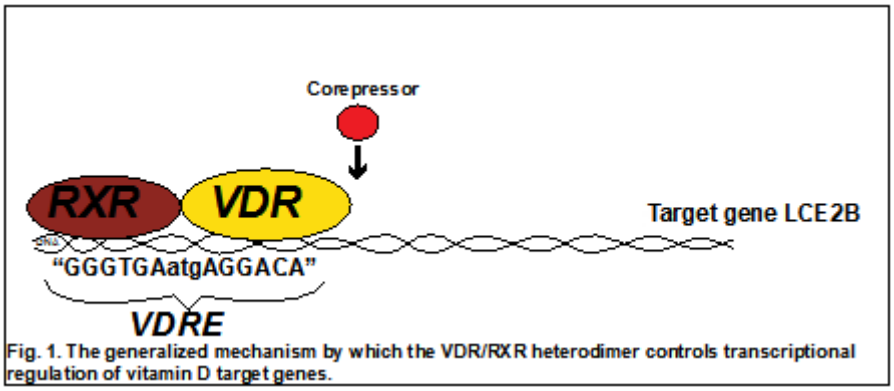


Fig. 1. The generalized mechanism by which the VDR/RXR heterodimer controls transcriptional regulation of vitamin D target genes.

IV. Discussion

- In Exp I, we found that LCE2e.3-1 and LCE2e.3-2 both had no significant activity when Vit-D is added to HEK293.
- In Exp II, we repeated the first experiment and had the same results.
- In Exp III, we tried different cell line, the COS-7, to measure the activity of LCE2e.31 and LCE2e.3-2 when Vit-D is added, and we had the same results.
- In Exp IV, we chose different ligand, Curcumin, that will bind to the same VDRE and had the same result.
- From the previous work, we can hypothesis that the LCE2e.3-1 and LCE2e.3-2 is Vit-D Independent (This is yet to be proved by further experiments).

V. Conclusions

- LCE2B.e3 is not active in our assay in two different cell lines and using two different VDR ligands (1,25D and curcumin)
- Unliganded VDR/RXR might be repressive
- The effect of 1,25D may differ depending on the cell line