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TITLE: Loss of ZDHHC-Mediated Scribble Palmitoylation Disrupts Cell Polarity and Promotes Prostate Cancer Progression

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CONTRACTING ORGANIZATION: Massachusetts General Hospital , Boston, MA

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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.

15. SUBJECT TERMS
None listed.

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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, one of which is the androgen receptor (AR), the key driver of prostate cancer. Loss of ZDHHC7 in PCa promotes SCRIB mislocalization. We will elucidate the mechanisms of ZDHHC7-mediated SCRIB and AR palmitoylation in regulating SCRIB mislocalization and cell polarity and AR protein stability, respectively, in prostate cancers. We will determine the roles of the ZDHHC7-mediated SCRIB and AR 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), androgen receptor, protein degradation

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 aberrant AR signaling and the progression of the disease. As shown below in the proposed SOW and our last annual report, we have elucidated how ZDHHC7 regulates Scribble and AR palmitoylation in prostate cancer cells. We further showed that loss of ZDHHC7 reduces SCRIB palmitoylation and thus activates YAP signaling which induces the expression of NEPC

markers. On the other hand, ZDHHC7-mediated AR palmitoylation results in AR protein degradation and that loss of ZDHHC7 increases AR signaling. In the last year of funding, we have made new progresses to examine ZDHHC7 protein levels in human PCa specimens and demonstrate that AR palmitoylation by ZDHHC7 leads to reduced AR protein stability, without affecting its cellular localization. The revised SOW (highlighted in red) has been approved by the scientific officer in 2019.

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	100%
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	100%
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	100%
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	Months			

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).	24-28	Dr. Wu	Dr. Yu	80%
Subtask 2: revised plan: will test how ZDHHC7 interacts with AR protein.	28-30	Dr. Wu	Dr. Yu	90%
Subtask 3: revised plan: will test how ZDHHC7 regulates AR protein stability.	30-33	Dr. Wu	Dr. Yu	80%
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	60%
Subtask 2: **revised plan: will also test the association of ZDHHC7 with PSA recurrence in PCa**	30-32		Dr. Yu	90%
Subtask 3: **revised plan: will test the expression of AR in correlation with ZDHHC7 in PCa by IHC.**	30-33		Dr. Yu	90%
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 revised Aims 2 and 3 (revised SOW approved in 2019) 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, following our discovery of AR as a novel substrate of ZDHHC7 in the AR-positive prostate cancer cell lines, in the last funding period, we focused on investigating the consequences of AR palmitoylation. In contrast to some reports of AR palmitoylation in the literature, we found that it does NOT affect AR cellular localization. On the other hand, we found that AR palmitoylation broadly reduces AR protein levels in cytoplasm, nuclei, as well as cell membrane. Mechanistically, this is due to AR palmitoylation catalyzed by ZDHHC7 decreased its protein half-life and stability, likely through proteasome pathways.

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 first examined public database for ZDHHC7 mRNA expression at different stages of PCa. Using optimized working conditions, we performed immunochemistry (IHC) analyses of ZDHHC7 using tissue microarrays (TMA) containing tissue sections from prostate tumors at various stages of progression.

Results: Analyses of multiple PCa dataset revealed that ZDHHC7 is down-regulated in primary PCa as compared to benign adjacent or normal prostate tissues (**Figure 1**). ZDHHC7 tends to get further down-regulated in CRPC, which however is not as consistent across datasets.

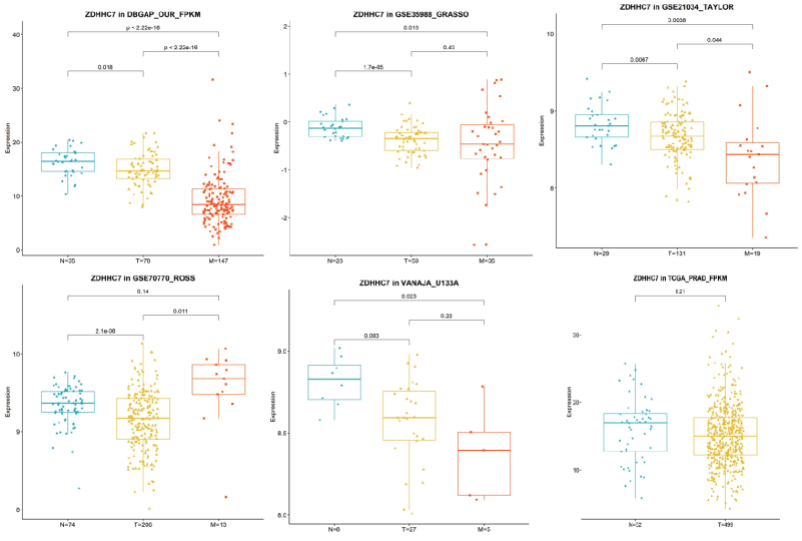

Figure 1. The expression of ZDHHC7 mRNA is decreased in PCa as compared to normal prostate tissues in multiple publically available gene expression profiling datasets.

Moreover, IHC analyses of ZDHHC7 protein using multiple TMA revealed mostly membrane and some cytoplasmic staining of ZDHHC7. The level of ZDHHC7 staining appears down-regulated in PCa as compared to benign adjacent prostate tissues, although the difference is not statistically significant (**Figure 2**). Surprisingly, in contrast to its mRNA expression, ZDHHC7 protein level seems to increase from primary PCa to CRPC and ZDHHC7 is ultimately lost as the disease progresses from CRPC to NEPC (AR-/NE+). Conclusion: ZDHHC7 is expressed in normal prostate. It is decreased in PCa as compared to benign tissues. Further investigation of additional TMA samples is required to determine whether the changes are significant.

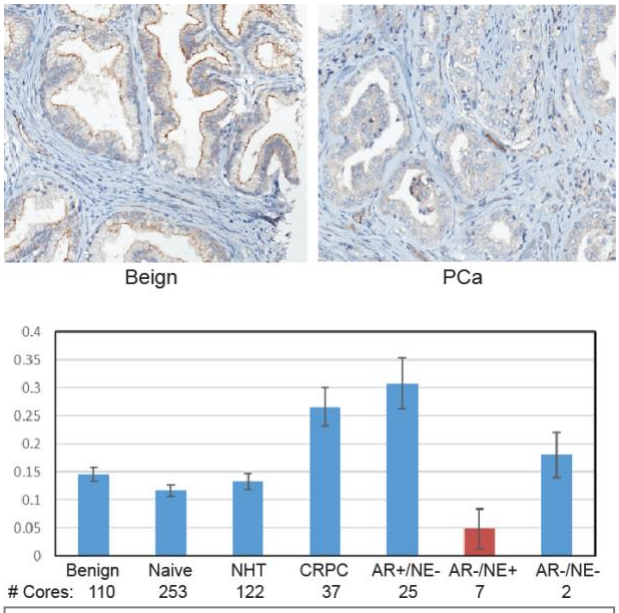

Figure 2. IHC staining of ZDHHC7 in TMA of PCa tissues. Top: representative staining in benign and cancerous prostate tissues. Bottom: IHC scores overall diverse PCa.

Major Task 6: Determine the activation of downstream oncogenic pathways upon ZDHHC7 knockdown.

Subtask 2: Examine the downstream signaling activities of YAP and AR .

Subtask 3: YAP and AR nuclear localization and transcriptional activities.

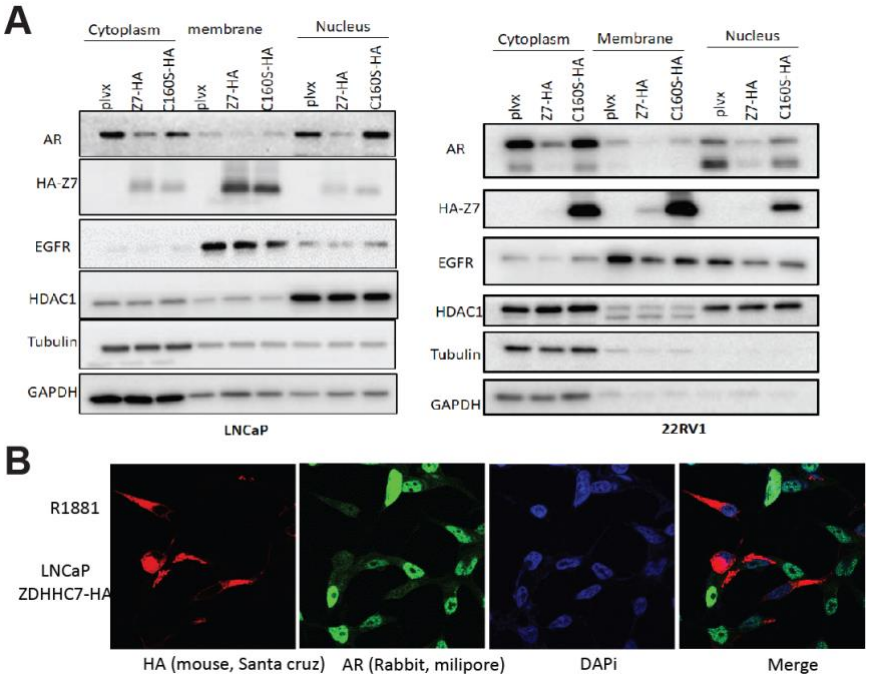

Figure 3. ZDHHC7 does NOT regulate AR protein translocation. LNCaP (left) and 22Rv1 (right) cell lysates were separated into fractions of cytoplasm, membrane, and nucleus and then analyzed by WB. EGFR, HDAC1, and Tubulin/GAPDH are respectively markers for membrane, nuclear, and cytoplasmic proteins.

Methods: Cell lysates were isolated from PCa cells and subjected to fractionation to separate membrane, nuclear, and cytoplasmic fractions, which were subsequently subjected to WB analyses.

Results: We have shown that AR is a substrate of ZDHHC7-mediated palmitoylation. It has been previously reported in the literature that palmitoylated AR is translocated from nuclei to the membrane. To examine if this is true for ZDHHC7-mediated AR palmitoylation in PCa cells, we performed WB analyses of cytoplasmic, membrane, and nuclear fractions of LNCaP and 22Rv1 cells with overexpression of ZDHHC7 and its enzymatic dead mutant C160S.

Surprisingly, we did NOT observe any increase of AR in the membrane fractions. Rather, we noticed greatly reduced AR in all cellular compartments in the cells with overexpression of ZDHHC7, but not C160S (**Figure 3A**). Interestingly, ZDHHC7 was also found to decrease AR variant that are expressed in the 22Rv1 cells in a manner similar to the full length AR protein. In addition, immunofluorescent staining confirmed drastically reduced, undetectable amount of AR in ZDHHC7-infected and –expressing cells, while ZDHHC7-negative cells stained very strongly for nuclear AR **(Figure 3B**).

We analyzed YAP localization and YAP-target gene expression in DU145 cell line with stable ZDHHC7 knockdown (KD). Consistent with our hypothesis, we found that YAP activation signatures are significantly upregulated, which also overlap with known NEPC markers by RNA-seq (Fig. 4A). We have confirmed that *HMMR*, *TOP2A*, *CENPE* and *FSCN1* expression levels are indeed increased by qRT-PCR analysis (Fig. 4B). Gene set enrichment analysis (GSEA) also confirmed that stable KD of ZDHHC7 leads to YAP/TAZ-TEAD direct target gene activation (Fig. 4C). Knocking down or knocking out (KO) ZDHHC7 in DU145 cells leads to downregulation of p-YAP, activation of p-AKT, which is consistent with the function of SCRIB mislocalization-induced downstream signaling (Fig. 4D-E). Finally, we analyzed the localization of YAP and SCRIB in the ZDHHC7 KD cells, and confirmed that loss of ZDHHC7 leads to SCRIB cytoplasmic localization and YAP nuclear localization (Fig. 4F).

Conclusion: Our data suggest that ZDHHC7-mediated AR palmitoylation reduced AR protein level without affecting its subcellular localization. The discrepancy of our findings with that of published literature will be further investigated.

We confirmed that in late stage of CRPC cell line (DU145), loss of ZDHHC7 leads to SCRIB mislocalization, and activation of YAP.

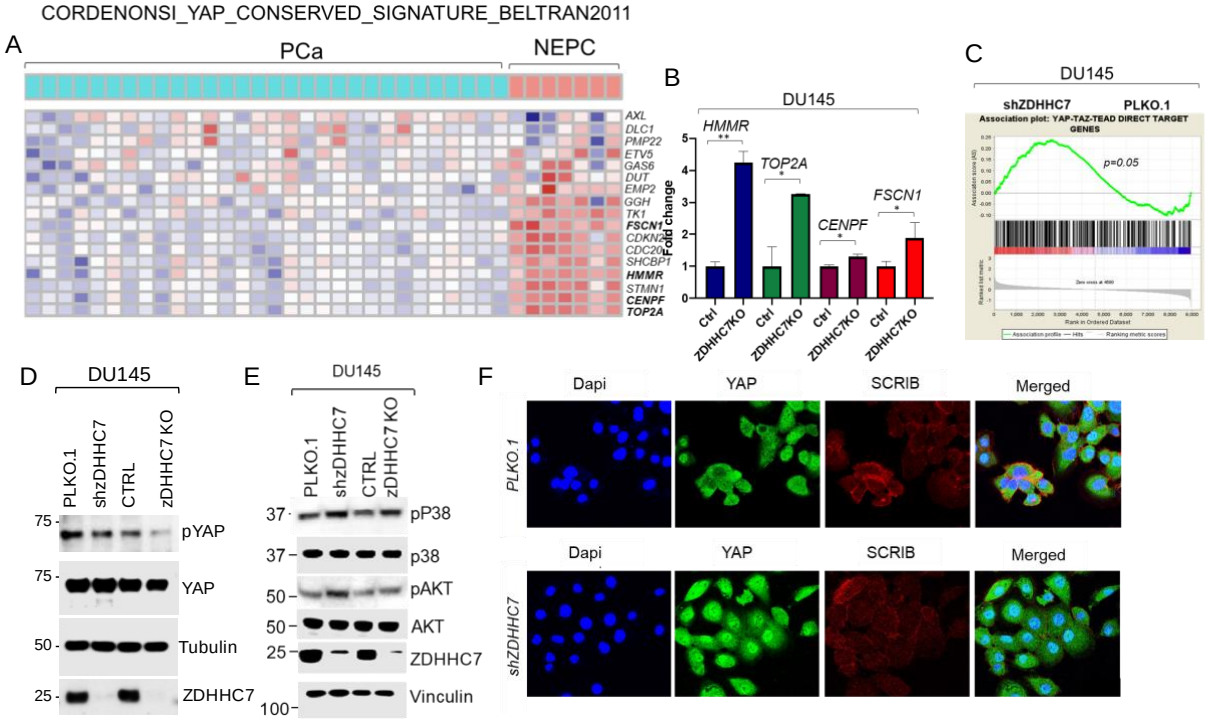

Figure 4. Loss of ZDHHC7 in DU145 leads to activation of YAP. (A) RNA-seq analysis reveals NEPC markers are among the upregulated YAP target genes. (B). Knockout of ZDHHC7 leads to upregulation of NEPC genes (HMMR, TOP2A, CENPF and FSCN1) by q-RT-PCR. (C) Gene set enrichment analysis shows that loss of ZDHHC7 leads to upregulation of YAP/TAZ-TEAD direct target genes. (D-F) Loss of ZDHHC7 leads to downregulation of p-YAP and induction of p-AKT. (F) Immunofluorescent staining showed mislocalization of SCRIB and enchanced YAP nuclear location upon ZDHHC7 knock down.

Aim 3: 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 2: will test how ZDHHC7 interacts with the AR protein.

Methods: Various domain-deletion mutants of AR were cloned to contain an HA-tag. They were co-transfected with Flag-tagged ZDHHC7 into 293T cells. Cell lysate was subjected to IP using anti-HA antibody followed by WB by ZDHHC7 antibody. In a reciprocal co-IP, 293T cells expressing WT or AR-V7 were subjected to IP by Flag antibody followed by WB detection using anti-AR or anti-ZDHHC7 (Z7).

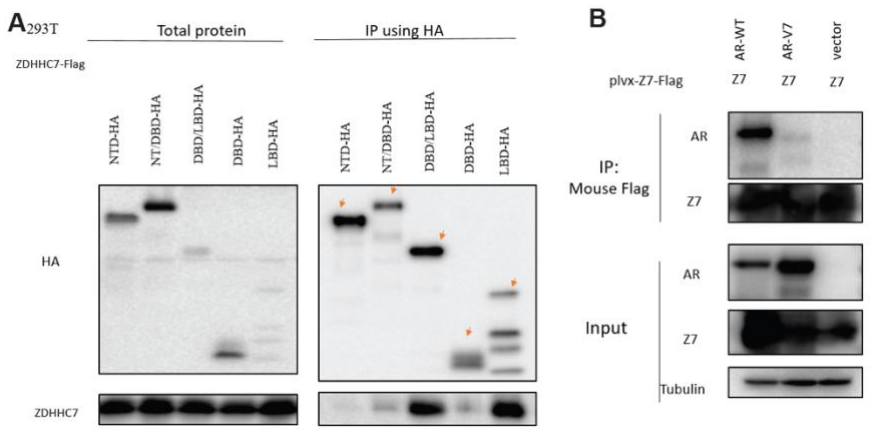

Figure 5. ZDHHC7 interacts with the LBD domain of AR protein. **(A)** 293T cells were co-transfected with HA-AR deletion mutants and ZDHHC7. The lysates were analyzed by HA IP followed by WB. (**B**). 293T cells were co-transfected with HA-AR-WT or V7 and Flag-ZDHHC7 and subjected to co-IP.

Results: To determine which domain of the AR protein interacts with ZDHHC7, co-IP was performed and showed that AR deletion mutants containing the LBD are able to interact with ZDHHC7 (**Figure 5A**). In good agreement with this, PCa-relevant AR-V7 variant that lacks the LBD was incapable of interacting with ZDHHC7 (**Figure 5B**).

Conclusion: We found that the LBD of AR is required for its interaction with ZDHHC7.

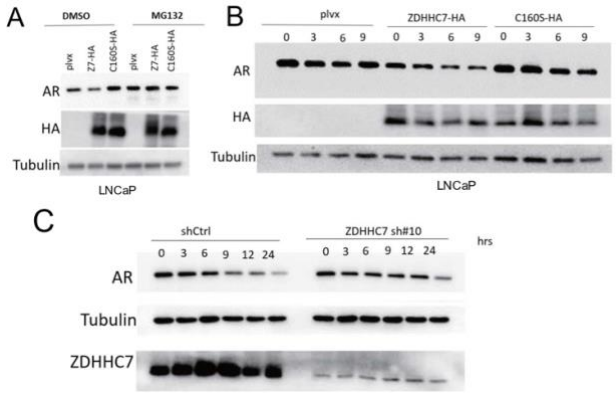

Figure 6. ZDHHC7 regulates AR protein stability. LNCaP cells with indicated gene expression were treated with (**A**) MG132 and (**B-C**) cycloheximide (CHX) before WB analyses.

Subtask 3: will test how ZDHHC7 regulates AR protein stability.

Methods: To determine protein stability, LNCaP cells with indicated gene expression were pre-treated with proteasome inhibitor 20uM MG132 before WB. To evaluate AR protein half-life, indicated LNCaP cells were treated with CHX, an mRNA translation inhibitor to suppress new protein synthesis, over a time-course before WB analyses.

Results: Our data show that overexpression of ZDHHC7, but not its palmitoylation mutant C160S led to reduced AR protein levels, which can be rescued by pre-treatment of proteasome inhibitor

MG132 (**Figure 6A**). Overexpression of ZDHHC7, but not C160S, greatly decreased AR protein half-life (**Figure 6B**), whereas ZDHHC7 knockdown prolonged AR protein half-life (**Figure 6C)**.

Conclusion: ZDHHC7 degrades AR protein through the proteasome pathway, thereby reducing AR protein half-life.

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

Postdoctoral fellows and graduate students 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 institutions that faculty, staff, pre and postdoctoral trainees, and graduate students are encouraged to attend.

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.

Yu lab research scientist Drs. Zhuoyuan Lin, Shivani Agarwal, and lab technical Elif Yagci have learned various techniques in molecular and cellular biology and genetics, especially those related to protein palmitoylation. Through interaction with our collaborator Dr. Xu Wu's laboratory in Harvard University, they have also acquired substantial knowledge about chemistry 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 and TRAMP-C2 cells with ZDHHC7 overexpression, and will correlate the changes with integrative analyses of PCa patient

specimens. We will evaluate YAP activation gene signature for enrichment in PCa cells with ZDHHC7 de-regulation probed by RNA-seq.

In terms of ZDHHC7 regulation of AR stability, we will identity any additional lysine sites (other than C807) in AR that may be palmitoylated by ZDHHC7. We will decipher which E3 ligase is involved in the degradation of AR protein after palmitoylation. We will perform RNA-seq and AR ChIP-seq in cells with ZDHHC7 overexpression or knockdown to determine how they affects AR signaling. Functionally, we will investigate the role of ZDHHC7 in PCa progression using cell lines and pre-clinical mouse xenograft model. 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. Ultimately, we plan to submit two manuscripts: ZDHHC7 regulation 1) of YAP in NEPC and 2) of AR in AR+ prostate cancer.

4. IMPACT:

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 PCa development and NEPC conversion, which could have significant impact in drug discovery and cancer research. We also observed an important link between ZDHHC7 and AR, suggesting AR as a substrate of ZDHHC7 and a potential tumor suppressive role of ZDHHC7 in AR+ prostate cancer.

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 NCI NIDDK Astellas Innovation fund

Name:	Carla Guarino
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	N/A

Nearest person month worked:	6.0
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?

Yes.

NEW

1R01DK127180-01 (Mao, PI) 12-01-20 – 11-30-25
0.3 cal mths
NIH/NIDDK total cost
(direct costs annually)
Dependence for TEAD transcription factors in intestinal development and polyposis
Role: Co-investigator (WU)
The major goal of the project is to understand the roles and mechanism of TEAD regulation during intestinal mesenchymal development and hamartomatous polyposis.

1R01DK127207-01 (Mao, PI) 2-01-20 – 1-31-25 0.3 cal mths
NIH/NIDDK total cost
total direct cost annually)
Mechanism of YAP/TAZ crosstalk with Wnt signaling
Role: Co-investigator (WU)
The major goal of the project is to dissect the molecular mechanisms underlying the signaling crosstalk between the Hippo/YAP and Wnt pathways.

INACTIVE
MRA-Samuel M. Fisher Memorial
Established Investigator Award (Wu) 5-15-17 – 11-14-20
Melanoma Research Alliance (MRA) accumulative total cost
($125,000 total direct annually)
Targeting TEAD autopalmitoylation in YAP-dependent uveal melanoma

The major goal of this project is to study the role of TEAD/YAP complex in GNAQ/GNA11 mutant uveal melanoma

Industry sponsored research agreement (Wu) 6-27-17 – 12-31-20
Astellas Venture Management LLC accumulative total cost
total direct annually, including subcontract to CRO Aurigene Technology)
TEAD autopalmitoylation collaboration project
The major goal of this project is to screen chemical libraries and to carry out pre-clinical drug discovery by targeting TEAD proteins for cancers through a CRO company (Aurigene Technology).

What other organizations were involved as partners?
Dr. Yu at Northwestern University

8. SPECIAL REPORTING REQUIREMENTS

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

9. APPENDICES:

None.