Epigenetic heterogeneity of osteosarcoma reveals distinct cellular states driven by enhancer regulation

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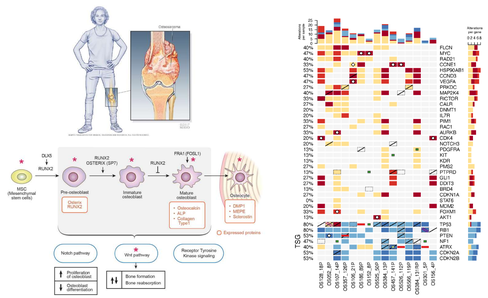
Osteosarcoma (OS) is characterized by high genomic complexity and heterogeneity

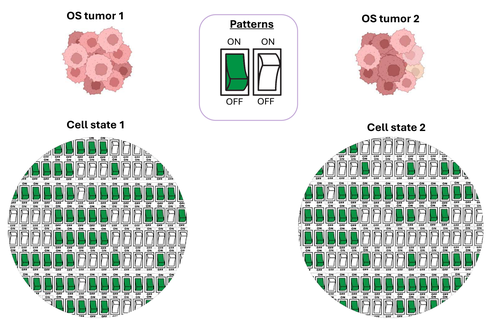


Young, E. P., Lopez-Fuentes E. *et al.* (2024). Cold Spring Harb Perspect Med.

Sayles *et al.* 2019. *Cancer Discovery*

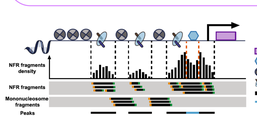
Osteosarcoma cells are thought to originate from the malignant transformation of cells within the osteoblastic lineage. The progressive differentiation stages of osteoblasts can be followed by the expression of specific proteins.

What is the Epigenetic landscape of Osteosarcoma?

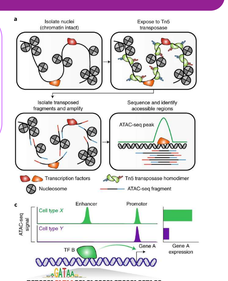


Mapping chromatin accessibility allows for identification of distinct cellular states

ATACseq (Assay for Transposase Accessible Chromatin with high-throughput sequencing) is a rapid and low-input method for mapping chromatin accessibility genome-wide by inserting sequencing adapters into accessible regions of the genome

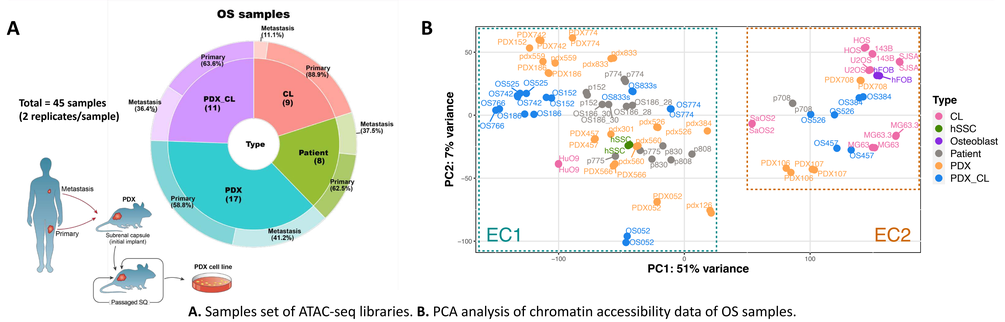


ChromVar: Schep, A. N., et al. (2017). *Nat Methods* **14**(10): 975-978.



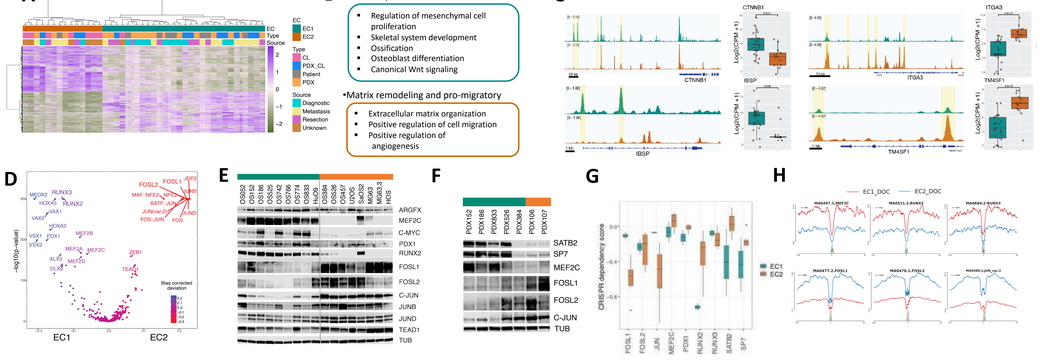
Grandi, F.C., *et al.* (2022). *Nat Protoc*

Osteosarcoma shows two epigenetically distinct cellular states defined by chromatin accessibility



A. Samples set of ATAC-seq libraries. **B.** PCA analysis of chromatin accessibility data of OS samples.

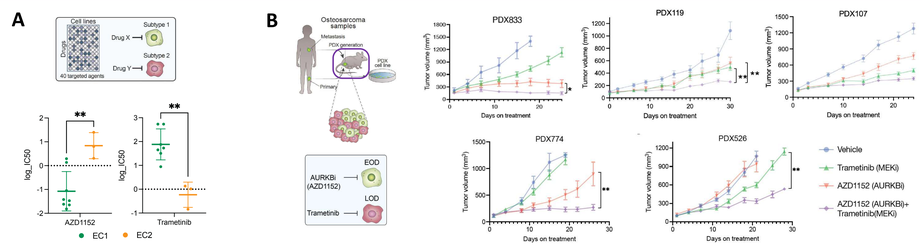
Differential chromatin accessibility between clusters is regulated by a specific TF binding activity



A. Differential analysis of chromatin accessibility between epigenetic clusters (EC). **B.** Enriched pathways. **C.** IGV snapshot of differential open genomic regions & gene expression. **D.** TF binding motif analysis by ChromVar. **E-F.** Protein abundance of EC-specific TFs in CLs panel **(E)** and PDXs panel **(F)**. **G.** CRISPR dependency score. **H.** TF footprint defined by Hint-ATAC.

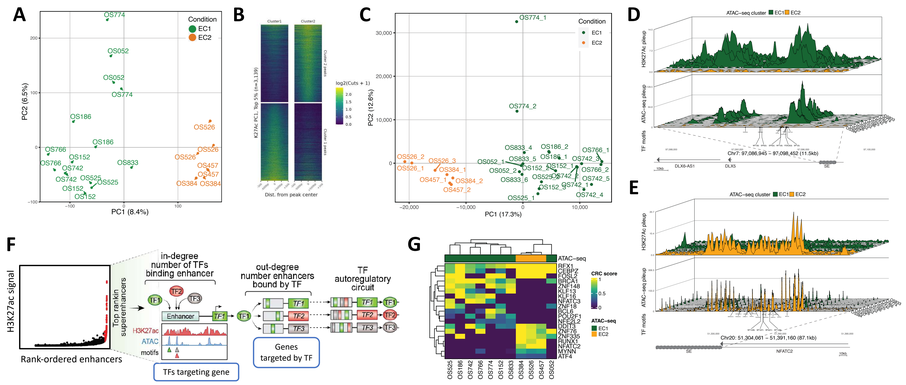
Differential drug response is correlated with the two epigenetically different cellular states

We evaluated a drug response dataset generated across more than 40 different targeted agents for this same panel of PDX-cell lines (Sayles *et al.* in preparation).



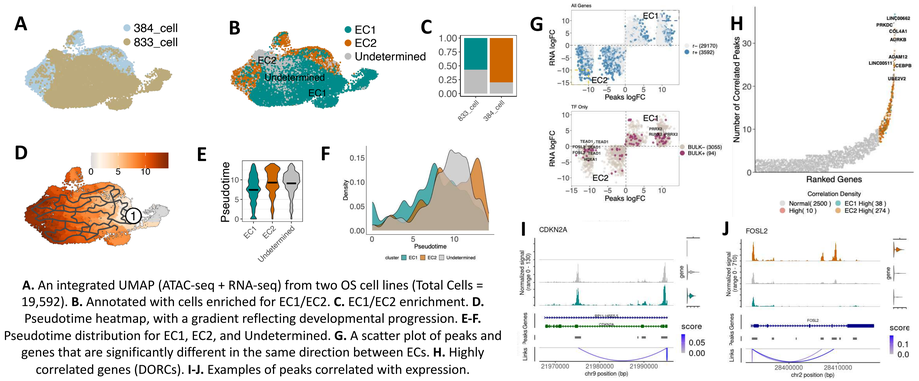
A. Drugs with differential drug response in the PDX-CL panel. **B-C.** EC-specific drug response *in vivo* tested in the PDXs.

Epigenetic clusters are driven by enhancer regulation (H3K27ac)



A. PCA of the top 10K H3K27ac data. **B.** Differential enhancer regulation per EC. **C.** PCA of the top 1000 SEs with the highest variance. **D.** EC1-specific SE in the DLX5-DLX6 loci. **E.** EC2-specific SE in the NFATC2 locus. **F.** Schematic of enhancer-based CRC analysis. **G.** Heatmap of clique enrichment scores for the TFs associated with top enhancers per EC.

EC2 shows a later developmental progression

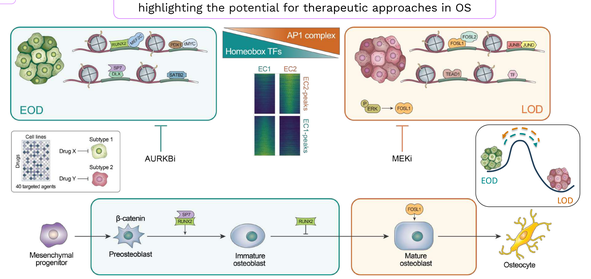


A. An integrated UMAP (ATAC-seq + RNA-seq) from two OS cell lines (Total Cells = 19,592). **B.** Annotated with cells enriched for EC1/EC2. **C.** EC1/EC2 enrichment. **D.** Pseudotime heatmap, with a gradient reflecting developmental progression. **E-F.** Pseudotime distribution for EC1, EC2, and Undetermined. **G.** A scatter plot of peaks and genes that are significantly different in the same direction between ECs. **H.** Highly correlated genes (DORCs). **I-J.** Examples of peaks correlated with expression.

Conclusions

Epigenetic Osteosarcoma model

ECs show differential drug responses and differential prognoses, highlighting the potential for therapeutic approaches in OS



ACKNOWLEDGEMENTS

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