Computational Methods for Exploring NELFE's Role in HCC

[1] Gwyneth Deng, [2,3] Kasonde Chewe, [2] Hien Dang – Hutchins Science Jefferson Program
[1] The Lawrenceville School, [2] Thomas Jefferson University, [3] Drexel University

Introduction

Hepatocellular Carcinoma (HCC) occurs when malignant tumors grow on the liver and has many risk factors including HBV infection and old age. It is the most common type of primary liver cancer and is often seen in Western countries, East Asia, and Northwest Africa regions.

One gene associated with the development of HCC is the Negative Elongation Factor E (NELFE), a proto-oncogene encoding important RNA-binding proteins in the multi-subunit NELF complex responsible for RNA polymerase II pausing during DNA transcription. NELFE is also heavily involved in the regulation and enhancement of MYC signaling, another proto-oncogene coding for transcription factors.

The dysregulation of both NELFE and MYC genes may lead to the progression of tumors. However, as MYC controls many essential cellular processes and cannot be targeted, NELFE appears to be a promising target for advancing HCC treatments.

Research Aims

What is the effect of knocking out NELFE in HCC cell lines?

What is the effect of knocking down NELFE on RNA expression?

Hypothesis

NELFE plays a crucial role in chromatin modulation, and the loss of NELFE negatively affects chromatin accessibility in HCC cell lines, which leads to reduced gene expression, RNA expression, and tumor progression.

Methods

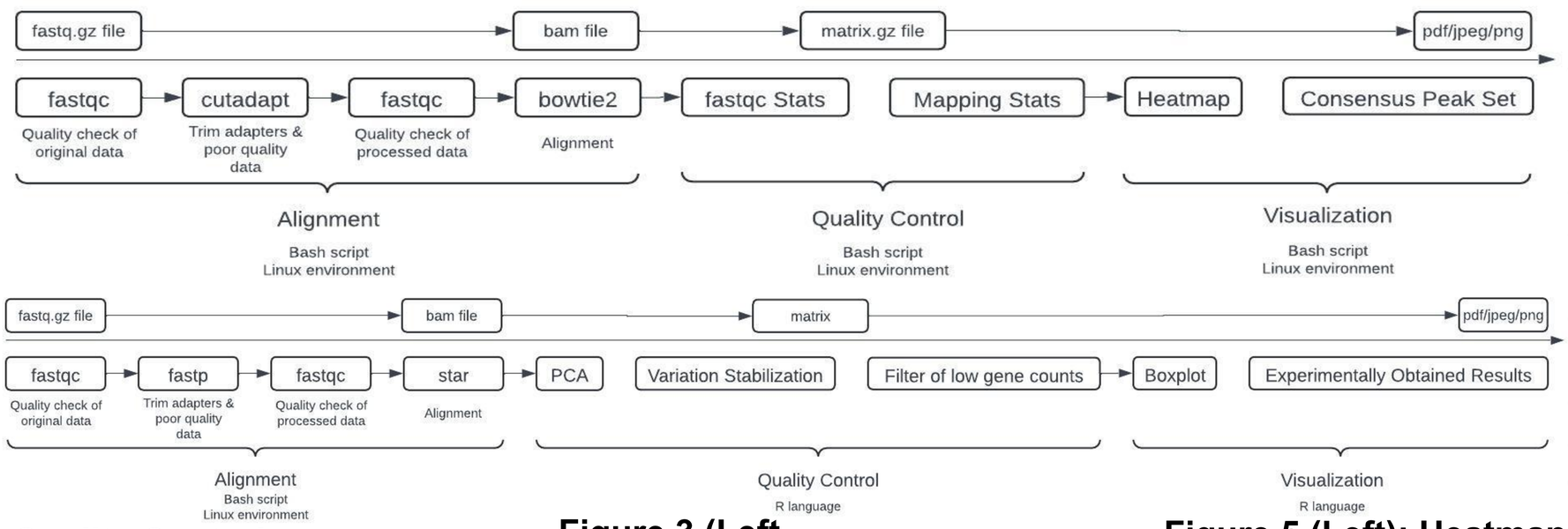

Figure 1 (Left Up): ATAC-Seq Pipeline
Figure 2 (Left Down): RNA-Seq Pipeline

ATAC-Seq is conducted using Tn5 transposase tagmentation and pair-ended sequencing to determine chromatin accessibility; in RNA-Seq, the RNA reads are converted to a library of cDNA pieces, ligated with adapters, and sequenced according to a reference transcriptome profile to assess mRNA expression. Data analysis is then performed in RStudio to align sequencing reads, check the quality of experiment data, and visualize results.

Quality Control

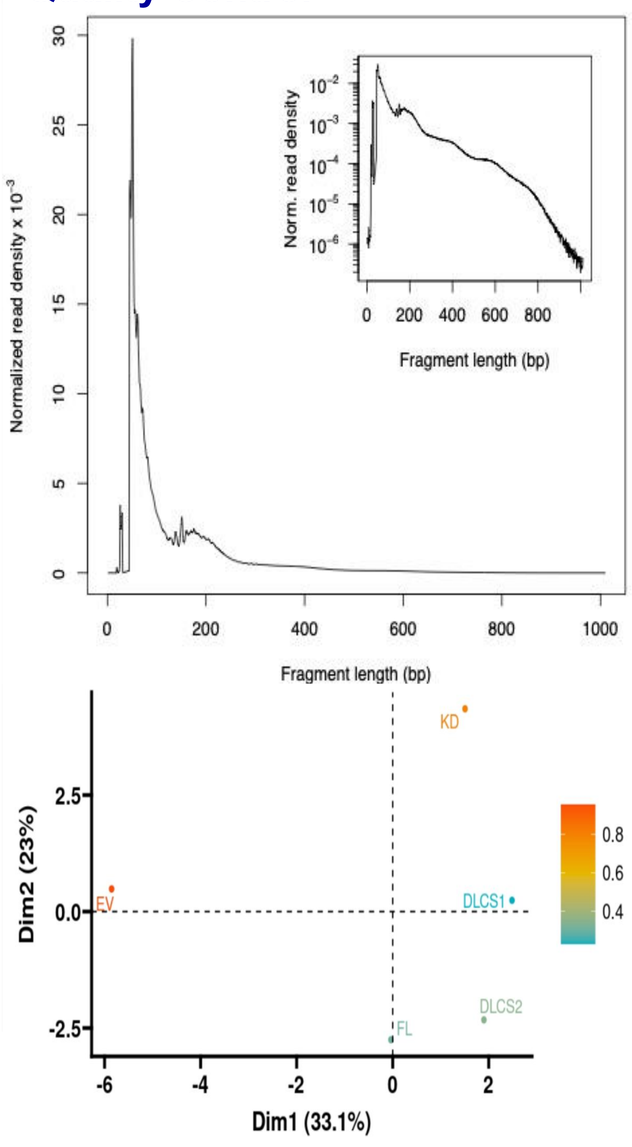

Figure 3 (Left Up): ATAC-Seq Fragment Size Distribution Plot

The majority of the fragments are less than 147 bp (size of a nucleosome), which indicates that the transpose indeed cut the reads into smaller parts of acceptable quality.

Figure 4 (Left Down): RNA-Seq Principal Component Analysis (PCA)

The biological replicates of each experiment condition demonstrate high similarity as they cluster together.

Results

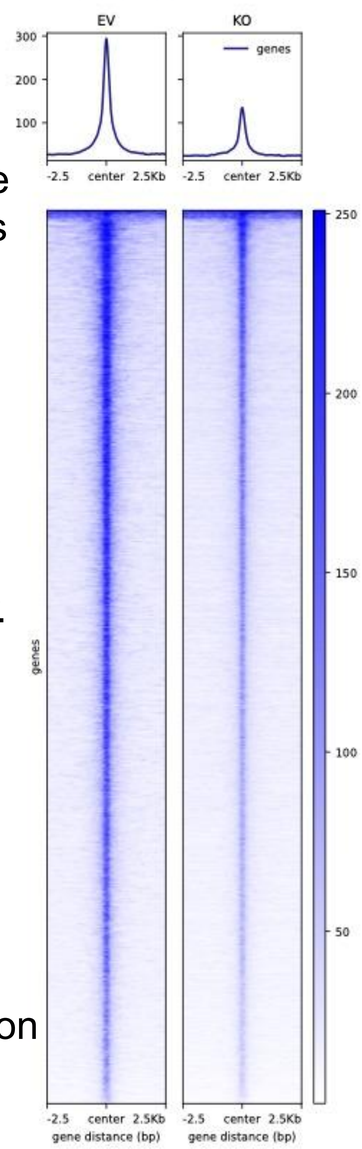

Figure 5 (Left): Heatmap Produced from ATAC-Seq

EV (endogenous vector with NELFE present) generates a stronger signal than KO (NELFE knocked out by CRISPR) as the absence of NELFE causes chromatin to close and inhibits sequence replication, gene expression, and signal generation.

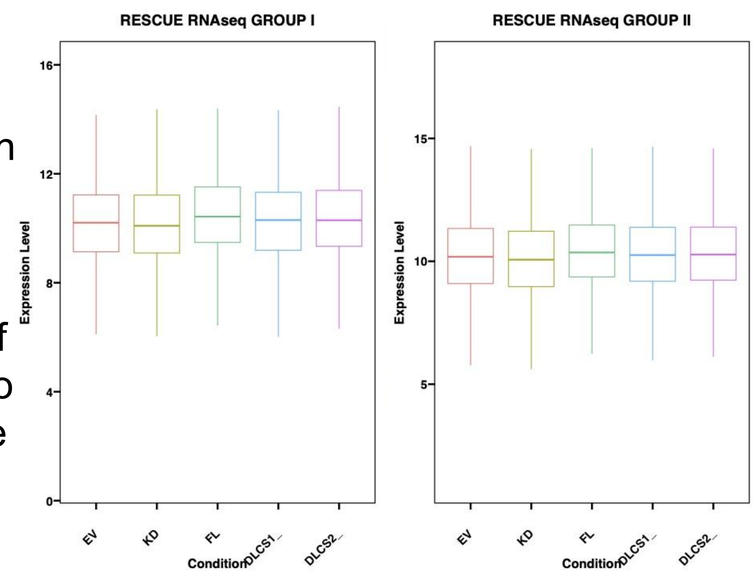

Figure 6 (Right): Boxplots Produced from RNA-Seq in Reference to Tumor Sizes in Mice

KD (NELFE knocked down by the mAID system) shows a lower expression level than EV while the overexpression models of NELFE (FL, DLCS1, and DLCS2) have higher expression levels, suggesting that NELFE is positively correlated with gene expression level, RNA expression level, and tumor sizes in mice with HCC.

Conclusions

Knocking out NELFE leads to more compact chromatin in HCC cell lines, which is correlated to a broader downregulation of genes possibly related to liver cancer.

As NELFE plays an important role in chromatin accessibility modulation, knocking down NELFE reduces gene expression, RNA expression, and hence tumorigenesis.

References

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., Szcześniak, M. W., Gaffney, D. J., Elo, L. L., Zhang, X., & Mortazavi, A. (2016). A survey of best practices for rna-seq data analysis. *Genome Biology, 17*(1). https://doi.org/10.1186/s13059-016-0881-8

Dang, H., Pomyen, Y., Martin, S.P. *et al.* NELFE-Dependent MYC Signature Identifies a Unique Cancer Subtype in Hepatocellular Carcinoma. *Sci Rep* 9, 3369 (2019). https://doi.org/10.1038/s41598-019-39727-9

Yan, F., Powell, D. R., Curtis, D. J., & Wong, N. C. (2020). From reads to insight: A hitchhiker's guide to atac-seq data analysis. *Genome Biology*, *21*(1). https://doi.org/10.1186/s13059-020-1929-3