ChIP-seq and RNA-seq Analysis Unveils Molecular Mechanisms Underpinning Pancreatic Cancer Stem Cell Characteristics

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Introduction

ChIP-sequencing (ChIP-seq) and **RNA-sequencing (RNA-seq)** are cutting-edge technologies widely used to explore gene regulation and expression patterns. ChIP-seq allows for identification of DNA regions bound by specific proteins, while RNA-seq provides a comprehensive view of transcriptomic profiles. CD Genomics specializes in providing high-quality ChIP-seq and RNA-seq services to support clients' research needs. This study utilized our ChIP-seq platform to analyze protein-DNA interactions in ABCG2+ L3.6pl tumorspheres, along with RNA-seq to profile transcriptomic changes under specific treatment conditions. Our comprehensive workflow, including chromatin shearing, library preparation, sequencing, and bioinformatics analysis, ensures reliable and reproducible results for the advancement of molecular research.

Methods

CHIP-sequencing

- ABCG2+ L3.6pl tumorsphere cells were used for chromatin immunoprecipitation (ChIP) with antibodies (anti-PHF5A, anti-PHF14, anti-KMT2A), followed by DNA sequencing to investigate the interaction between the target proteins and DNA in CD Genomics.

RNA-sequencing

- RNA-seq was performed on MM-102-treated ABCG2+ L3.6pl cells to assess transcriptomic changes upon KMT2A inhibition in CD Genomics. Cells were treated with a suboptimal MM-102 concentration (50 μM) for 5 days to preserve viability.

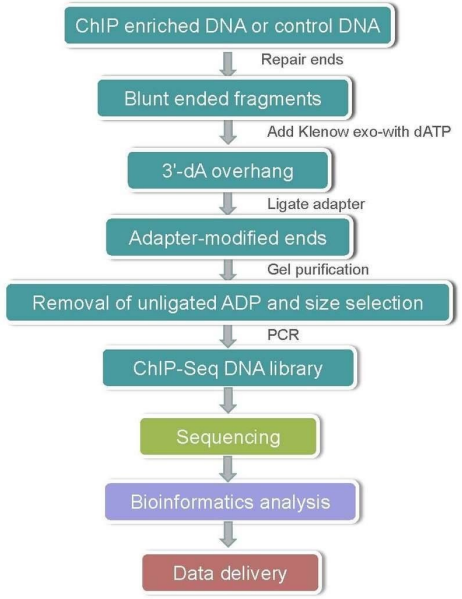
Data Analysis

- BCL convert for data processing and conversion.
- Cleaning: Fastp for raw read cleaning, FastQC for quality check.
- Mapping: Burrows-Wheeler Aligner (ChIP-seq) and STAR (RNA-seq) for read mapping to the human genome hg38.
- Peak Calling/Quantification: MACS2 for ChIP-seq peak calling, featureCounts for RNA-seq quantification.
- Differential Analysis: DiffBind for ChIP-seq differential binding, DESeq2 for RNA-seq differential expression.
- Functional Enrichment: GSEA with fgsea, g:GOSt for over-representation analysis.

Workflow and Results

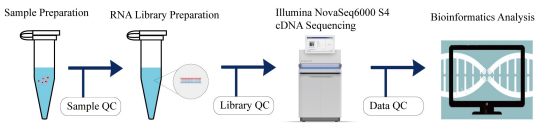
ChIP-Seq workflow in CD Genomics

CD Genomics offers high-quality **ChIP-Seq** services for genome-wide analysis of DNA-protein interactions. Our ChIP-Seq workflow includes chromatin immunoprecipitation, library preparation, high-throughput sequencing, and bioinformatics analysis such as peak calling, motif prediction, and functional annotation. With minimal sample requirements and advanced sequencing platforms, these services support applications in gene regulation, histone modification, and disease research, delivering comprehensive data and analysis tailored to client needs.



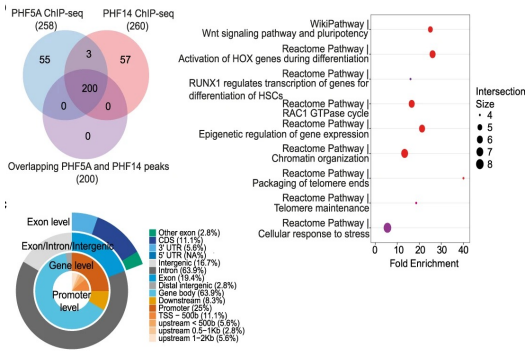
RNA-Seq workflow in CD Genomics

The **RNA-Seq** workflow includes sample preparation, RNA library preparation, sequencing with Illumina NovaSeq6000 or PacBio systems, and rigorous quality control (QC) at every stage—sample QC, library QC, and data QC—ensuring reliable results. Bioinformatics analysis follows, encompassing de novo assembly, gene annotation, differential expression analysis, and novel transcript discovery.

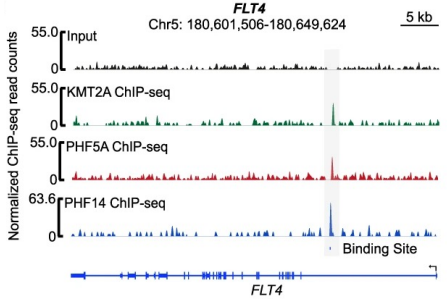


Mapping PhF5A and PHF14 Binding by CHIP-seq

Using mass spectrometry, the authors identified a physical interaction between PHF5A and PHF14 in L3.6pl and L3.6sl monolayer cell cultures. This was subsequently validated through **ChIP-seq** experiments provided by CD Genomics, which confirmed the co-binding sites of these two proteins in ABCG2+ L3.6pl tumorspheres. The results revealed shared binding sites across approximately 200 genes. Furthermore, the study uncovered the genomic distribution of the common DNA binding sites occupied by PHF5A and PHF14, with 63.9% of the target sites located within gene bodies, 25% in promoter regions, and 8.3% downstream of genes. These binding sites are associated with key biological processes, including stem cell self-renewal, activation of HOX genes during differentiation, transcriptional regulation, and chromatin remodeling.

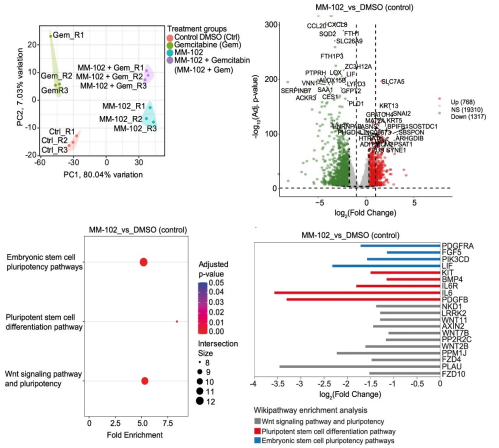


The authors further explored the relationship between KMT2A, PHF5A, and PHF14 by mapping their genomic occupancy in ABCG2+ L3.6pl tumorspheres using **ChIP-seq** analysis. They specifically focused on the genomic binding of KMT2A, PHF5A, and PHF14 at the FLT4 gene in ABCG2+ L3.6pl tumorspheres. ChIP-seq tracks highlighted common peak regions at the introns of FLT4, indicating the co-binding of these proteins at this locus. This finding suggests that KMT2A, PHF5A, and PHF14 may play a role in the regulation of FLT4, which is potentially involved in the regulation of cancer stemness and pluripotency.



RNA-seq Reveals KMT2A Regulation in PCSCs

The authors conducted an **RNA-seq** analysis to explore how KMT2A epigenetically regulates PCSCs, with a focus on transcriptomic changes following KMT2A inhibition with MM-102. For analysis RNA was extracted from ABCG2+ L3.6pl cells treated with a suboptimal concentration of MM-102. Results revealed that MM-102 monotherapy significantly upregulated 768 genes and downregulated 1317 genes in comparison to the control. When combined with gemcitabine, 668 genes were upregulated and 1193 genes downregulated. Gene expression changes induced by MM-102, either alone or in combination with gemcitabine, were distinct from those caused by gemcitabine alone, as shown by principal component analysis (PCA). Pathway enrichment analysis of differentially expressed genes (DEGs) highlighted the downregulation of genes related to Wnt signaling, pluripotent stem cell differentiation, and embryonic stem cell pluripotency in MM-102-treated cells, whereas gemcitabine treatment upregulated these pathways. These findings suggest that KMT2A inhibition plays a key role in the regulation of cell stemness and pluripotency in PCSCs.



Conclusion

- This study highlights the power of advanced **ChIP-seq** and **RNA-seq** technologies in uncovering the molecular mechanisms that regulate pancreatic cancer stem cell (PCSC) characteristics.
- Through our comprehensive **ChIP-seq** service, the identification of co-binding sites between PHF5A and PHF14 in ABCG2+ L3.6pl tumorspheres provided valuable data on the genomic distribution of these binding sites.
- Furthermore, our **RNA-seq** platform enabled a deep analysis of KMT2A regulation, showcasing how its inhibition influences gene expression in PCSCs and highlighting the involvement of key pathways.

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REFERENCES
Mouti, Mai Abdel et al.*Nature communications* vol. 14,1 5685. 14 Sep. 2023

We can also provide other epigenomics and transcriptomics related services to achieve your research purposes, like cut&tag, ATAC-seq, lncRNA sequencing.