Targeting histone lysine demethylase KDM4A in Aggressive Variant Prostate Cancer

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ABSTRACT

Background: Despite advancement in treatment, prostate cancer remains the second leading cause of death among men. Prostate cancer patients treated with potent second-generation anti-androgen inhibitors such as enzalutamide and abiraterone inevitably develop therapeutic resistance and progress to castration-resistant prostate cancer (CRPC). Neuroendocrine prostate cancer (NEPC), which has a median survival of 7 months after initial diagnosis, represents one of the most lethal forms of CRPC. In contrast, castration-resistant prostate adenocarcinoma, the more common subtype of CRPC, has a median survival of 13 to 31 months, depending on the organ sites of metastasis. NEPC is characterized by attenuated androgen receptor (AR) signaling, the expression of neuroendocrine lineage markers (e.g., synaptophysin), uncontrolled hyperproliferation, and widespread metastasis (e.g., bone, liver, and lung). *De novo* NEPCs are rare (2%-5%); the majority arises as a mechanism of resistance from prostate adenocarcinoma treated with potent AR pathway inhibitors (ARPIs). The widespread use of ARPIs in non-metastatic CRPC and hormone-sensitive metastatic tumors has led to an increase in the incidence NEPC. Due to the lack of life-prolonging systemic therapies, there is an urgent need to better understand the mechanisms underlying the pathogenesis of NEPC.

Recent evidence suggests that epigenetic dysregulation is a hallmark of NEPC. Among the various epigenetic regulatory mechanisms, histone lysine methylation, which is balanced by writers (histone lysine methyltransferase [KMT]) and erasers (histone lysine demethylases [KDM]), plays an important role in development and cancer, including prostate. However, whether KDMs play any roles in NEPC progression is unknown.

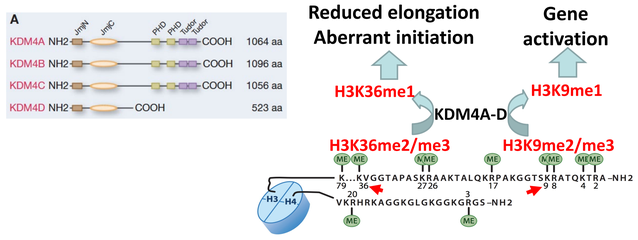

Figure 1. Structure of the KDM4 family proteins and schematic mechanism of enzymatic activity of the family (Reference 1 & 2).

Methods: By perturbation of KDM4A expression (overexpression, knockdown and knockout) and inhibition of KDM4A functions with small-molecule inhibitors in multiple model systems *in vitro* and *in vivo*, we will determine the function of KDM4A and the potential regulatory pathway of KDM4A in AVPC.

RESULTS

KDM4A is overexpressed in NEPC

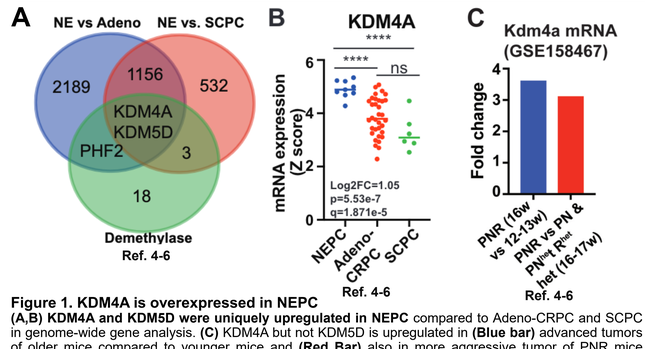

Figure 1. KDM4A is overexpressed in NEPC
(A,B) KDM4A and KDM5D were uniquely upregulated in NEPC compared to Adeno-CRPC and SCPC in genome-wide gene analysis. **(C)** KDM4A but not KDM5D is upregulated in **(Blue bar)** advanced tumors of older mice compared to younger mice and **(Red Bar)** also in more aggressive tumor of PNR mice compared to PN and PNR^{het} mice.

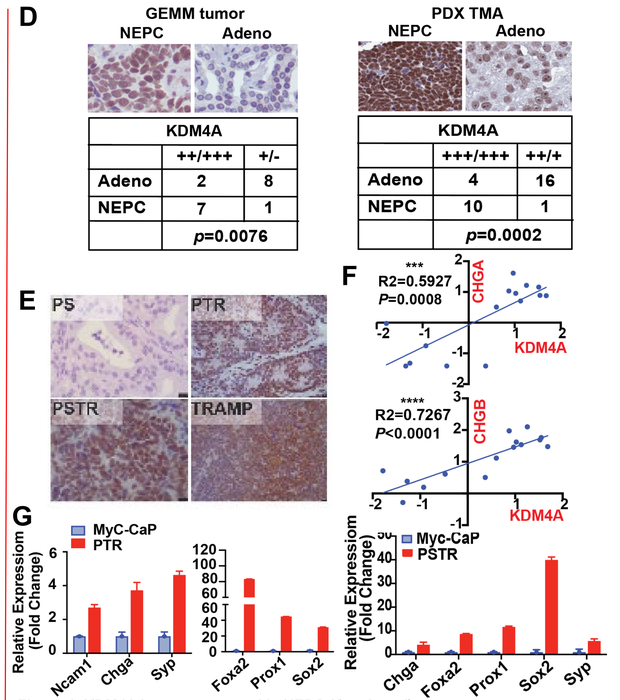

Figure 1. KDM4A is overexpressed in NEPC (Continued)
IHC results shows that KDM4A is overexpressed in **(D) (Left)** mouse NEPC but not in Adenocarcinoma. Similar result is also observed in **(D) (Right)** patient-derived xenograft tissue microarray. **(E)** KDM4A is highly expressed in tumor of 3 of the NEPC generated GEMM model (PTR, PSTR and TRAMP) compared to the adenocarcinoma model (PS). **(F)** KDM4A has a strong correlation with multiple neuroendocrine (NE) markers, including CHGA and CHGB. **(G)** Both mouse cell lines derived from the NEPC models **(Left)** PTR and **(Right)** PSTR have upregulated NEPC markers compared to Adeno cell line MYC-CAP.

KDM4A KD or KO impairs NEPC growth *in vitro* and *in vivo*

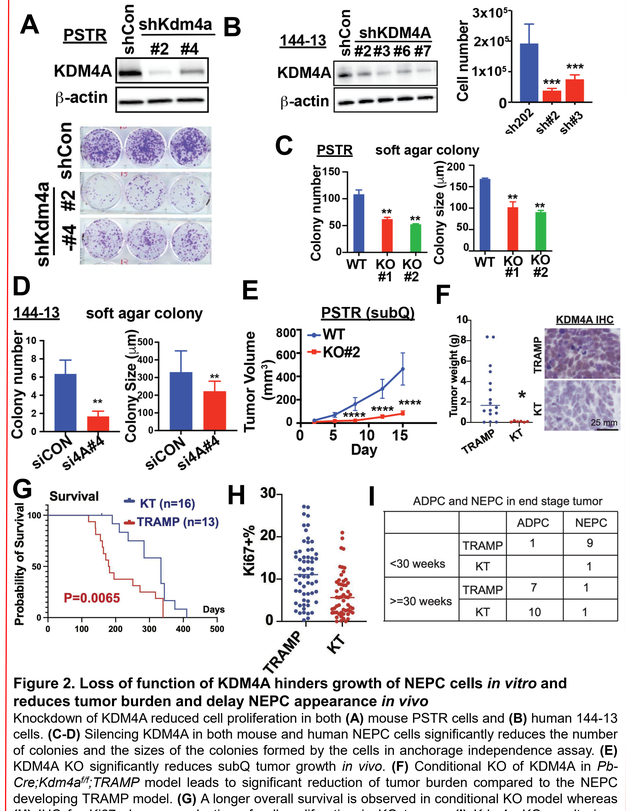

Figure 2. Loss of function of KDM4A hinders growth of NEPC cells *in vitro* and reduces tumor burden and delay NEPC appearance *in vivo*
Knockdown of KDM4A reduced cell proliferation in both **(A)** mouse PSTR cells and **(B)** human 144-13 cells. **(C-D)** Silencing KDM4A in both mouse and human NEPC cells significantly reduces the number of colonies and the sizes of the colonies formed by the cells in anchorage independence assay. **(E)** KDM4A KO significantly reduces subQ tumor growth *in vivo*. **(F)** Conditional KO of KDM4A in *Pb-Cre;Kdm4a$^{f/f}$;TRAMP* model leads to significant reduction of tumor burden compared to the NEPC developing TRAMP model. **(G)** A longer overall survival is observed in conditional KO model whereas **(H)** IHC for Ki67 shows a reduction of cell proliferation in KO tumors. **(I)** Kdm4a KO results in a reduction of NEPC incident in older mice.

KDM4 inhibitors suppressed NEPC growth and induce apoptosis *in vitro*

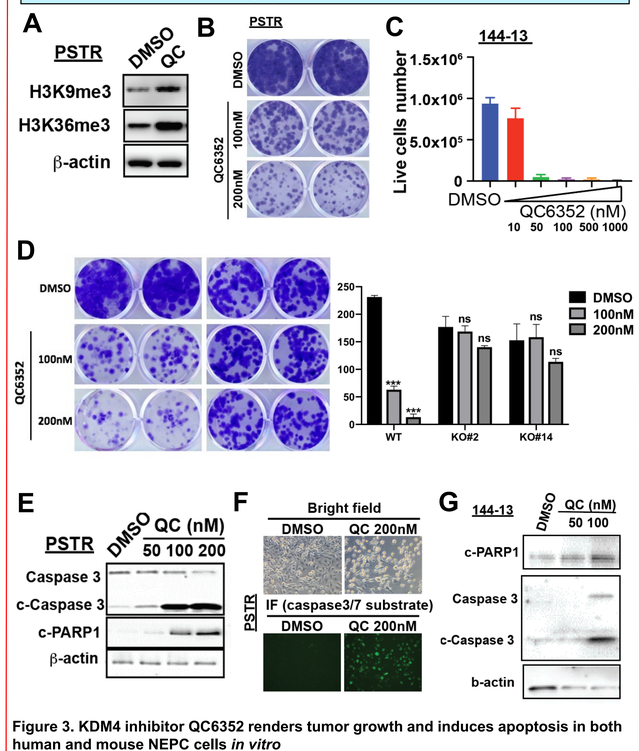

Figure 3. KDM4 inhibitor QC6352 renders tumor growth and induces apoptosis in both human and mouse NEPC cells *in vitro*
(A) QC6352 treatment drastically increases the level of both KDM4A substrates, H3K9me3 and H3K36me3. QC treatment drastically reduces proliferation of both **(B)** mouse PSTR and **(C)** human 144-13 NEPC cells in a dose dependent manner. **(D)** KO of KDM4A abrogates PSTR response to QC6352. **(E,G)** QC6352 treatment increases c-Caspase3 and c-PARP expression in PSTR and 144-13 cells. **(F)** Significant increase in caspase3/7 signal in QC treated cells compared to control.

KDM4 inhibitor hinders NEPC progression *in vivo*

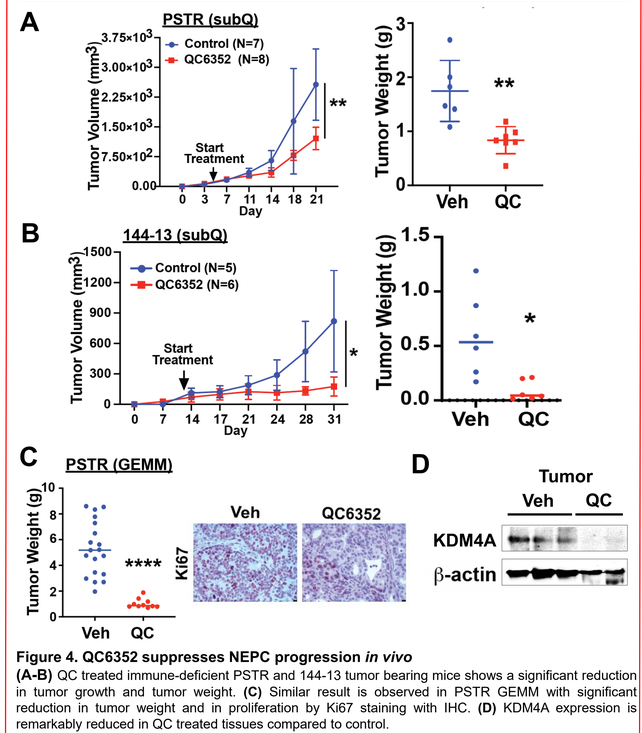

Figure 4. QC6352 suppresses NEPC progression *in vivo*
(A-B) QC treated immune-deficient PSTR and 144-13 tumor bearing mice shows a significant reduction in tumor growth and tumor weight. **(C)** Similar result is observed in PSTR GEMM with significant reduction in tumor weight and in proliferation by Ki67 staining with IHC. **(D)** KDM4A expression is remarkably reduced in QC treated tissues compared to control.

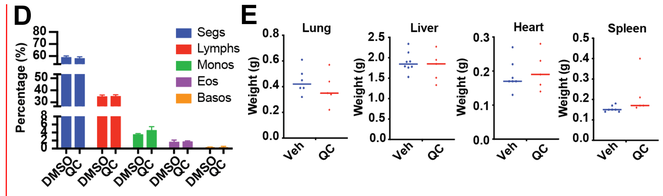

Figure 4. (Continued). QC6352 suppressed NEPC progression *in vivo*
(D) No observable differences between cell types in whole white blood count and **(E)** major organs weight, suggesting QC6352 has no concernable adverse effects of QC6352 on mice.

KDM4A regulates MYC expression in NEPC cells

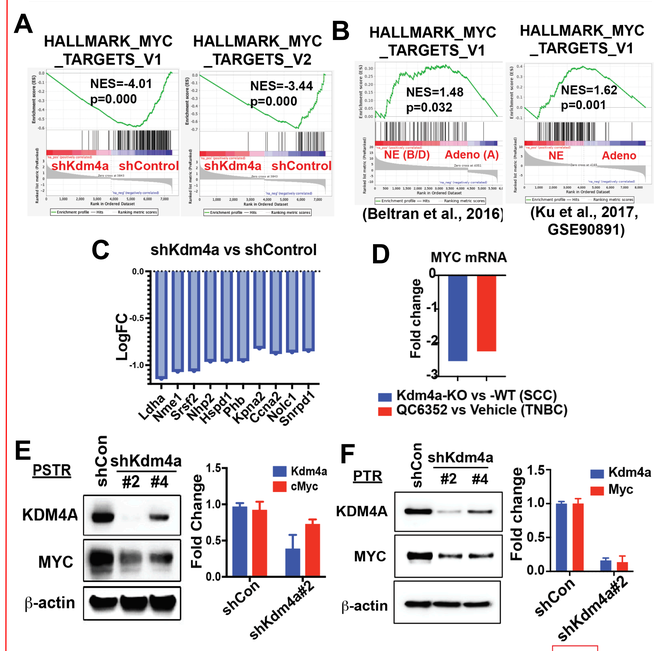

Figure 6. KDM4A regulates MYC pathways in NEPC
(A-B) GSEA on RNA-seq of Kdm4a-KD and –control PTR cells identifies MYC signatures as the top downregulated pathways in Kdm4a KD cells. On the other hand, MYC pathways are the top hallmark pathways activated in both human and mouse NEPC. **(C)** MYC target genes are significantly downregulated in Kdm4a-KD cells. **(D)** MYC mRNA is downregulated in mouse squamous cell carcinoma cells with Kdm4a KO compared to control cells and in QC6352 treated triple-negative breast cancer cells. Both protein and mRNA expressions of MYC in Kdm4a KD **(E)** PSTR and **(F)** PTR cells are reduced significantly when compared to control cells. **(G)** KD KDM4A in 144-13 cells drastically reduced MYC protein expression. **(H)** CHIP-seq in 144-13 cells shows that KDM4A binds to MYC promotor, suggesting an active MYC transcription in NEPC cells.

CONCLUSIONS

Our findings demonstrated that histone lysine demethylase KDM4A plays an undeniable role in the progression of NEPC in prostate cancer through regulating MYC and the downstream pathways. Targeting KDM4A can potentially be an effective therapeutic option for combating NEPC. In the future, we will further delineate and confirm the detailed mechanism of which KDM4A regulates MYC, be it by directly binding to the MYC promotor or its function as a transcriptional co-activator. We will also explore the therapeutic potential to leverage the combination of silencing KDM4A by either RNAi or QC6325 treatment with chemotherapy, with the aim to reduce side effects of chemotherapy on patients.

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