# The CHD1-KDM Axis in Prostate Cancer


**Kaitlyn A. Tremble[1,2], Haoyan Li[1], Di Zhao[1]**

[1]MD Anderson Cancer Center, Houston, Texas [2]Baylor University, Waco, Texas


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## Introduction

Chromodomain-helicase-DNA-binding protein 1 (CHD1) is an ATP-dependent chromatin remodeler involved in gene transcription and organismal development [1]. Homozygous CHD1 deletion leads to genomic instability as well as cell phenotypic alterations and is the second most common deletion found in human prostate cancer (PCa) [2-4]. KDM1/3/4 protein family are histone demethylases that target H3K9 and H3K36 [5]. High expression of KDMs can lead to the downregulation of H3K9me3 and subsequent abnormal gene activation, promoting tumorigenesis in prostate cancer (*Fig. 1*) [6, 7]. ML324 is a small-molecule selective inhibitor of KDM4s which reduces PCa cell proliferation [8-10]. Here, we study the crosstalk between CHD1 and KDM4 in PCa.

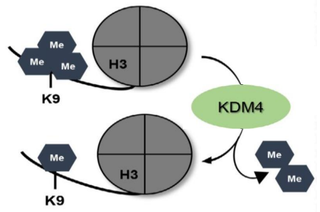


Fig. 1 Alteration of H3K9 methylation states by KDM4 influences transcriptional regulation and chromatin dynamics [7].

## Methods

Epigenetics compound library screening was performed using LNCaP WT cells and isogeneic CHD1 knockout cells, cell viability was then measured using CellTiter-Glo® Luminescent Cell Viability Assay. The small compounds were ranked based on the sensitivity in WT versus CHD1 knockout cells. For top candidates, the IC50 was determined by measuring cell viability in multiple PCa cell lines. RNA levels were measured using qPCR and RNA array. ChIP-seq was used to assess CHD1 regulation of KDM4A and KDM4D. Protein expression and histone markers were measured by Western Blot and immunohistochemistry.

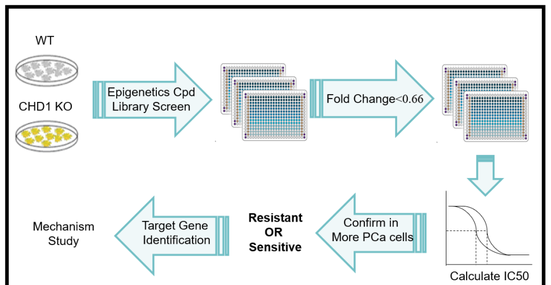


Fig. 2 Epigenetics compound library screening process to determine drug responsiveness in CHD1 loss prostate cancer.

## Results

Through epigenetics compound library screening, ML324, a small-molecule selective inhibitor of KDM4, ranked as the most sensitive compound in WT versus CHD1 knockout LNCaP cells (*Fig. 3A*). IC50 analyses in multiple PCa cell lines with or without CHD1 deletion confirmed that CHD1 loss makes PCa cells less sensitive to KDM4 inhibitor ML324 (*Fig. 3B-C*). Mechanistically, we found that CHD1 deletion reduces the expression of KDM4A and KDM4D both *in vitro* (*Fig. 4*) and *in vivo* (*Fig. 5*). KDM4A and KDM4D regulate the demethylation of H3K9, but trimethylation levels of H3K9 did not change with CHD1 loss *in vivo* (*Fig. 5*). However, CHD1 deletion upregulates KDM1A and KDM3A (*Fig. 6*), which also regulates the demethylation of H3K9. This may compensate for the reduced KDM4A/D, resulting in the maintenance of H3K9me3 levels.

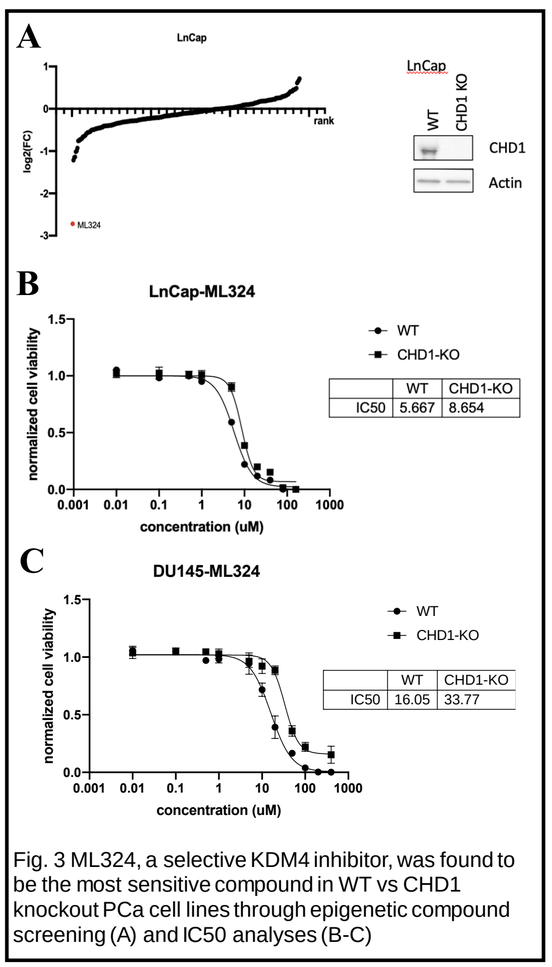


Fig. 3 ML324, a selective KDM4 inhibitor, was found to be the most sensitive compound in WT vs CHD1 knockout PCa cell lines through epigenetic compound screening (A) and IC50 analyses (B-C)

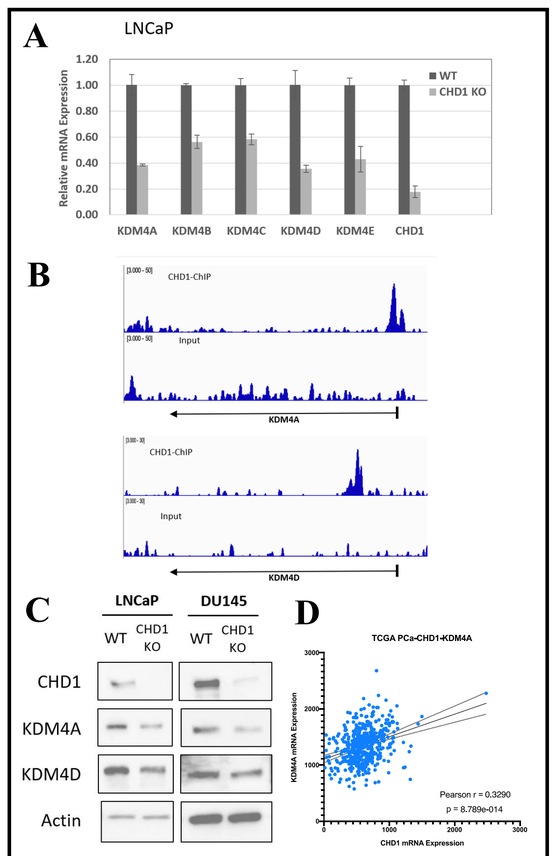


Fig. 4 CHD1 regulates KDM4A and KDM4D levels. qPCR shows mRNA expression in LNCaP WT vs CHD1 KO cells (A). ChIP-seq shows that CHD1 binds to the promoter regions of KDM4A and KDM4D (B). Western Blot shows decreased KDM4A and KDM4D protein levels in CHD1 KO lines (C). TCGA database shows that KDM4A and CHD1 levels are highly correlated.

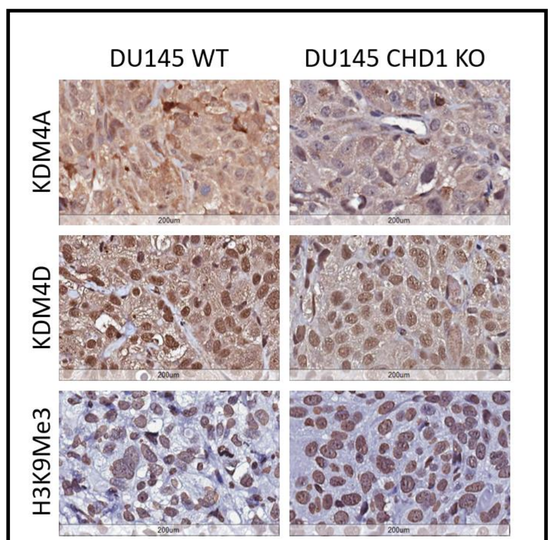


Fig. 5 Immunohistochemistry of DU145 WT and CHD1 knockout tumors show a decrease in KDM4A and KDM4D, however no apparent change in H3K9me3

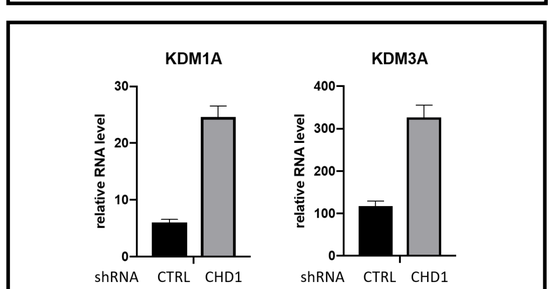


Fig. 6 KDM1A and KDM3A RNA levels are upregulated upon CHD1 deletion, show via RNA microarray.

## Conclusions

As a chromatin remodeler, CHD1 has been found to be involved in transcription regulation by reading H3K4me3. Here, our study uncovered a novel function of CHD1 in modulating KDMs. CHD1 loss inhibits KDM4A/D while promoting KDM1A and KDM3A, resulting in the maintenance of H3K9me3 levels. Future studies will focus on exploring this relationship as well as the identification of target genes and downstream pathways of the CHD1-KDM axis to better understand their function in PCa development and progression.

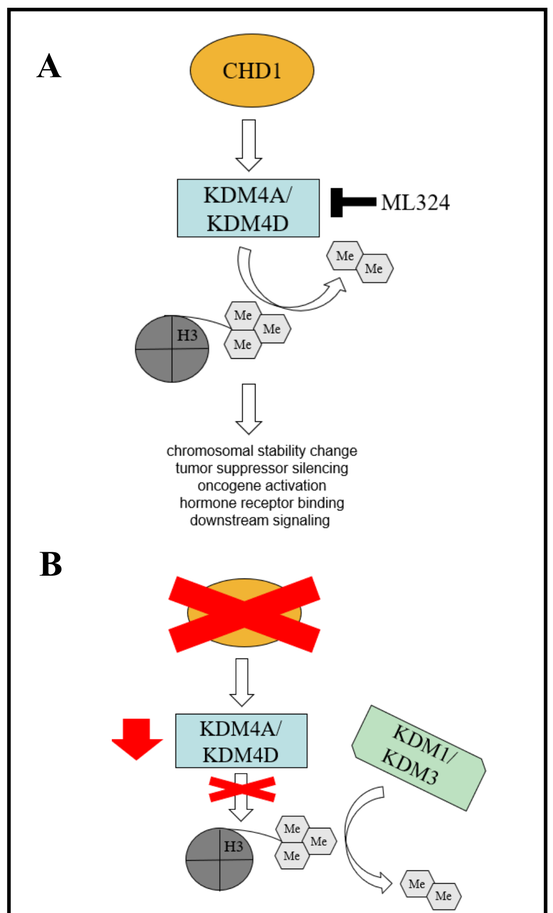


Fig. 7 Proposed mechanism for CHD1-KDM axis.

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