MP29-01

DNA replication stress by the loss of male specific histone demethylase ‘KDM5D’ in aggressive prostate cancer

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Introduction and Objectives

- KDM5D is a male-specific Lysine-Specific Demethylase encoded on the Y chromosome. (Yq11.223).
- We previously reported a deletion of KDM5D in prostate cancer cell lines (Komura et al, PNAS, 2016).
- The objecctive in this study is to further explore the biological characteristics of KDM5D deficient prostate cancer and assess clinical implication.

Fig 1. Genes transcipted on Y Chromosome are Rare, and Exhibits Modest Clinical Impact with its Decreased Exression Except for KDM5D in Prostate Cancer.

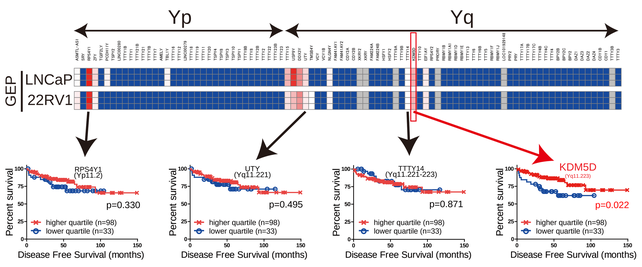
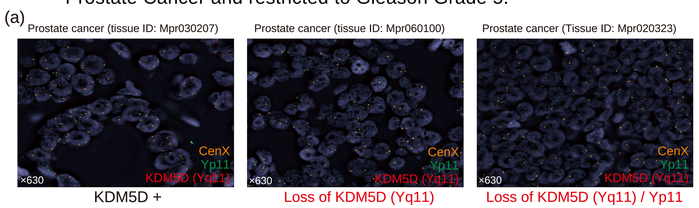


Fig 1: GEP (gene expression profiling) of the transcriptome on Y chromosome in LNCaP and 22RV1 prostate cancer cell lines. Lower panel shows the Kaplan-Meier curves for the disease specific survival (DFS) in 131 primary prostate cancer patients in Taylor’ s cohort according to RNA expression level of indicated genes. Log rank test was performed to evaluate the difference of the survivals.

Fig 2. Copy Number Loss of KDM5D is Observed in More Than 10% of Prostate Cancer and restricted to Gleason Grade 5.

(a)



(b)

The summary of FISH analysis in TMA.

Variables	Number of cases	Positive for Loss of KDM5D (%)
Normal prostate	7	0
Adjacent normal prostate	7	0
Adenocarcinoma	75	8 (11%)
Tumor Grading		
T1	2	0
T2	37	5 (14%)
T3	20	1 (5%)
T4	3	0
Not known	13	2 (15%)
Stage		
I	3	0
II	32	5 (16%)
III	14	1 (8%)
IV	12	0
Not known	14	2 (13%)
Gleason Grade		
3	9	0
4	30	1 (12%)
5	29	7 (88%)
Not known	7	0

Fig 2:
(a) Result of Fluorescence in situ hybridization (FISH) analysis using a 3-color KDM5D (Yq11: red) / Yp11 (green) / X chromosome (CenX) (orange) probe mix in formalin-fixed paraffin-embedded (FFPE) tissue sections (T195c and PR1921a; US Biomax). The Yp11 probe was included to detect/distinguish loss of KDM5D due deletions versus the loss of entire Y-chromosome. CenX probe (a centromeric repeat plasmid specific to the X chromosome) served as the control for hybridization (in situations with complete loss of Y-chromosome, 0 copies). A minimum of 50-100 nuclei per case were evaluated. To minimize truncation artifacts, only nuclei with at least one control signal were considered. A case was considered to exhibit loss of KDM5D if >90% of tumor cells in a minimum of 2~5 adjacent or non-adjacent fields (63X) showed absence of Red (KDM5D) signal.

(b) The summary of FISH in tissue microarray (TMA) revealed that loss of KDM5D is only detected in prostate cancer, not in normal tissue. Note that the deletion was restricted to primary gleason’ s score of 5 implying an aggressive fature of this subset with the loss of KDM5D.

Fig 3. Orthotopic Xenograft Model Showed Aggressive Phenotype of the loss of KDM5D in Prostate Cancer.

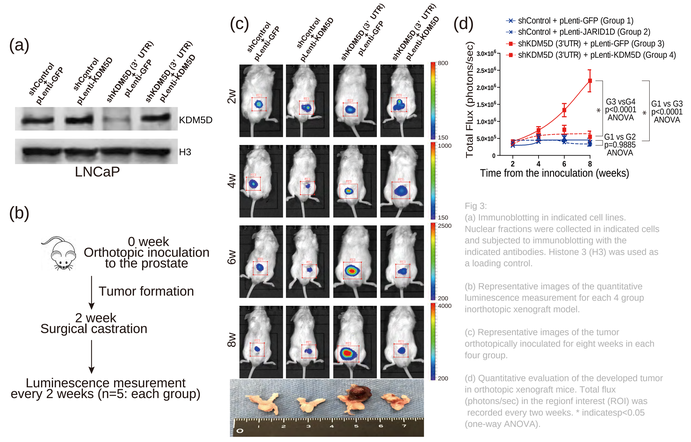


Fig 3:
(a) Immunoblotting in indicated cell lines. Nuclear fractions were collected in indicated cells and subjected to immunoblotting with the indicated antibodies. Histone 3 (H3) was used as a loading control.

(b) Representative images of the quantitative luminescence measurement for each 4 group inorthotopic xenograft model.

(c) Representative images of the tumor orthotopically inoculated for eight weeks in each four group.

(d) Quantitative evaluation of the developed tumor in orthotopic xenograft mice. Total flux (photons/sec) in the regionf interest (ROI) was recorded every two weeks. * indicatesp<0.05 (one-way ANOVA).

Fig 4. KDM5D is an Essential Co-regulator of Transcription Factors for Cell Cycle by Regulation of H3K4metylation Pattern in the Promotor Region.

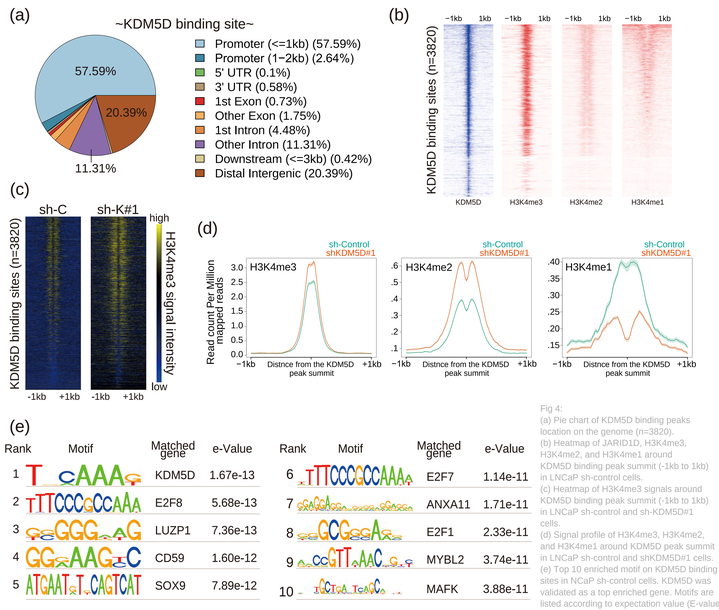


Fig 4:
(a) Pie chart of KDM5D binding peaks location on the genome (n=3820).
(b) Heatmap of JARID1D, H3K4me3, H3K4me2, and H3K4me1 around KDM5D binding peak summit (-1kb to 1kb) in LNCaP sh-control cells.
(c) Heatmap of H3K4me3 signals around KDM5D binding peak summit (-1kb to 1kb) in LNCaP sh-control and sh-KDM5D#1 cells.
(d) Signal profile of H3K4me3, H3K4me2, and H3K4me1 around KDM5D peak summit in LNCaP sh-control and shKDM5D#1 cells.
(e) Top 10 enriched motif on KDM5D binding sites in NCaP sh-control cells. KDM5D was validated as a top enriched gene. Motifs are listed according to expectation value (E-value).

Fig 5. LNCaP (intact KDM5D) and LNCaP-104R2 (KDM5D loss) as a Experimental Model for RNAseq Validation.

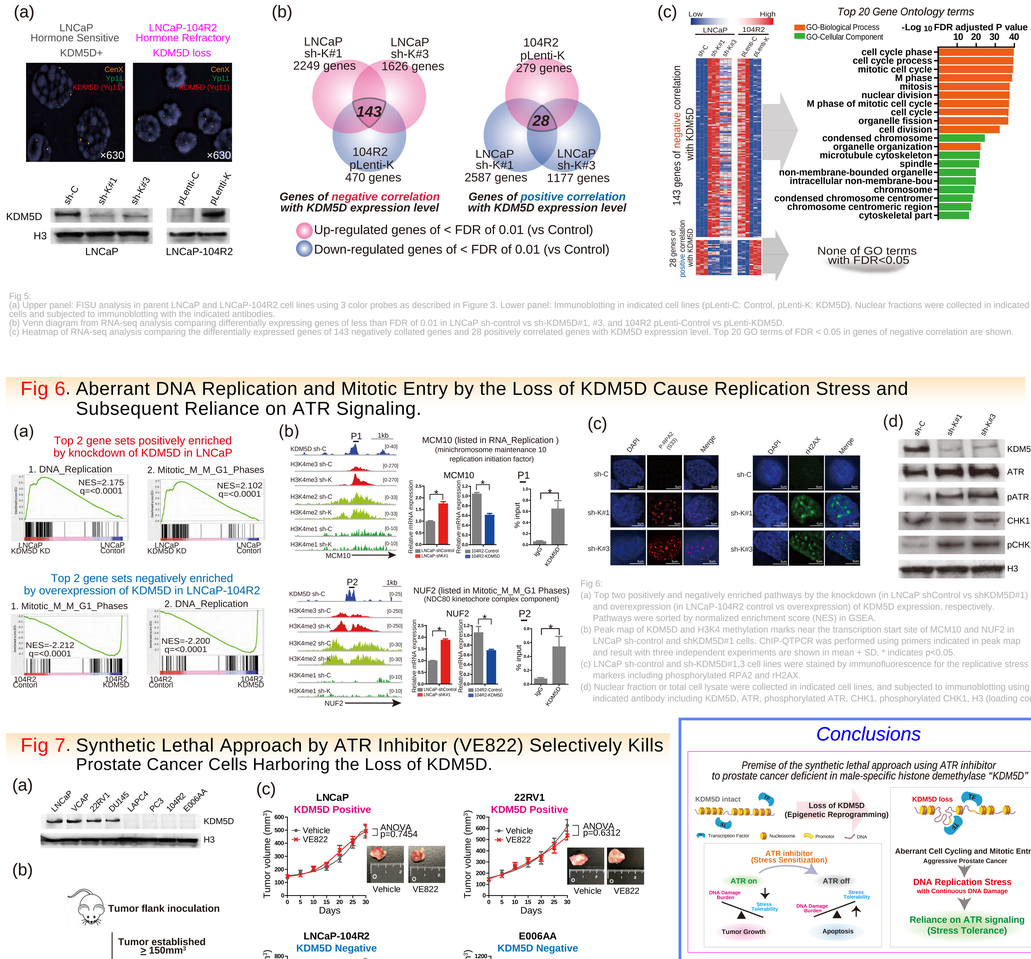


Fig 5:
(a) Upper panel: FISU analysis in parent LNCaP and LNCaP-104R2 cell lines using 3 color probes as described in Figure 3. Lower panel: Immunoblotting in indicated cell lines (pLenti-C: Control, pLenti-K: KDM5D). Nuclear fractions were collected in indicated cells and subjected to immunoblotting with the indicated antibodies.
(b) Venn diagram from RNA-seq analysis comparing differentially expressing genes of less than FDR of 0.01 in LNCaP sh-control vs sh-KDM5D#1, #3, and 104R2 pLenti-Control vs pLenti-KDM5D.
(c) Heatmap of RNA-seq analysis comparing the differentially expressed genes of 143 negatively collated genes and 28 positively correlated genes with KDM5D expression level. Top 20 GO terms of FDR < 0.05 in genes of negative correlation are shown.

Fig 6. Aberrant DNA Replication and Mitotic Entry by the Loss of KDM5D Cause Replication Stress and Subsequent Reliance on ATR Signaling.

Fig 6:
(a) Top two positively and negatively enriched pathways by the knockdown (in LNCaP shControl vs shKDM5D#1) and overexpression (in LNCaP-104R2 control vs overexpression) of KDM5D expression, respectively. Pathways were sorted by normalized enrichment score (NES) in GSEA.
(b) Peak map of KDM5D and H3K4 methylation marks near the transcription start site of MCM10 and NUF2 in LNCaP sh-control and shKDM5D#1 cells. ChIP-QTPCR was performed using primers indicated in peak map and result with three independent experiments are shown in mean + SD. * indicates p<0.05.
(c) LNCaP sh-control and sh-KDM5D#1,3 cell lines were stained by immunofluorescence for the replicative stress markers including phosphorylated RPA2 and rH2AX.
(d) Nuclear fraction or total cell lysate were collected in indicated cell lines, and subjected to immunoblotting using indicated antibody including KDM5D, ATR, phosphorylated ATR, CHK1, phosphorylated CHK1, H3 (loading control).

Fig 7. Synthetic Lethal Approach by ATR Inhibitor (VE822) Selectively Kills Prostate Cancer Cells Harboring the Loss of KDM5D.

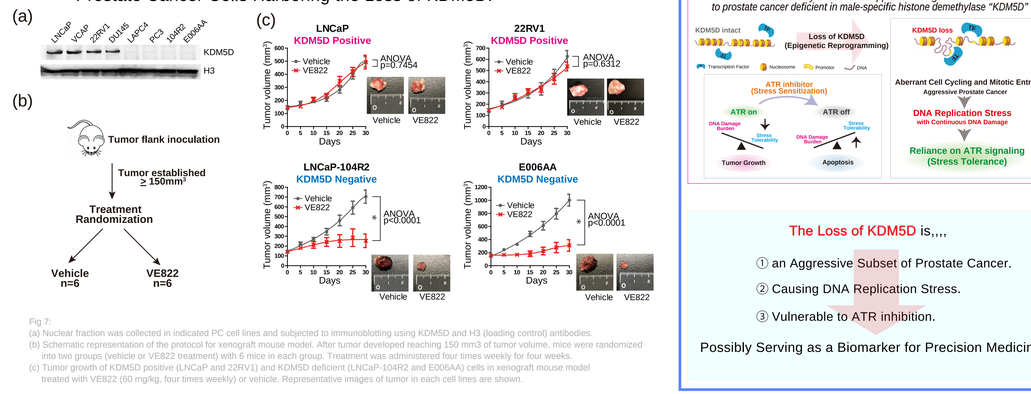


Fig 7:
(a) Nuclear fraction was collected in indicated PC cell lines and subjected to immunoblotting using KDM5D and H3 (loading control) antibodies.
(b) Schematic representation of the protocol for xenograft mouse model. After tumor developed reaching 150 mm3 of tumor volume, mice were randomized into two groups (vehicle or VE822 treatment) with 6 mice in each group. Treatment was administered four times weekly for four weeks.
(c) Tumor growth of KDM5D positive (LNCaP and 22RV1) and KDM5D deficient (LNCaP-104R2 and E006AA) cells in xenograft mouse model treated with VE822 (60 mg/kg, four times weekly) or vehicle. Representative images of tumor in each cell lines are shown.

Conclusions

Premise of the synthetic lethal approach using ATR inhibitor to prostate cancer deficient in male-specific histone demethylase “KDM5D”

The Loss of KDM5D is,,,,

① an Aggressive Subset of Prostate Cancer.

② Causing DNA Replication Stress.

③ Vulnerable to ATR inhibition.

Possibly Serving as a Biomarker for Precision Medicine.