

# Characterization Of Chromatin Accessibility In Hepatocellular Carcinoma Cells Using Single Cell ATAC Sequencing

## Background

- Hepatocellular carcinoma (HCC) is a heterogeneous malignancy that is highly resistant to therapy.
- Cancer stem cells (CSCs) are known to play an important role in driving tumor evolution and therapeutic resistance.
- Open DNA regions are more transcriptionally active and predict the location of enhancers and promoters.

Hypothesis: Chromatin accessibility patterns can identify unique regulatory element dependencies in both clonal and sub-clonal populations of HCC cell lines.

# Methods

- Single-cell RNA sequencing (scRNA-seq) and scATAC-seq were done using the 10X Chromium platform.
- Peak calling from scATAC-seq data was done with MACS2 in concordance with the published Satpathy et al.<sup>1</sup> pipeline.
- Graph-based clustering, UMAP reduction and marker selection for both scRNA-seq and scATAC-seq were done using Seurat v3. • Transcription factor motif enrichment was called using ChromVAR.
- Gene activity scores were calculated using Cicero.

Figure 1: Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) is an innovative technology that can assess genome-wide chromatin (Q) 🔊 accessibility<sup>2</sup>.



# Results I

### scATAC Sequencing Statistics of 4 HCC Cell Lines

Cell Line	Number of Cells	Median Unique Fragments per Cell	Median Number of Peaks per Cell	Fraction of Reads in Peaks
MHCC97H	4,052	16,692	10,168	0.53
HUH1	4,866	16,162	8,720	0.48
HEP3B	3,344	15,858	8,086	0.44
HUH7	4,051	16,423	9,081	0.48



Promoter (<=1kb) (20.34%)</p> Promoter (1–2kb) (6.62%) Promoter (2–3kb) (4.75%) **5**' UTR (0.23%) **3**' UTR (2.21%) 1st Exon (1.28%) Other Exon (2.93%) 1st Intron (12.59%) Other Intron (23.09%) Downstream (<=300) (0.98%)</p> Distal Intergenic (24.99%)

Figure 2: Peak Annotation Pie Chart. Each color represents a different annotated region of the genome for which peaks were found to overlap.

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Results II

# scATAC-seq Can Distinguish Cell Line of Origin and Identify Differences in Stemlike Characteristics of Individual Cells



**Distinct Transcription Factors are Active in Each Cell Line That Regulate Proliferation and Differentiation** 



**Figure 4**: **A.** Heatmap of scATAC-seq ChromVAR bias-corrected deviations (activity score) of cell line specific transcription factors (N=86). B. Transcription factor binding motifs of top marker transcription factors for each cell line. C. Violin plot of FOS::JUN ChromVAR activity score. **D**. Violin plot of FOS gene expression calculated from scRNA-seq profiles of 10,566 HCC cells.

Figure 5: A. Genome tracks of aggregate scATAC-seq data at the MET locus, a target gene of the AP-1 (FOS::JUN) transcription factor complex. **B.** Violin plot of MET gene expression calculated from scRNA-seq profiles of HCC cells.



Figure 3: A. UMAP projection of scATAC-seq profiles for 16,313 HCC cells. Each color represents 1 of 4 cell lines. Each dot represents 1 cell. **B.** UMAP projection colored by lognormalized gene activity scores for PROM1, calculated using Cicero.



Figure 6: A. UMAP projection of scATAC-seq profiles for MHCC97H cells. Each color represents 1 of 5 clusters. Cluster 4 separates from all other clusters and is highlighted in red. Each dot represents 1 cell. B. Dot plot of ZNF384 ChromVAR activity in MHCC97H cells aggregated by cluster. C. Dot plot of ZNF384 gene expression calculated from scRNA-seq. Dot size represents percent of cells with ZNF384 ChromVAR activity/expression. Color represents average ChromVAR activity or expression.





**Figure 7**: Genome tracks of aggregate scATAC-seq data at the MMP1 locus, a target gene of the ZNF384 transcription factor. A. MHCC97H cells aggregated by cluster. **B.** All cells aggregated by cell line.

- scATAC-seq identifies unique transcription factor dependencies for proliferation and differentiation regulation.
- HEP3B and HUH7 chromatin accessibility patterns promote stemlike characteristics, such as PROM1 (CD133) expression.
- MHCC97H specifically utilizes AP-1 to regulate proliferation. which regulates the transcription of extracellular matrix genes
- A sub-clonal population of MHCC97H has high ZNF384 activity, involved in migration and metastasis.

### **References:**

- <sup>1</sup> Satpathy A, et al. Nature Biotech. 2019; 37:925-936.



Results III

### ZNF384 Target Gene MMP1 Promoter has Heterogeneously Open Chromatin in MHCC97H, which is not Found in Other Cell Lines

# Summary

<sup>2</sup> Buenrostro J, et al. Curr Protoc Mol Biol. 2016;109: 21.29.1–21.29.9.



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