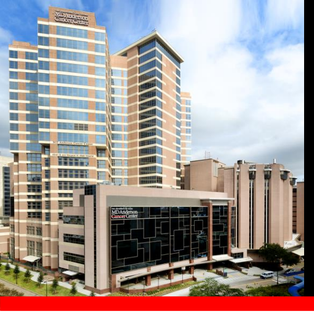

Ultraconserved Enhancers Roles in Cancer

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Background

Ultraconserved elements (UCEs) are DNA segments with 100% conservation between the orthologous genomes of human, mouse and rat, and high conservation in other species.
UCEs are involved in cancer studies in multiple ways:

- ❑ **UCEs as enhancers:** mutated UCEs can alter expression of neighboring gene(s).
- ❑ **UCEs as prognostic factors:** mutations in UCEs can affect response to treatment and overall survival.

Through mutational screen and CRISP-Apf screen of cell proliferation in a previous study from Calin's group[4], **UCE_2272, UCE_1943** and **UCE_11409** were found important for human cancers.

- ❖ This study looks into the enhancing ability of these three UCE regions in 293T/17 cells and the subsequent effect of the identified enhancer on its neighboring genes.

Hypothesis

Because UCE_2272, UCE_1943, and UCE_11409 were selected as enhancers in mouse tissue according to Vista Enhancer Browser (https://enhancer.lbl.gov/), and UCE_11409 was identified as enhancer in 293T/17 cell line in previous study[4],

- ➢ We hypothesize that the three UCEs are expected to show enhancer activity in 293T/17 cells by Luciferase reporter assay.

Methods

A. Luciferase reporter assay was performed to determine potential enhancer activity in 293T/17 cells.

i. **Gene amplification and cloning:** UCE_2272, UCE_11409 and UCE_1943 genomic regions were amplified from normal human male DNA and both sense and antisense direction constructs were cloned in pGL3-control-TK plasmid (Promega) downstream luciferase sequence.

Methods (continued)

II. **DNA extraction:** Plasmids were extracted using HiSpeed Plasmid Purification (QIAGEN)

III. **Cell transfection:** Plasmids were transfected in 293T/17 cell line using lipofectamine.

IV. **Luciferase Assay:** Dual Luciferase Reporter Assay System was used 48 hours after transfection and luciferase data were normalized using Renilla luciferase activity.

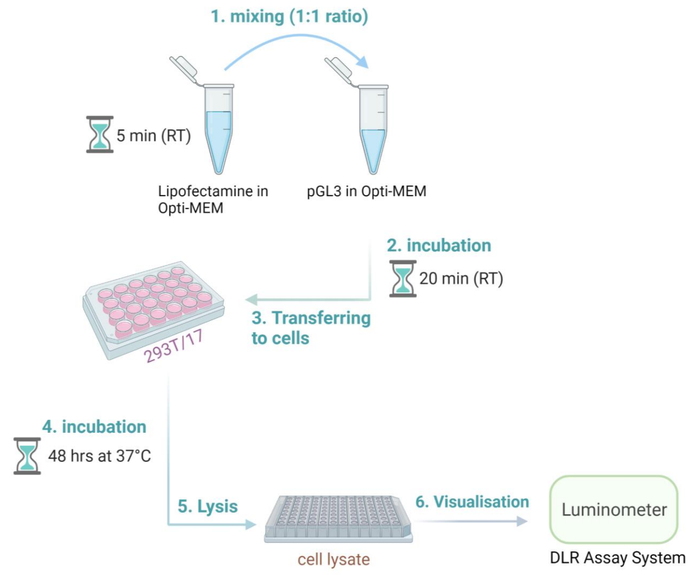


Figure 1. Luciferase assay steps

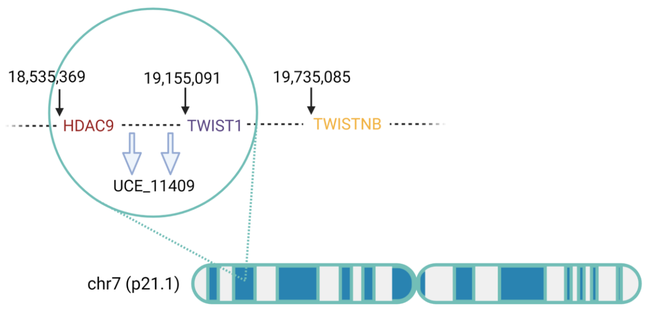


Figure 2. Relative location of UCE_11409 and neighboring genes – hg19 Assembly

Methods (to be performed)

B. After determining the enhancer region in vitro, the expression of the neighboring genes will be assessed:

I. UCE_11409^{KO} clones were generated in DLD-1 cells using CRSIPR AsCpf1.

II. UCE_11409 neighboring genes were determined using Vista enhancer browser (https://enhancer.lbl.gov/).

III. RT-qPCR will be performed on mutated UCE_11409 DLD-1 cells as well as DLD-1 wild type cells.

IV. TaqMan probes (ThermoFisher Scientific) will be used to analyze mRNA expression of HDAC9, TWIST1 and TWISTNB (UCE_11409 neighboring regions).

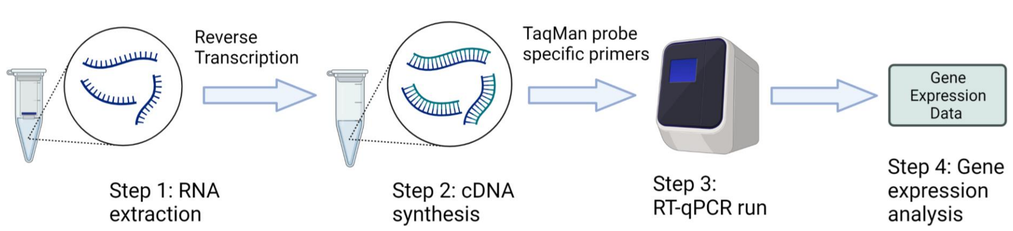
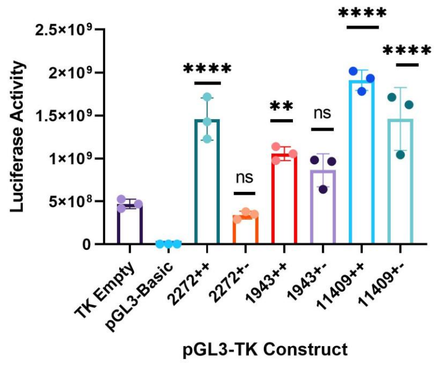


Figure 3. RT-qPCR steps for gene expression analysis

Results

1. Luciferase Assay



Luciferase activity data obtained were normalized using Renilla activity and compared to the respective empty vector (pGL3-control-TK). UCE_2272 and UCE_1943 showed only a partial increase in Luciferase activity, which is not sufficient at this time to prove that these genomic regions are enhancers. UCE_11409 however, showed increased luciferase activity in both sense and antisense directions, suggesting its enhancer role in 293T/17 cells.

2. Gene Expression

HDAC9, TWIST1, and TWISTNB were found to be neighboring genes of UCE_11409 using vista browser (https://enhancer.lbl.gov/).
Results of the RT-qPCR for determining the effect of this UCE region on the m-RNA of its target genes are pending at this time.

Conclusions

UCEs can function as enhancer to regulate gene expression in cancer. Based on data collected so far, we did not identify UCE_2272 and UCE_1943 as enhancers at this time. UCE_11409 was identified as enhancer in 293T/17 cells and its effect on its neighboring genes is currently being studied.

References

1) (Figures) Created with BioRender.com
2) enhancer.lbl.gov. https://enhancer.lbl.gov/. Accessed July 23, 2021.
3) Fabris L, Knutsen E, Nguyen TH, et al. Ultraconserved duet: DNA and RNA co-regulation of p16-E2F1 signaling. In submission. 2021.
4) Ivan C, Dragomir MP, Peng X, et al. The Mutational Landscape of Ultraconserved Elements in Human Cancers. In submission 2021.