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14. ABSTRACT

Progression and metastasis of prostate cancers (PCs) are major therapeutic challenges, with the underlying mechanisms remaining unclear. The rise of AR-negative neuroendocrine prostate cancer (NEPC) cells is recently recognized as a major mechanism. The apical-basal polarity of epithelial cells plays critical roles in regulating normal cell migration and proliferation in prostate. Loss of cell polarity leads to tissue disorganization, uncontrolled proliferation and migration, hallmarks of prostate cancer progression and metastasis. ZDHHC7-mediated palmitoylation of Scribble is critical for cell polarity and metastasis. In the second year of the study, we found that loss of ZDHHC7 is significant in NEPC samples. We have generated ZDHHC7 knockout cell lines with various prostate cancer progression stages. We found that loss of ZDHHC7 led to loss of SCRIB palmitoylation, and activation of YAP signaling, and up-regulation of NEPC markers. We will further test the hypothesis that ZDHHC7 loss and SCRIB depalmitoylation contributes to prostate cancer cell progression through converting castration-resistant prostate cancer cells to NEPC phenotype.

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1. INTRODUCTION:

Prostate cancer (PCa) is a commonly diagnosed cancer in American men. However, a majority of these cancers recur and develop resistance to treatments. The apical-basal polarity of epithelial cells plays critical roles in regulating epithelial cell functions, including differentiation, migration, proliferation, and apoptosis, and is essential for normal development and tissue homeostasis. Loss of cell polarity leads to tissue disorganization, uncontrolled proliferation, epithelial-to-mesenchymal transition (EMT), and migration, which are hallmarks of progression of PCa. SCRIB has been characterized as an essential regulator of cell polarity, tumorigenesis and metastasis. SCRIB is frequently amplified and overexpressed in multiple human cancers, including PCa. Amplified, but mislocalized SCRIB could function as an oncogenic factor. Therefore, the mechanism that regulates SCRIB membrane localization might be an important molecular switch, critical for PCa progression. We identified that ZDHHC7 is the major palmitoyl acyltransferase regulating SCRIB. Loss of ZDHHC7 decreases SCRIB palmitoylation and lead to its mislocalization, activation of the oncogenic YAP pathway, and cell invasion. The overall objective of this project is to define the roles of cell polarity regulator SCRIB in PCa cell progression, and how misregulation of SCRIB palmitoylation contributes to the disease. We hypothesized that: loss of cell polarity plays major roles in prostate cancer progression, and the signal transduction network involving ZDHHC7, SCRIB and the downstream YAP, MAPK or PI3K/AKT pathways promotes prostate cancer progression. ZDHHC7 functions as a potential tumor suppressor in PCa cells, and restricts the downstream oncogenic factors. Loss of ZDHHC7 in PCa promotes SCRIB mislocalization. We will elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers, determine the roles of the ZDHHC7-mediated SCRIB palmitoylation in prostate cancer progression using preclinical *in vitro* and *in vivo* models, and evaluate their expression in primary specimens, and identify the regulator(s) of SCRIB de-palmitoylation in prostate cancer cells, and to validate it as new therapeutic target for prostate cancer therapeutics.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Cell polarity, palmitoylation, Scribble, ZDHHC7, prostate cancer, metastasis, neuroendocrine prostate cancer (NEPC)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overall objective of this project is to define the roles of cell polarity regulator SCRIB in PCa cell progression, and how mis-regulation of SCRIB palmitoylation and loss of ZDHHC7 contributes to the progression of the disease. As shown below in the proposed SOW and our last annual report, we have elucidated the regulation of ZDHHC7 in Scribble palmitoylation, localization in prostate cancer cell lines. In the third year of funding, we have made new progresses to show that loss of ZDHHC7 in prostate epithelial cells and prostate cancer cells leads to loss of SCRIB palmitoylation, enhance YAP activation, and induce the expression of neuroendocrine prostate cancer (NEPC) markers. In addition, we found that the up-regulation of

NEPC markers are due to YAP activation. Inhibition of YAP-TEAD transcription complex could block the NEPC marker expression in prostate cancer cells. NEPCs are recently recognized as progression from AR-resistant prostate cancers with strong invasive and metastatic potentials. We will further investigate with loss of ZDHHC7 lead to NEPC conversion and prostate cancer progression. We also show a link between ZDHHC7 and androgen receptor (AR) that might convey an important role of ZDHHC7 in the majority of prostate cancers that are driven by AR. The revised SOW (highlighted in red) has been approved by the scientific officer last year.

Due to the COVID-19 pandemic crisis and our research facility shutdown, the progress of the project is significantly delayed. We have requested a no-cost extension (NCE) for this project until July 2021, and will continue to work on the project as proposed.

Aim 1. To elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers	**Timeline**	**Site 1**	**Site 2**	**completion**
Major Task 1: Evaluate the palmitoylation levels of SCRIB in prostate cancer cells	Months			
Subtask 1: evaluate the palmitoylation levels of SCRIB in different prostate cancer cell lines	1-3	Dr. Wu		100%
Subtask 2: evaluate the localization of SCRIB in different prostate cancer cell lines	1-6	Dr. Wu	Dr. Yu	100%
Major Task 2: Establish that loss of ZDHHC7 leads to loss of SCRIB palmitoylation and its mislocalization in prostate cancer cells.				
Subtask 1: evaluate the ZDHHC7 expression levels in prostate cancer cell lines by western blot	3-6		Dr. Yu	100%
Subtask 2: generate ZDHHC7-stably knockdown or knockout cell lines using shRNA or CRISPR/Cas9-mediated knockout methods	6-10	Dr. Wu	Dr. Yu	100%
Subtask 3: evaluate the SCRIB palmitoylation levels in ZDHHC-deleted PC cells	9-12	Dr. Wu		100%
Subtask 4: evaluate the SCRIB localization status in prostate cancer cell lines at different stages and in ZDHHC-deleted cell lines	9-12	Dr. Wu	Dr. Yu	100%
Major Task 3: Determine that SCRIB mislocalization leads to loss of cell polarity in prostate cancer cells				
Subtask 1: examine the expression of cell junction markers in prostate cancers	12-15		Dr. Yu	50%
Subtask 2: examine cell junction markers in ZDHHC7-stably knockdown or knockout PC cells	12-15	Dr. Wu		80%
Subtask 3: express SCRIB C4/10S mutant in prostate epithelial cell lines or benign cells and evaluate the cell polarity markers	15-18	Dr. Wu		50%
Aim2: To determine the roles of the ZDHHC7-mediated SCRIB palmitoylation in prostate cancer progression using preclinical in vitro and in vivo models, and evaluate their expression in primary specimens.				
Major Task 4: Evaluate the expression levels of				

ZDHHC7 and SCRIB in localized and metastasized prostate cancer specimens				
Subtask 1: perform IHC of SCRIB in PC samples	12-18		Dr. Yu	80%
Subtask 2: Perform IHC of ZDHHC7 in PC samples	12-18		Dr. Yu	80%
Major Task 5: Determine the tumor suppressor roles of ZDHHC7 in prostate cancer cell lines in vitro and in vivo				
Subtask1: will examine the effects of re-expression of ZDHHC7 in prostate cancer cell lines	18-24	Dr. Wu		March 2020
Subtask2: test the tumorigenesis potential of these cell lines in vivo in tumor initiation (sub-cu xenograft model) and metastasis models	20-26		Dr. Yu	20%
Subtask 3: generate stable knockdown or knockout cell lines of ZDHHC7 in benign cell line; as well as androgen dependent cell lines	18-24	Dr. Wu	Dr. Yu	May 2020
Subtask 4: evaluate cell growth, colony formation, cell invasion in ZDHHC7-deleted cells	24-28	Dr. Wu		80%
Subtask 5: test ZDHHC7-deleted cells in vivo	24-30		Dr. Yu	20%
Major Task 6: Determine the activation of downstream oncogenic pathways upon ZDHHC7 knockdown or expression of SCRIB palmitoylation deficient mutant, and the effects result in EMT, cell migration and metastasis in vitro and in vivo. (revised plan: we will also test androgen receptor (AR) pathway genes and NEPC markers)				
Subtask 1: generate prostate cancer cells (LNCAP, C4-2B, 22RV1 and PC3) with expression of SCRIB WT or C4/10S mutant	24-30	Dr. Wu	Dr. Yu	60%
Subtask 2: examine the downstream signaling activities of MAPK, AKT and YAP. Western blots of p-MEK, p-ERK, p-AKT, and p-YAP will be studied. (revised plan: will also test AR pathway genes and NEPC markers: Sox2, NMyc, BRN2 etc.)	30-33	Dr. Wu	Dr. Yu	80%
Subtask 3: YAP nuclear localization and transcriptional activities will be evaluated by co-focal imaging or qRT-PCR of downstream target genes (CTGF, Cyr61 etc.) (revised plan: will also test AR pathway genes and NEPC markers expression by qRT-PCR)	33-36	Dr. Wu	Dr. Yu	70%
Subtask 4: evaluate the effects of EMT, cell migration and metastasis in vitro and in vivo. (revised plan: will also evaluate AR pathway genes and NEPC conversion)	30-36	Dr. Wu	Dr. Yu	60%
Aim3: To validate inhibition of SCRIB depalmitoylation or inhibition of downstream pathways (YAP, MEK, AR etc.) as potential new therapeutic opportunities in NEPC				

Major Task 7: Recently published literature have shown APT2 is regulating SCRIB depalmitoylation. This task will be re-focused on validating APT2 in NEPC conversion.	Months			
Subtask 1: Will validate whether overexpression of APT2 promotes NEPC conversion) (Preliminary data showed AR as a substrate of ZDHHC7, which will be tested here).	24-28	Dr. Wu	Dr. Yu	50%
Subtask 2: revised plan: will test how ZDHHC7 interacts with AR protein.	28-30	Dr. Wu	Dr. Yu	50%
Subtask 3: revised plan: will test how ZDHHC7 regulates AR protein stability.	30-33	Dr. Wu	Dr. Yu	50%
Subtask 4: Will test whether knockdown of APT2 could inhibit SCRIB downstream signaling)	30-33	Dr. Wu		10%
Major Task 8: revised plan: will test AR as a substrate of ZDHHC7 and the effects on AR signaling; will test ZDHHC7-SCRIB-YAP-Sox2 axis contribute to NEPC conversion	Months			
Subtask 1: **revised plan: will test the expression of AR, ZDHHC7, and PSA in PCa cell lines**	28-32		Dr. Yu	40%
Subtask 2: **revised plan: will also test the association of ZDHHC7 with PSA recurrence in PCa**	30-32		Dr. Yu	80%
Subtask 3: **revised plan: will test the expression of AR in correlation with ZDHHC7 in PCa by IHC.**	30-33		Dr. Yu	80%
Subtask 4: generate lentiviral shRNA constructs for stable knockdown or tet-inducible shRNA constructs for inducible knock-down YAP/TAZ, APT2 etc.	28-32	Dr. Wu		30%
Subtask 5: evaluate whether it can inhibit EMT, cancer cell proliferation, cell migration and induces apoptosis in these cells. Synthesize tool inhibitors and test compound in vitro, **Will also test the roles of ZDHHC7 regulation of AR in PCa; will also evaluate NEPC conversion)**	32-36	Dr. Wu	Dr. Yu	20%
Subtask 6: carry out in vivo experiments to validate that knock down or knockout of the depalmitoylating enzyme could inhibit prostate cancer cell growth in vivo. **Will test the roles of ZDHHC7 regulation of AR in PCa. Will test whether inhibition of APT2 or YAP pathways could block NEPC tumor growth)**	32-36	Dr. Wu	Dr. Yu	10%

What was accomplished under these goals?

As shown in the above SOW table, our work has focused on Aims 2 and 3 in the last funding period. We have developed ZDHHC7 knockout methods using CRISPR/Cas9 system and knockdown using shRNA systems. We have generated stably ZDHHC7 KO cells using PrEC, DU145 and BPH cells. We found that loss of ZDHHC7 leads to SCRIB depalmitoylation in prostate cancer cell lines. More importantly, we identified that ZDHHC7 loss leads to conversion

to neuroendocrine prostate cancer cells (NEPC), which was a novel and unexpected finding. NEPCs are developed from castration resistant prostate cancers with high metastasis and drug resistance. Our finding is highly important, showing that loss of ZDHHC7 might contribute to NEPC development, which is also consistent with our initial hypothesis. We have also demonstrated that YAP activation is driving the NEPC marker expression. ZDHHC7 loss promotes "stemness" markers of NEPCs, Sox2 and EZH2 expression, and promotes cell growth and migration. More importantly, we found that an inhibitor (MGH-CP1), which blocks TEAD-YAP interaction, is able to block NEPC conversion.

In addition, distinct from the above findings of ZDHHC7 in AR-negative NEPC cells, we found that AR is a novel substrate of ZDHHC7 in the AR-positive prostate cancer cells. ZDHHC7-mediated palmitoylation of AR reduces its protein stability and results in AR protein degradation. ZDHHC7 thus exhibit tumor suppressive roles in AR-positive prostate cancer.

Aim 1. To elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers.
Major Task 1: Completed in prior years.
Major Task 2: Completed in prior years.
Major Task 3: Completed at 50-80% in prior years. No progress in the past funding period.

Aim 2: To determine the roles of the ZDHHC7-mediated SCRIB palmitoylation in prostate cancer progression using preclinical in vitro and in vivo models, and evaluate their expression in primary specimens.

Major Task 4: Evaluate the expression levels of ZDHHC7 and SCRIB in localized and metastasized prostate cancer specimens

Methods: We analyzed mRNA levels of SCRIB and ZDHHC7 in different grade PCa including benign, ADPC, CRPC and NEPC using publically available PCa dataset and evaluated association between ZDHHC7 expression and patient survival. Meanwhile we optimized the working conditions of ZDHHC7 and SCRIB antibody for immunofluorescence (IF) in PCa cell line LNCaP and immunochemistry (IHC) in future.

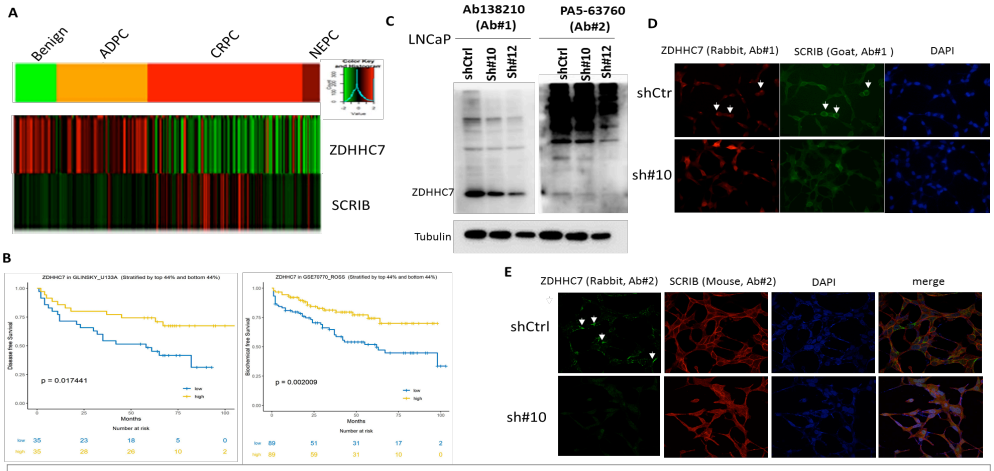

Figure 1. The expression of ZDHHC7 is decreased during PCa progression. (A) Bioinformatic analysis showing that ZDHHC7 and SCRIB expression in bening, ADPC, CRPC, and NEPC. (**B**) Patients has high ZDHHC7 expression has good outcome. (**C**) Test ZDHCC7 Ab#1 and Ab#2 for WB in LNCaP cells with ZDHHC7 knockdown. (**D-E**) ZDHHC7 and SCRIB IF in LNCaP cells with ZDHHC7 Knockdown.

Results: As shown in **Figure 1A**, we found that ZDHHC7 mRNA expression levels are significantly decreased as PCa progresses from ADPC to CRPC and NEPC, while the mRNA levels of SCRIB was the highest in CRPC. Interestingly, low level of ZDHHC7 is associated with poor survival (**B**). We then optimized

ZDHHC7 and SCRIB antibody for IF and IHC in future by using antibody from different vendors with ZDHHC7 knockdown in LNCaP cells. As shown in **Figure 1C**, both ZDHHC7 antibodies work for WB, but Ab#1 of ZDHHC7 shows nonspecific staining in IF (**D**). Ab#2 of ZDDHC7 works well for IF, being consistent with misallocated SCRIB after ZDHHC7 knockdown (**E**).

Conclusion: Our results showed that the expression of ZDHHC7 was decreased during PCa progression and patients has low ZDHHC7 has worse outcomes. We are investigating the availabilities of tissue microarrays in order to examine the proteins levels of ZDHHC7 and its downstream targets in PCa.

Major Task 5: Determine the tumor suppressor roles of ZDHHC7 in prostate cancer cell lines in vitro and in vivo

Subtask1: will examine the effects of re-expression of ZDHHC7 in prostate cancer cell lines

Subtask 3: generate stable knockdown or knockout cell lines of ZDHHC7 in benign cell line as well as androgen-dependent cell line.

Subtask 4: evaluate cell growth, colony formation, cell invasion in ZDHHC7-deleted cells

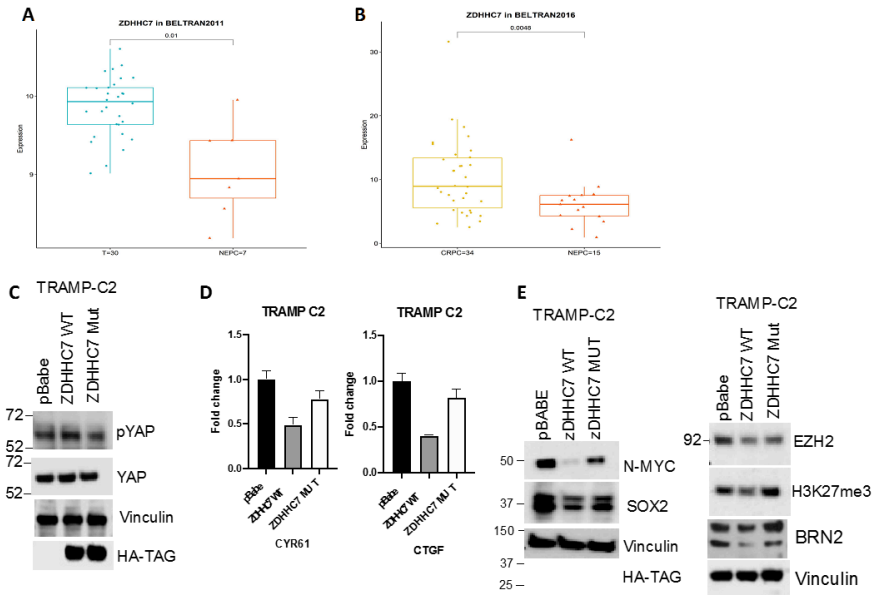

Figure 2. ZDHHC7 is a tumor suppressor of neuroendocrine prostate cancer. (A-B) Bioinformatic analysis has shown that ZDHHC7 expression levels are significantly lower in NEPC samples, compared to other prostate cancer samples in two datasets. (**C-D**) Expression of ZDHHC7 wild type, but not the catalytically inactive mutant could suppress YAP and the expression of YAP target genes (CTGF and Cyr61). **(E)** ZDHHC7 expression suppressed NEPC markers (N-myc, Sox2 and EZH2) expression in TRAMP-C2 cells.

Methods: We first analyzed the RNA expression profiles of ZDHHC7 in androgen dependent and independent prostate cancer samples and in NEPC cells from published datasets. We have generated lentiviral vectors carrying wild type ZDHHC7 or the catalytically inactive mutant (C160S). As reported in the last progress report, we evaluated the SCRIB palmitoylation levels in these cells. In the last funding period, we have examined the NEPC markers in NEPC TRAMP-C2 cells. In addition, we have generated shRNA-stably KD of ZDHHC7 in prostate cancer cell lines, including DU145, and studied its growth and migration

Results: As shown in **Figure 2A** and **B**, we found that ZDHHC7 expression levels are significantly decreased in NEPC samples. We then re-express of ZDHHC7 WT, and the C160S in to DU154 and TRAMP-C2cells. We found that ZDHHC7 WT, but not the C160S mutant could suppress YAP activation (Fig. 1C), expression of YAP target genes (Fig. 1D) and the expression of NEPC markers, including Sox2, N-myc and EZH2 etc. (**Fig. 2E**).

Conclusion: Our results showed that ZDHHC7 is a potential tumor suppressor of NEPC conversion. Loss of ZDHHC7 might facilitate NEPC conversion.

Subtask2: test the tumorigenesis potential of these cell lines in vivo in tumor initiation and metastasis models.
Subtask 5: test ZDHHC7-deleted cells in vivo
Methods: PC-3M cells were inoculated through intraprostatic implantation or tail vein to examine its dissemination in the mouse body and establishment of metastasis.
Results: We have found that inoculation of PC-3M cells through intraprostatic implantation, which involves abdominal surgery, is time-consuming and the metastases were largely limited to the abdomen. Tail vein injection is much more practical and we expect it to yield more metastasis to distant organs. This experiment is still ongoing.
Conclusion: we are establishing a second model (tail vein injection) to study metastasis.

Major Task 6: Determine the activation of downstream oncogenic pathways upon ZDHHC7 knockdown or expression of SCRIB palmitoylation deficient mutant, and the effects result in EMT, cell migration and metastasis in vitro and in vivo. (revised plan: we will also test androgen receptor (AR) pathway genes and NEPC markers)

Subtask 1: generate prostate cancer cells (LNCAP, C4-2B, 22RV1 and PC3) with expression of SCRIB WT or C4/10S mutant
Subtask 2: examine the downstream signaling activities of MAPK, AKT and YAP. Western blots of p-MEK, p-ERK, p-AKT, and p-YAP will be studies (revised plan: will also test AR pathway genes and NEPC markers: Sox2, NMyc, BRN2 etc.)
Subtask 3: YAP nuclear localization and transcriptional activities will be evaluated by co-focal imaging or qRT-PCR of downstream target genes (CTGF, Cyr61 etc.) (revised plan: will also test AR pathway genes and NEPC markers expression by qRT-PCR)

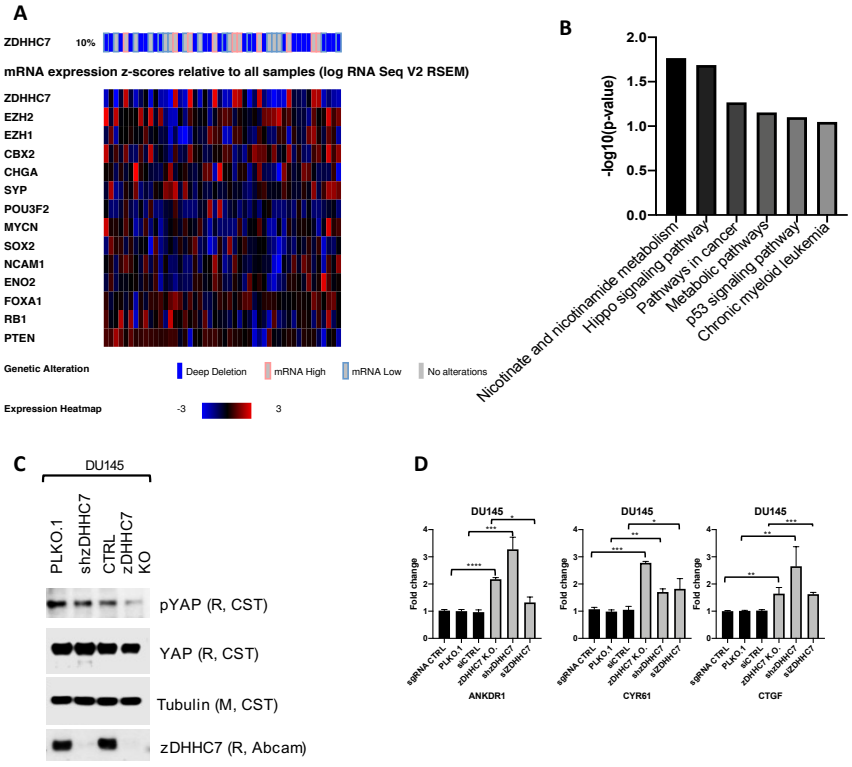

Figure 3. Stable knockdown of ZDHHC7 promotes YAP activation. (A) RNA-seq analysis of DU145 cells with shRNA knockdown of ZDHHC7. Heatmap of RNA expression of prostate cancer related genes. **(B)** Pathway analysis of the RNA-seq data showed that Hippo pathway is among the top altered pathways. **(C)** Western blot analysis of KD or KO cells showed that p-YAP level is inhibited, suggesting that YAP is activated in ZDHHC7 KD cells. **(D)** YAP target genes (ANKDR1, Cyr61 and CTGF) expression is induced in ZDHHC7-KD cells.

Methods: We have generated lentiviral vectors carrying shRNA targeting ZDHHC7, and have generated stable KD of ZDHHC7 in LNCaP, C4-2B, PC3, and DU145. We analyzed the gene expression profiles of DU145 (ZDHH7 KD) cells using RNA-seq, and followed up by q-RT-PCR. In addition, we carried out western blot analysis of various YAP target genes and NEPC marker. Further, we analyzed AR signaling pathway of AR positive cell line LNCaP and C4-2B cells with ZDHCC7 knockdown or ZDHHC7 and C160S mutant overexpression by WB and RT-PCR.

Results: As shown in Figure 3A, we have carried out RNA-seq experiments using DU145 cells with stable ZDHHC7 knockdown. We have analyzed the gene expression profile of various prostate cancer related genes. In addition, Hippo pathway is one of the top pathways altered in the KD cells (**Fig. 3B**). We have also analyzed p-YAP levels and YAP target genes and confirmed that ZDHHC7 KD activates YAP (**Fig. 3C-D**).

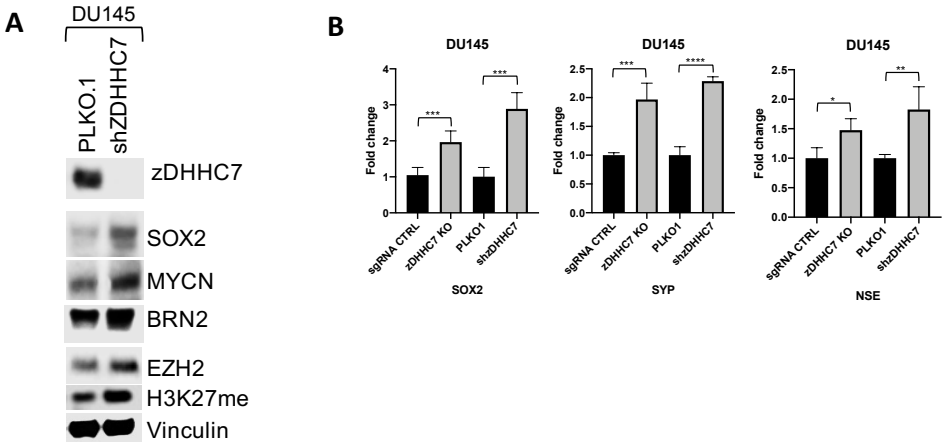

Figure 4. (A) Knockdown of ZDHHC7 promotes the expression of NEPC mediators (Sox2. N-myc, BRN2, EZH2) in DU145 cells by western blot. **(B)** NEPC markers expression are elevated in ZDHHC7 KD cells by RT-PCR.

We further analyzed the NEPC markers using western blot and RT-PCR in the AR-negative DU145 cells with ZDHHC7 knockdown (**Fig. 4A-B**). We found that recently reported NEPC mediators, including Sox2, N-Myc, EZH2 and EZH2-catalyzed H3K27me3 are markedly increased in ZDHHC7 KD cells. In addition, qRT-PCR analysis confirmed up-regulated expression of SOX2 and NEPC markers SYP and NSE in DU145 cells with ZDHHC7 knockdown.

In addition to AR negative PCa cells, we also performed ZDHHC7 WT or C160S mutant overexpression in the AR-positive LNCaP and C4-2B cells, representing androgen-dependent PC (ADPC) and castration-resistant prostate cancer (CRPC), respectively (**Fig.5**). We found ZDHHC7 WT overexpression decreased AR target genes such as PSA, TMPRSS2, and KLK2 expression in both cell lines. Importantly, C160S mutation of ZDHHC7 was not able to inhibit AR and AR target gene transcription in

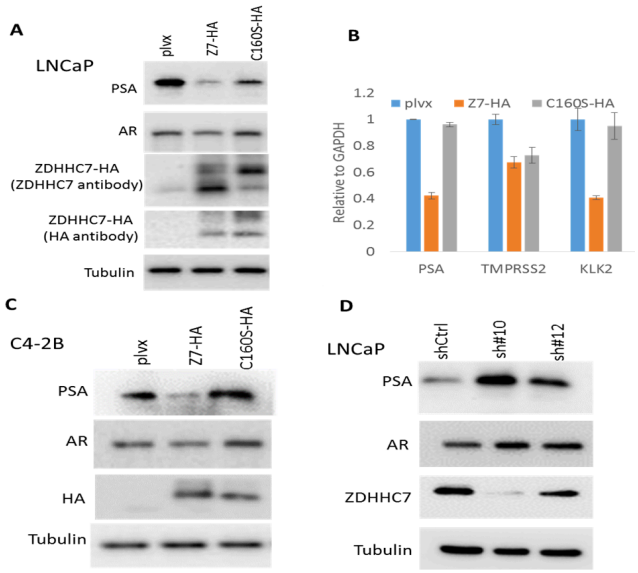

Figure 5. ZDDHC7 regulates AR signaling in AR positive PCa cells. (A-B) Overexpression of ZDHHC7 WT decreases PSA protein level (**A**) and transcription of PSA, TMPRSS2 and KLK2 (**B**). **(C)** Overexpression of ZDHHC7 WT decreases PSA protein level in C4-2B cells. (**D**) Knockdown of ZDHHC7 increases PSA protein level in LNCaP cells.

both cell lines (**Fig. 5A-C**). This data strongly suggest that ZDHHC7 decreases AR expression through palmitoylation-dependent mechanisms. Meanwhile, we also carried out ZDHHC7 knockdown using shRNAs in LNCaP cells. Accordingly, ZDHHC7 knockdown slightly increased AR and drastically increased AR target gene PSA expression (**Fig. 5D**).

Conclusion: Our results showed that loss of ZDHHC7 promotes YAP activation and neuroendocrine prostate cancer conversion in DU145 cells (AR-negative cells). In addition, ZDHHC7 reduces AR level and inhibits AR signaling in AR-positive PCa cells.

Aim3: To validate inhibition of SCRIB depalmitoylation or inhibition of downstream pathways (YAP, MEK, AR etc.) as potential new therapeutic opportunities in NEPC

Major Task 7: Recently published literature have shown APT2 is regulating SCRIB depalmitoylation. This task will be re-focused on validating APT2 in NEPC conversion.
Subtask 1: Will validate whether overexpression of APT2 promotes NEPC conversion) (Preliminary data showed AR as a substrate of ZDHHC7, which will be tested here).
Subtask 2: revised plan: will test how ZDHHC7 interacts with AR protein.
Subtask 3: revised plan: will test how ZDHHC7 regulates AR protein stability.

Methods: We performed co-immunoprecipitation (Co-IP) to investigate interaction between ZDHHC7 and AR in LNCaP PCa cell line. Further, we carried out Click-it assay to test whether AR is substrate of ZDHHC7 in 293T cells with AR and ZDHHC7 WT and C160S overexpression. Meanwhile, we used ubiquitination assay and M132 pre-treatment assay to test AR protein degradation mediated by ZDHHC7-induced palmitoylation in LNCaP cells with ZDHHC7 WT and C160S overexpression.

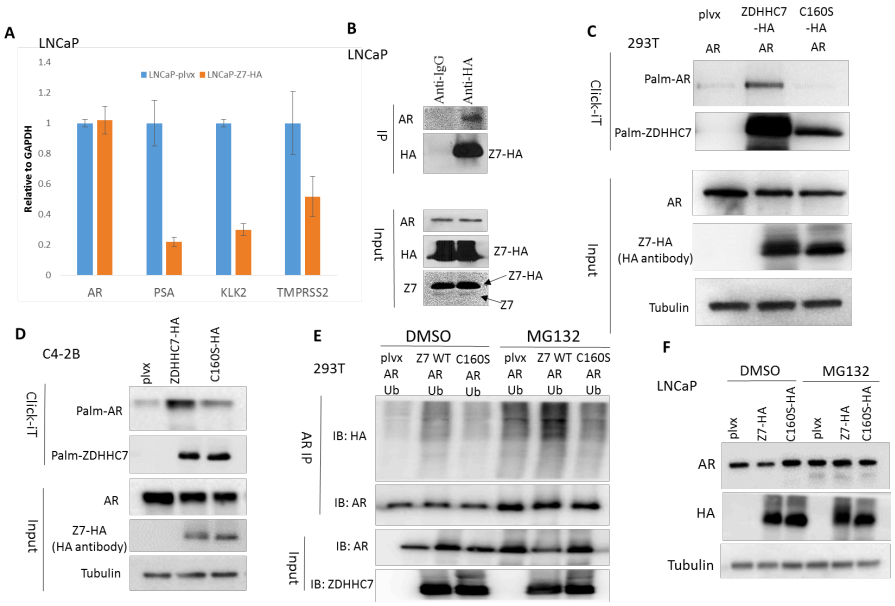

Figure 6. The palmitoylation of AR mediated by ZDHHC7 promotes AR degradation in PCa cells. (A) ZDHHC7 overexpression does not affect AR transcription in LNCaP cells. (**B**) Co-IP western blot shows ZDHHC7 interacts with AR in LNCaP cells. **(C-D)** Click-it assay shows AR is palmitoylated by ZDHHC7 WT but not C160S in both 293T cells and PCa cell line C4-2B. (**E**) ZDHHC7 WT but not C160S overexpression increases AR ubiquitination in 293T cells. (**F**) MG132 pre-treatment is able to block ZDHHC7 WT induced AR degradation in LNCaP cells.

Results: As ZDHHC7 WT, but not its palmitoylation-disabling mutant C160S, decreased AR protein level, we wondered whether ZDHCC7 regulates AR at transcription or post-transcription level. As shown in **Figure 6A**, ZDHHC7 overexpression does not affect AR transcription, suggesting that ZDHHC7 might regulate AR protein through post-

transcriptional mechanisms. To test this, we first confirmed that ZDHHC7 interacts with AR in PCa cells (**Fig. 6B**). Secondly, we demonstrated that AR was palmitoylated by ZDHHC7 WT, but not C160S, in both 293T cells and PCa cell line C4-2B using Click-IT assay (**Fig. 6C-D)**. As it has been shown protein stability could be regulated by palmitoylation, we hypothesized that palmitoylated AR is not stable. As shown in **Figure 6E**, the ubiquitination of AR was dramatically increased with ZDHHC7 WT, but not C160S, co-expression in 293T cells. Importantly, ZDHHC7-mediated AR degradation was blocked by treatment of proteasome inhibitor MG132 (**Fig. 6F**). Taken together, these data suggest that ZDHHC7 mediates AR degradation through palmitoylation and proteasome degradation pathway.

Conclusion: Our results showed that AR is a new substrate of ZDHHC7 and AR palmitoylation by ZDHHC7 promote AR degradation.

Major Task 8: revised plan: will test AR as a substrate of ZDHHC7 and the effects on AR signaling; will test ZDHHC7-SCRIB-YAP-Sox2 axis contribute to NEPC conversion.
Subtask 2: ***revised plan: will also test the association of ZDHHC7 with PSA recurrence in PCa***

Method: we examined ZDHHC7 expression in human prostate cancer profiling dataset and associated its expression with clinical outcome.
Results: we found that high ZDHHC7 expression is protective in that patients with high-ZDHHC7 have better PSA recurrence free survival. This is consistent with the observation that ZDHHC7 decreases AR protein and thus PSA level.
Conclusion: our data show that in AR-positive prostate cancer, ZDHHC7 is a natural inhibitor of AR, exhibiting tumor suppressive roles.

What opportunities for training and professional development have the project provided?

Postdoctoral fellows and graduate students at the Cutaneous Biology Research Center (CBRC) receive in depth training within their PI's laboratory. They gain first hand experience in state of the art experimental approaches. As an important part of training, fellows are continuously challenged and prompted to prepare early written drafts of ongoing work, in which initial results are already framed within clearly formulated working hypotheses. Research goals are achieved through direct personal discussions with their PI supervisors, as well as other colleagues in the laboratory and more formal weekly lab meetings. There are a number of seminars and tutorials, held at various locations throughout the institution and Boston area that faculty, staff, pre and postdoctoral trainees, and graduate students are encouraged to attend. Each Tuesday at CBRC, a tutorial is presented by a staff member (or pre or postdoctoral fellow or graduate student) on work in progress or review of fields that are being considered for expansion. The CBRC Seminar Series is presented monthly. World-renowned scientists from a variety of specialties are invited to spend the day meeting with faculty, having a private lunch with fellows and students and giving a seminar that is attended by staff throughout the institution.

Within our research groups, the PI provides continuous supervision, advice, and guidance related to ongoing research projects in the laboratory as well as career counseling and personal development.

This project has provided training and professional development of the postdoctoral fellow in Wu lab, Carla Guarino. Dr. Guarino gained significantly progresses in their molecular and cell biology, and chemical biology. Through interactions with our collaborator Jindan Yu's lab, she has also learned techniques to culture and test prostate cancer cell lines. We focused on improving her understanding to cell polarity and prostate cancer progression, and improving their technical skills of various experiments. I believe she is becoming more independent in terms of experimental design and interpretation. Postdoctoral fellow Dr. Song Tan have learned various techniques in molecular and cellular biology and genetics. Through interaction with our collaborator Dr. Xu Wu's laboratory in MGH/Harvard, he has also acquired substantial knowledge about chemistry, in particular palmitoylation, and have learned some relevant skills/assays such as Click-IT.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

As we planned in the original and revised SOW, we will continue to evaluate the role of ZDHHC7 and SCRIB in prostate cancer cell progression. We will carry out in-depth analysis of RNA-seq results of DU145 with ZDHHC7 KD. We are also obtaining RNA-seq data from TRAMP-C2 cells with ZDHHC7 overexpression, and will correlate the changes. We will evaluate the mechanism that link YAP activation to NEPC conversion. In terms of ZDHHC7 regulation of AR stability, we will identity which site in AR is palmitoylated and which E3 ligase involved in AR degradation which mediated by ZDHHC7. Further we will perform ZDHHC7 and SCRIB IHC in different stage PCa patients samples. We also will investigate the role of ZDHHC7 in PCa progression using pre-clinical mouse xenograft model.

In addition, in preliminary studies, we found that YAP-TEAD inhibitor could block NEPC conversion in vitro. We will further evaluate the effects of these inhibitors in NEPC cell growth, metastasis etc. during the NCE period of the project.

We plan to examine the functional consequence of ZDHHC7 regulation of AR signaling in prostate cancer. We will perform in vitro and in vivo functional assays to delineate the roles of ZDHHC7 in AR-positive and AR-negative PCa growth and metastasis. We also plan to examine human prostate cancer tissues to demonstrate the clinical relevance of these pathways. Lastly, we plan to submit two manuscripts: ZDHHC7 regulation 1) of YAP in NEPC and 2) of AR in AR+ prostate cancer.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

We have demonstrated that a chemical approach using palmitoylation reporters to detect Scribble palmitoylation in prostate cancer cell lines, and correlated with its mislocalization. In addition,

we found that loss of ZDHHC7 promotes CRPC and NEPC conversion, which could have significant impact in drug discovery and cancer research.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

No major changes since previous SOW

Changes that had a significant impact on expenditures

No change on the expenditure.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

No change.

Significant changes in use or care of human subjects

No change.

Significant changes in use or care of vertebrate animals

No change.

Significant changes in use of biohazards and/or select agents
No change.

6. PRODUCTS:

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Xu Wu
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1624-0143
Nearest person month worked:	3
Contribution to project:	Dr. Wu has supervised the research, designed the experiments and interpreted the results
Funding support:	MGH Institutional fund Melanoma Research Alliance NCI NIDDK Astellas Innovation fund

Name:	Carla Guarino
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	12
Contribution to project:	Dr. Guarino has carried out the studies of Scribble in prostate cancers. She has developed the biochemical methods to detect Scribble palmitoylation in prostate cancers.
Funding support:	None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No.

What other organizations were involved as partners?
Dr. Yu at Northwestern University is a partnering PI of this award.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Dr. Yu at Northwestern University will submit a separate report.

9. APPENDICES:

None.