Dynamics of Histone Post-translational Modification in Squamous Differentiation and Tumorigenesis

Eesha Alladin[1,2], Chao Lu[1,2]

[1]Department of Genetics and Development, [2]Herbert Irving Cancer Center, Columbia University, New York, NY

Abstract

Epigenetics involves changes in cellular function that do not alter the DNA sequence.[1] Key epigenetic mechanisms include DNA methylation, histone modifications, nucleosome remodeling, and non-coding RNAs.[1] Post-translational modifications (PTMs) of histones, occurring on specific amino acids such as lysine and arginine, are critical for regulating gene expression and cellular processes.[2] In cancers like head and neck squamous cell carcinomas (HNSCC), mutations in enzymes such as NSD1 and KMT2D, which facilitate histone PTMs, are prevalent.[3] Recent studies have identified histone gene mutations in HNSCC, leading to genome-wide alterations in histone PTMs.[3] This project focuses on histone PTMs, specifically investigating the spatial variation in levels of different histone PTMs in tumor tissues. Through immunofluorescence (IF), QuPath, and PRISM, histone acetylation and methylation levels can be quantified to demonstrate cross-talk between different histone marks. In particular, in sarcomas with the H3.3K36M mutation, there is a global loss of H3K36me2/3 and an increase in H3K27me3, indicating cross-talk between these marks.[3] In HNSCC, however, the role of NSD1-mediated H3K36me2 remains under-explored. This study aims to identify H3K36me2 levels, its opposing mark H3K27me3, and other histone PTMs in tissue samples to uncover novel regulatory interactions.

Introduction

- Histone PTMs occur on specific amino acids such as lysine and arginine.
  - Facilitates how tight/loose DNA will wrap around histone

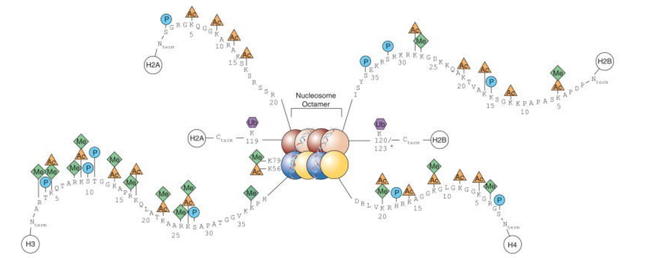


Figure 1 depicts acetylation/methylation PTMs on lysine and arginine residues on different histone marks

- Enzymes are involved in histone PTMs.[4]
  - Writers- enzymes that add a group
  - Erasers- enzymes that remove a group
  - Readers- effector proteins that recognize specific marks & recruit proteins, altering the function of the mark.
- In cancers like head and neck squamous cell carcinoma (HNSCC), mutations in enzymes such as KMT2D and NSD1, which facilitate histone PTMs, are prevalent.[3]

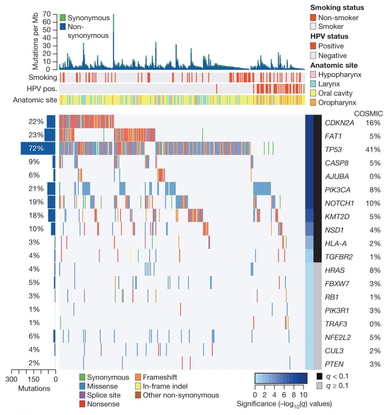


Figure 2 shows tumor sequences in patients diagnosed with HNSCC and identifies the prevalence of KMT2D and NSD1.

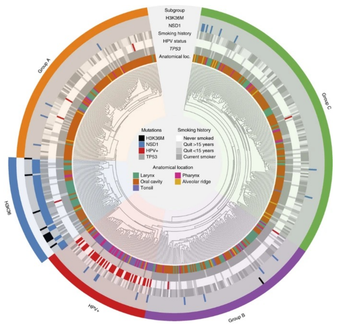


Figure 3 shows in the blue region an NSD1 mutation in patients diagnosed with HNSCC. The black regions directly above represent mutations within the histones themselves. This causes impairment of H3K36methylation in patients with HNSCC.

Methods and Materials

- Cancer cells were injected into the tip of the tongue in mice. Tumors were dissected, cut into tissue slices, and blocked in paraffin.
- Immunofluorescence (IF) employs primary and secondary antibodies to stain normal and cancerous cells with DAPI, GFP, and RFP.

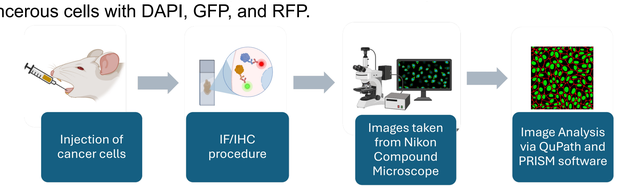


Figure 4 displays an overview of the methods

- Images were quantified by cell segmentation via QuPath software

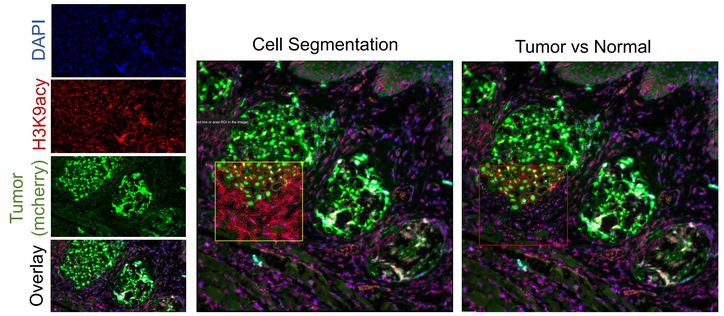


Figure 5 depicts the three stains utilized, which include DAPI, H3K9acytelation, and tumor. This gives an overview of the cell segmentation processes.

Results

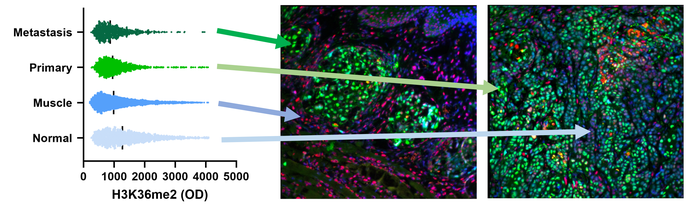
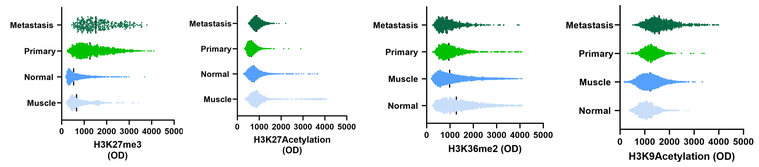


Figure 6 identifies regions for how the data was organized for this project

	H3K27me3	H3K27acy	H3K36me2	H3K9acy
Basement				
Primary				
Metastasis				

Table 1 organizes IF imaging in 3 distinct regions of the tissue that is specific to the following histone marks: H3K27me3, H3K27acy, H3K36me2, and H3K9acy.

Results



	H3K27me3	H3K27acy	H3K36me2	H3K9acy
	Gene Silence	Active Transcription	Active Transcription in gene body	Active Promoters
Metastasis	↑	Slight change	↓	↑
Primary	↑	Slight change	↓	↑
Normal	↓	Slight change	↑	↓
Muscle	↓	Slight change	↑	↓

Table 2 shows that tumor cells exhibit higher OD levels for H3K27me3, suggesting increased gene silencing compared to normal cells. In the case of H3K27acetylation, both tumor and normal cells display minimal changes, indicating similar levels of active transcription. For H3K36me2, tumor cells demonstrate slightly reduced gene body transcription activation relative to normal cells. Lastly, the data for H3K9acetylation indicates that tumor cells have more active promoters.

Summary & Future Direction

- Immunofluorescence was used to detect the signal for normal and tumor cells.
  - This helped to take images that were utilized for cell segmentation.
- Through QuPath, cell segmentation was performed to determine OD level data for normal and tumor cells
- PRISM was utilized to organize the data in graphs to make conclusions based on the data.
- Utilize CRISPR-Cas9 to knock out different histone proteins to look at the effect on oral epithelial
- Utilize CHIP-seq to identify which genes are turned on/off by histone mark

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