

AWARD NUMBER: W81XWH-16-1-0394

TITLE: Cotargeting of androgen synthesis and androgen receptor expression as a novel treatment for castration-resistant prostate cancer

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CONTRACTING ORGANIZATION: Purdue University
WEST LAFAYETTE, IN

REPORT DATE: August 2019

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE AUGUST 2019			2. REPORT TYPE Annual Report		3. DATES COVERED 1 Aug 2018 -31 July 2019	
4. TITLE AND SUBTITLE Cotargeting of androgen synthesis and androgen receptor expression as a novel treatment for castration-resistant prostate			5a. CONTRACT NUMBER W81XWH-16-1-0394		5b. GRANT NUMBER PC150697	
			5c. PROGRAM ELEMENT NUMBER		5d. PROJECT NUMBER	
			5e. TASK NUMBER		5f. WORK UNIT NUMBER	
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			8. PERFORMING ORGANIZATION REPORT NUMBER			
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			10. SPONSOR/MONITOR'S ACRONYM(S)		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
			13. SUPPLEMENTARY NOTES			
14. ABSTRACT Prostate cancer is the third leading cause of cancer death among American men in 2017. The majority of the death is due to the development of castration resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT). Despite the development and use of next generation anti-AR signaling inhibitors (ASI) such as abiraterone and enzalutamide, resistance to ASI remains the major clinical challenge. The proposed research is based on the finding that protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic activator of AR transcription. If PRMT5 targeting can inhibit or eliminate AR transcription, combining PRMT5 targeting with androgen synthesis inhibition should exhibit a better treatment effect for CRPC. During the past grant period, we have pICln as a novel cofactor to cooperate with PRMT5 to epigenetically activate AR expression and promote the growth of CRPC cells in vitro and xenograft tumors in mice. We will continue to extend this novel finding and complete proposed animal experiments in Aim 3 using a novel PRMT5 inhibitor during next grant period.						
15. SUBJECT TERMS CRPC, PRMT5, AR, AR-V7, epigenetics, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT U	18. NUMBER OF PAGES 46	19a. NAME OF RESPONSIBLE PERSON USAMRMC	
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)	

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1. Introduction

Prostate cancer is the third leading cause of cancer death among American men in 2017 [1], and the vast majority of these patients die of the development of castration resistant prostate cancer (CRPC), a lethal status of the disease [2-4]. The major mechanism underlying the development of CRPC is reactivation of the androgen receptor (AR), the driver of prostate cancer development and progression. AR reactivation mechanisms include AR overexpression (with or without AR gene amplification), AR mutations, AR splice variants, and androgen-independent activation of AR by AR modulators as well as de novo androgen synthesis in prostate cancer cells [3, 4]. In fact, abiraterone was approved by the FDA in 2011 for CRPC treatment because of its ability to inhibit CYP17A1, a critical enzyme involved in the de novo androgen synthesis in prostate cancer cells [5]. We have recently discovered that protein arginine methyltransferase 5 (PRMT5), an emerging epigenetic enzyme involved in epigenetic control of target gene expression [6-8], is overexpressed in prostate cancer tissues, and its expression positively correlates with the expression of AR [9]. Preliminary data strongly suggest that PRMT5 regulates prostate cancer cell growth through epigenetic control of AR expression. Based on these novel findings, *we hypothesize that co-targeting androgen synthesis and AR expression simultaneously will overcome the mechanisms of AR reactivation and provide an effective treatment for CRPC.* The goal of proposed research is to provide preclinical evidence that inhibiting androgen synthesis by abiraterone in combination with inhibiting or eliminating AR expression by PRMT5 targeting is an effective and novel therapeutic approach for CRPC treatment. We will use CRPC cells and patient derived xenograft (PDX) tumors to test our hypothesis *in vitro* and in mice. Completion of proposed research will provide preclinical evidence to guide the design of future clinical trials (*short-term impact*). If successful, this novel treatment will likely benefit all CRPC patients and ultimately reduce prostate cancer morbidity and mortality (*long-term impact*).

2. Keywords

PRMT5, epigenetics, AR, CRPC, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide

3. Accomplishments

3A. What were the major goals of the project? There are three major goals in this project as defined by three Specific Aims in the approved SOW.

Major Goal 1: To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines

Major Goal 2. To test whether PRMT5 targeting in combination with abiraterone shows a better killing effect in CRPC cells

Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice

3B. What was accomplished under these goals?

Major Goal 1: To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines (Months 1-12) Completed.

Goal 1A-C: As reported in the 2016-2017 annual report, we completed all goals in this major goal as following: (1) We demonstrated that inhibition of PRMT5 by BLL3.3 suppresses cell growth by down-regulating the expression of AR and AR-V7 in several CRPC cells; (2) Co-treatment of CRPC cells with BLL3.3 and abiraterone or enzalutamide is more effective in suppressing CRPC cell growth; (3) knockdown of PRMT5 also suppresses the growth of CRPC cells through down-regulation of AR-FL and AR-V7 expression; (4) regulation of AR-FL and AR-V7 in 22Rv1 cells is also through epigenetic regulation via dimethylation of H3R3 and both Sp1 and Brg1 are involved. In summary, we completed this major goal and confirmed that the regulatory mechanism of CRPC cell growth and the expression of AR-FL and AR-V7 is the same as we reported in hormone naïve prostate cancer cells (HNPC) LNCaP [9].

Goal 1D. PRMT5 is overexpressed in prostate cancer tissues and its nuclear expression correlates with AR expression in prostate cancer tissues: Completed

As reported in the 2017-2018 Annual Progress Report, we retrieved data from cBioPortal database and found that PRMT5 expression correlates with AR expression in metastatic prostate cancer. During the last funding period, we collaborated with Dr. Jiaoti Huang to perform IHC for the expression of PRMT5, AR and AR-V7 in 20 CRPC specimens. As shown in Fig. 1, PRMT5 expression positively correlates with AR expression in CRPC specimens. We also retrieved gene expression data from GEO datasets (8) and analyzed the correlation between PRMT5 expression and AR expression at the mRNA level. Our results show that PRMT5 expression also positively correlates with AR expression. These results suggest that PRMT5 indeed regulates AR transcription in both HNCP and CRPC tissues.

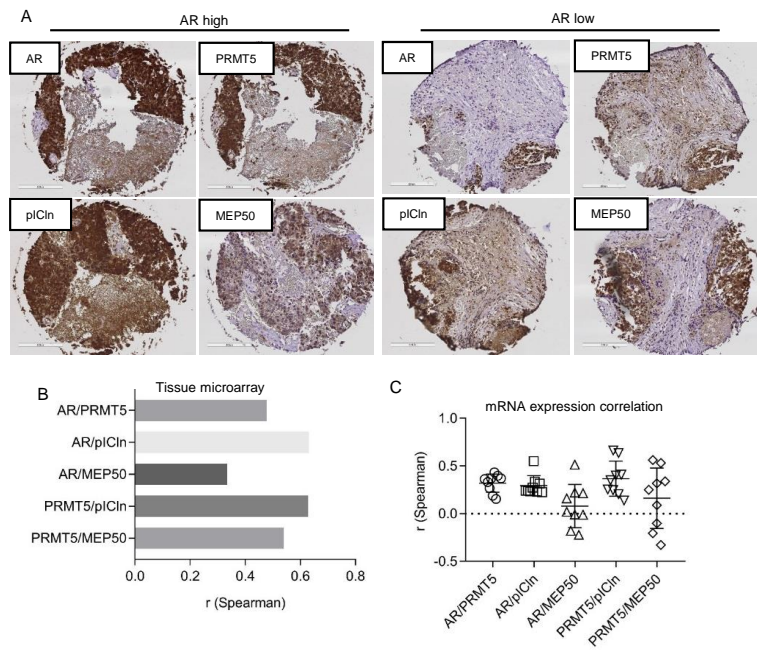


Figure 1. PRMT5/pICln expression correlates positively with AR in CRPC patients. **A, B,** AR, PRMT5, pICln, and MEP50 protein expressions were analyzed by IHC in metastatic CRPC tissue microarray (20 samples). **A,** Representative IHC images of AR, PRMT5, pICln, and MEP50 expression. **B,** The correlation analysis between the proteins (Spearman analysis). **C,** AR, PRMT5, pICln, and MEP50 mRNA expression data were obtained from 8 Gene Expression Omnibus (GEO) datasets, and the correlation analysis between the mRNAs (Spearman analysis) was performed.

Goal 1E. Biological evaluation of a novel PRMT5 inhibitor BLL3.3. Completed

As reported in the 2016-2017 annual report, we evaluated the effect of our PRMT5 inhibitor BLL3.3 and confirmed that BLL3.3 recapitulates the effect of PRMT5 knockdown in HNPC cells (Oncogene 2017, 3B-1-3).

Major Goal 2. To test whether PRMT5 targeting in combination with abiraterone shows a better killing effect in CRPC cells (Months 13-24). Completed

As reported in Major Goal 1 of the 2016-2017 annual report, we confirmed that co-treatment of LNCaP95 with BLL3.3 and abiraterone or enzalutamide is more effective in suppressing cell growth. We also confirmed the co-targeting effect in 22Rv1 cells, which was reported in the 2017-2018 Progress Report. Treatment of LNCaP95 with abiraterone did not induce expression of AR-V7 in our hands, and hence we did not pursue this.

Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice (Months 1-6 and 19-36) Partially completed

We are still waiting for PRMT5 inhibitors from our collaborator Dr. Chenglong Li to perform proposed *in vivo* experiments. Recently developed analogs showed better potency and PK properties. We anticipate that a potent inhibitor will be likely available for *in vivo* experiments during next grant period. As reported in the 2017-2018 Progress Report, we already demonstrated that knockdown of PRMT5 effectively suppressed xenograft tumor growth in mice. As presented below, we also confirmed that knockdown of pICln is also effective in suppressing xenograft tumor growth in mice.

Other Achievements

As reported in the 2017-2018 Annual Progress Report, we found that MEP50, the obligated cofactor of PRMT5, does not participate in the AR regulation by PRMT5. As PRMT5 also interacts with several proteins to epigenetically regulate gene transcription [8], we performed ChIP-qPCR to determine the binding of pICln, RioK1 and COPR5 to the AR proximal promoter region. Interestingly, we found that pICln, but not RioK1 and COPR5, bound to the AR promoter region (Fig. 2A). This unexpected result suggests that PRMT5 may cooperate with pICln to regulate AR transcription independently of MEP50.

To determine that pICln participates in the PRMT5 regulation of AR transcription, we established doxycycline-inducible knockdown cell lines in 22Rv1 and examined whether pICln knockdown has any impact on AR expression. As shown in Fig. 2B and 2C, knockdown of pICln inhibited expression of AR and AR-V7 at the protein level in 22Rv1 cells. Consistent with the epigenetic regulation of AR transcription by PRMT5, knockdown of pICln decreased the expression of AR and AR-V7 at the mRNA level (Fig. 2D) and significantly reduced the enrichment of H4R3me2s on the AR proximal promoter region (Fig. 2E) as observed for PRMT5 in HNPC and CRPC cells. To determine whether pICln also cooperates with PRMT5 to regulate AR transcription in HNPC cells, we similarly established Dox-inducible stable cell lines in LNCaP

cells. Indeed, knockdown of pICln decreased the expression of AR at the protein level (Fig. 2F and 2G) and the mRNA level (Fig. 2H).

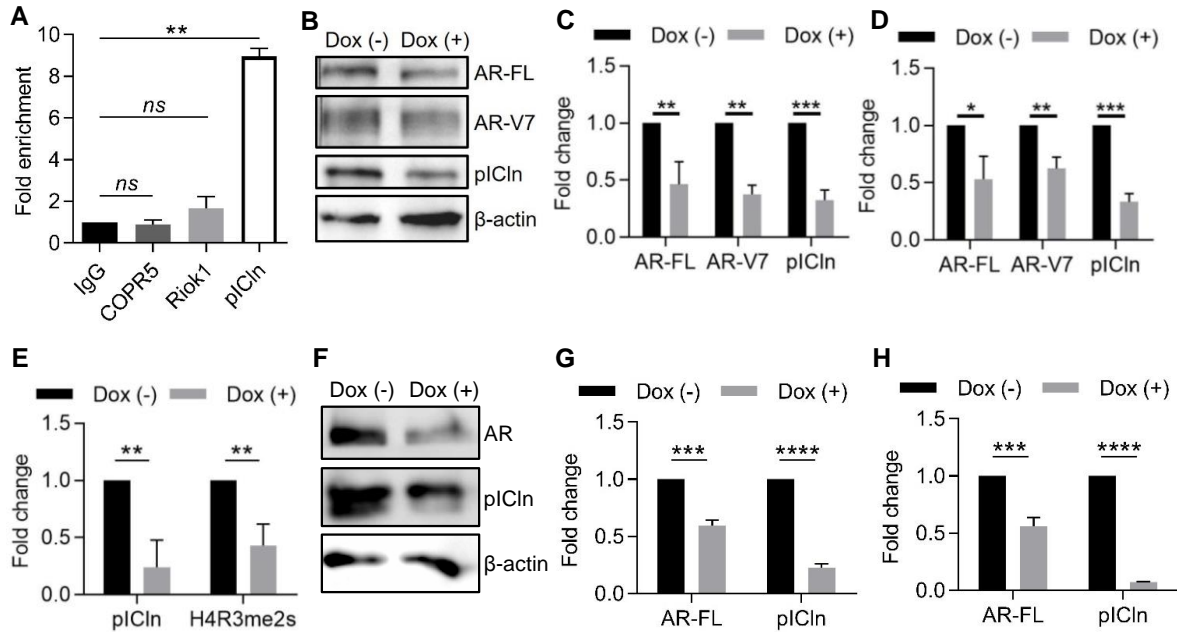


Figure 2. pICln binds to the AR promoter region and regulates transcription of AR and AR-V7 in CRPC cells. **A.** The antibodies were used to immunoprecipitate the indicated proteins for CHIP-qPCR analysis. The results are normalized the IgG control and mean \pm SD were obtained from 3 independent experiments for their enrichment on the proximate region of AR. **B.** 22Rv-1-shpICln stable cell line was treated with doxycycline (Dox) or without doxycycline (Dox-) for 6 days and total cell lysate was used for Western blotting of the indicated proteins. Shown are representative blots from one biological replicate. **C.** Quantified expression of AR-full length (AR-FL), AR-V7 and pICln from three independent biological replicates of **B.** **D.** Similar experiments were performed to determine the expression of AR-FL, AR-V7 and pICln at the mRNA level using RT-qPCR. **E.** CHIP-qPCR for determination of the enrichment of H4R3me2s in 22Rv-1-shpICln cells with or without Dox treatment. **F.** LNCaP-shpICln stable cell line was used to induce pICln knockdown for 6 days to determine the effect of pICln knockdown on the expression of AR-FL as described in **B.** **G.** Quantified expression of AR-full length (AR-FL) and pICln from three independent biological replicates of **F.** **H.** Similar experiments were performed as described in **F.** and the expression of AR-FL and pICln at the mRNA level was determined using RT-qPCR with (Dox+) or without (Dox-) pICln knockdown. All experiments were performed for three times and Student's *t*-test was used to determine the statistical significance between the control and experiment group. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, NS: not significant

We previously determined that nuclear PRMT5 expression positively correlates with the expression of AR in HNPC [9] (Fig 3A). To determine whether pICln also correlates with the expression of AR, we used the same TMA (72 cases) and found that pICln indeed correlates positively with the expression of AR (Fig. 3B). Furthermore, PRMT5 expression also correlates positively with pICln expression (Fig. 3C). In addition, we also analyzed the expression of PRMT5, pICln, MEP50 and AR at the mRNA level and found that the expression of PRMT5 and pICln, but not MEP50, correlates positively with the expression of AR (Fig. 1). Further, PRMT5 expression also positively correlates with pICln expression (Fig. 3D). These results together

strongly suggest that pICln may indeed cooperate with PRMT5 to regulate AR transcription in both HNPC and CRPC cells.

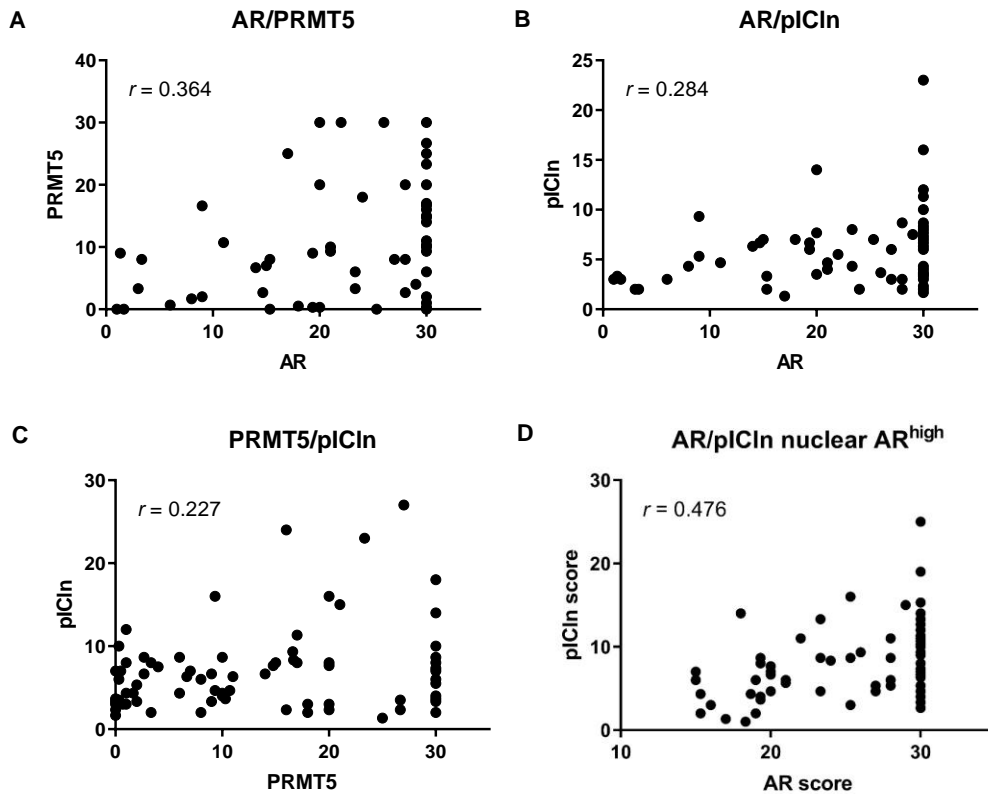


Figure 3. pICln expression correlates positively with the expression of AR in HNPC tissues. A-C. A tissue microarray constitutes 32 BPH and 40 prostate cancer tissues was used to study for PRMT5, AR, and pICln. The Spearman's correlation coefficient between the indicated two proteins was determined. **D.** The tissues were stratified based on higher AR expression and the expression of nuclear pICln was used to determine the Spearman's correlation coefficient with AR expression.

To evaluate whether knockdown of pICln also suppresses cell growth in CRPC cells, we performed MTT assays and observed that knockdown of pICln significantly inhibited cell growth (Fig. 4A). Further, we evaluated whether knockdown of pICln has any effect on xenograft tumor growth in mice. We injected 1×10^6 22Rv1 cells into hind leg of castrated male mice (6-9 weeks). After tumors grew to $\sim 100 \text{ mm}^3$, all mice were fed drinking water containing doxycycline (1 mg/ml) and tumor volume was measured twice a week. Compared to scrambled control cell line (SC), knockdown of pICln significantly inhibited tumor growth. IHC analysis confirmed pICln knockdown and down-regulation of AR expression.

Taken together, our mechanistic studies revealed an unexpected finding that pICln may function as a cofactor to cooperate with PRMT5 to epigenetically activate transcription of AR or AR splice variants in both HNPC and CRPC, and validated PRMT5 as a potential therapeutic target for treatment of both HNCP and CRPC.

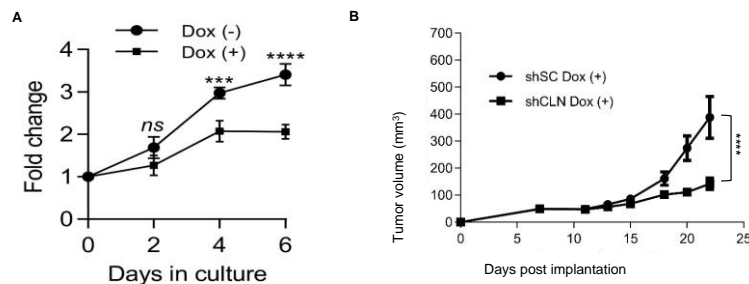


Figure 4: pICLn regulates the growth of 22Rv1 cells in vitro and in mice. **A.** 22Rv-1-shpICln (shCLN) cells were cultured in the presence of knockdown (Dox+) or without knockdown (Dox-), and cell growth was measured using MTT assay. Results are mean \pm SD from 3 independent experiment. **B.** 22Rv-1-shpICln (shCLN) or 22Rv-1shSC (shSC) cells (1×10^6) were injected subcutaneously in right flanks of surgically castrated male NRG mice. Tumor-bearing mice were treated with doxycycline in drinking water once tumors reached $\sim 100 \text{ mm}^3$. Tumor growth kinetics were determined and compared between groups (ANOVA; ****, $P < 0.0001$).

3C. What opportunities for training and professional development has the project provided?

3C-1. Research Trainings. During the grant period, the following four people have been involved in the project and received training (one-on-one research training).

Elena Beketova, a fourth year graduate student from our PULSe (Purdue University Life Science Umbrella) Program, has been working on the project. Elena was recruited to the lab in May 2016 after she completed one-year rotations. During the last grant period, she gave two poster presentations at the 2018 SBUR (Society of Basic Urological Research) conference and at the Midwest Chromatin and Epigenetics Meeting. She also received two travel awards (2018 Purdue University Women in Science Travel Award and Purdue University Center for Cancer Research Travel Award). Elena also received a Graduate Research Fellowship from the College of Pharmacy (2018-19) to provide partial support of her graduate research. She also served as a Judge for the 2019 Lafayette Science Fair. In addition, Elena presented in the lab meetings (6 times per year) and attended weekly cancer biology journal club in the Purdue University Center for Cancer Research. Other than these professional activities, I met with Elena on a weekly basis to discuss her research progress and plans for future research. I have been also working with her to prepare a manuscript for submission.

Jake Owens, a fifth year graduate student of MCMP (Medicinal Chemistry and Molecular Pharmacology) program was partially working on the project. His major role is to collaborate with Elena to generate necessary research materials and help with TCGA data analysis. Jake attended the 2019 Washington DC. Translational Science conference and received Blue Ribbon poster presentation award. In addition, Jake also gave poster presentations at the 2018 SBUR (Society of Basic Urological Research) conference, 2018 IBUR (Indiana Basic Urological Research) Symposium, 2018 MCMP Department Scientific Retreat, and 2018 Indiana CTSI Annual Meeting. In addition, Jake presented in the lab meetings (6 times per year) and attended weekly cancer biology journal club in the Purdue University Center for Cancer Research. Other than these

professional activities, I met with Jake on a weekly basis to discuss his research progress and plans for future research. Jake also worked with me on a manuscript, which is currently under review for *iScience*.

Xuehong Deng, a senior lab technician who has been working on the project, continued to work on the project and provided training and technical support to Elena Beketova and other lab members. She helped to generate several stable cell lines that can inducibly express shRNAs to knockdown PRMT5, MEP50 and pICln.

Jonathan Malola, a third year of pharmacy student in the Purdue University College of Pharmacy, has been working on the project under the supervision of Elena Beketova. He has been assisting Elena to construct some plasmids for the proposed research. In particular, he performed BiFC analysis and confirmed the interaction of PRMT5 with MEP50 and pICln.

3C-2. Conference presentations

Beketova E., Deng X., Hu C.D. (2018) Protein Arginine Methyltransferase 5 as a Potential Target for Treatment of Castration-Resistant Prostate Cancer. [Poster presentation](#) at the Society of Basic Urologic Research annual meeting.

Beketova E. (2018) Targeting PRMT5 as a novel approach for the treatment of prostate cancer. [Poster presentation](#) at Midwest Chromatin and Epigenetics Meeting, Purdue University

Owens, J.L., Deng, X., Beketova, E., Tinsley, S.L., Asberry, A. and Hu, C.D. (2019) PRMT5 acts as a master epigenetic regulator to promote repair of DNA damage and is a novel therapeutic target to improve cancer radiation therapy – [Poster presentation](#) at Washington D.C., Translational Science 2019 conference → Blue ribbon poster award

Owens, J.L. and Hu, C.D. (2018) PRMT5 is a novel therapeutic target to enhance cancer radiation therapy – [Poster presentation](#) for at Purdue University, College of Pharmacy Graduate Research Symposium (Jenkins/Knevel award for Outstanding Graduate Research in College of Pharmacy)

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 is a master epigenetic regulator of the DNA damage response and is a novel therapeutic target for prostate cancer radiosensitization – [Poster presentation](#) at Palm Springs CA, Society of Basic Urological Research (SBUR) annual meeting

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 is a master epigenetic regulator of the DNA damage response and is a novel therapeutic target for prostate cancer radiosensitization – [Poster presentation](#) at Purdue University, Indiana Basic Urological Research (IBUR) Symposium

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 as a putative therapeutic target for prostate cancer treatment – [Poster presentation](#) at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (2018) PRMT5 acts as a master epigenetic regulator to promote repair of DNA damage and is a novel therapeutic target to improve cancer radiation therapy – Poster presentation, Indiana CTSI 2018 annual meeting September

3D. How were the results disseminated to communities of interest?

N/A.

3E. What do you plan to do during the next reporting period to accomplish the goals?

Major Goal 1: We have already accomplished Major Goal 1 as we planned. In addition, we made additional accomplishments by establishing the positive correlation of PRMT5 expression with AR expression in metastatic prostate cancer tissues at the mRNA level and protein level. One major unexpected and exciting finding is that pICln, but not MEP50, binds to the proximate region of AR region and regulates AR transcription. These results together suggest that PRMT5 and pICln cooperate to epigenetically activate AR transcription in HNPC and CRPC cells. We will continue to extend this novel finding and determine how PRMT5 and pICln regulates cell growth (cell cycle analysis, apoptosis and etc) and evaluate whether knockdown of pICln also suppresses xenograft tumor growth in mice as we observed for PRMT5 knockdown. Since we encountered technical difficulty to stain AR-V7 in CRPC tissues, we were unable to establish the correlation between PRMT5 expression and AR-V7. During the next grant period, we will continue to collaborate with Dr. Jiaoti Huang group at Duke University to optimize the IHC condition and complete this experiment. We will also determine the mechanism by which cell growth is suppressed when PRMT5 or pICln is knocked down. We will also analyze xenograft tumor tissues to see if cell proliferation (Ki67 staining) or apoptosis (cleaved caspase 3) are involved.

Major Goal 2: We have completed all proposed experiments in Major Goal 2 and demonstrated that targeting of PRMT5 by either knockdown or inhibition in combination with abiraterone or enzalutamide is more effective. Preliminary results showed that treatment with abiraterone or enzalutamide did not have any effect on the expression of AR-FL and AR-V7.

Major Goal 3: We are waiting for PRMT5 BLL3.3 derivatives as potent PRMT5 inhibitors for *in vivo* evaluation. We will work closely with Dr. Chenglong Li at University of Florida to acquire his potent inhibitor for *in vivo* studies. Once available, we will start *in vivo* experiments. Alternatively, we will also evaluate whether a novel PRMT5 inhibitor JNJ-64619178 from Johnson & Johnson has any effect on PRMT5-mediated AR transcription. If so, we will use this inhibitor for *in vivo* experiments. If not, we will consider combining PRMT5 knockdown with abiraterone or enzalutamide to conduct proposed experiments.

4. Impact

4A. What was the impact on the development of the principal discipline(s) of the project?

Androgen receptor (AR) is the driver of prostate cancer development and progression and is the validated therapeutic target for prostate cancer treatment. Androgen deprivation therapy (ADT) by suppressing androgen levels or inhibiting the activity of AR is the primary treatment

option for metastatic disease. Unfortunately, AR reactivation via increased expression (gene amplification), mutation or expression of splice variants that are not responsive to conventional ADT is the underlying mechanisms of resistance to ADT. As such, patients inevitably develop into castration resistant prostate cancer (CRPC). The next generation anti-AR signaling inhibitors (ASI) abiraterone and enzalutamide remain ineffective. The findings from the past two years provide evidence that co-targeting of AR expression via PRMT5 knockdown and androgen synthesis via abiraterone or AR inhibition via enzalutamide is more effective in killing CRPC cells. As AR reactivation is the major mechanism underlying CRPC development, targeting PRMT5 could potentially overcome AR reactivation by eliminating AR transcription, particularly in combination with androgen synthesis inhibition or AR inhibition. Importantly, we also made unexpected and surprising finding that pICln, but not the canonic cofactor MEP50, may participate in epigenetic activation of AR transcription in prostate cancer cells. This raises a very interesting possibility that developing inhibitors specifically targeting the PRMT5/pICln interaction may offer a specific and unique approach to treat HNPC and CRPC.

4B. What was the impact on other disciplines?

Although it is generally thought that PRMT5 functions as an epigenetic repressor in multiple human cancers, the current report provides evidence that PRMT5 also functions as an epigenetic activator to activate AR transcription by symmetrically dimethylating H4R3 not only in hormone naïve prostate cancer but also in CRPC cells. This further confirm that AR reactivation is the mechanism of CRPC. As epigenetic regulation is a tissue-specific and complex process that involves formation of multiple protein complexes, identification of pICln as a potential cofactor of PRMT5 raises an interesting possibility that PRMT5/pICln may cooperate to epigenetically activate gene transcription whereas PRMT5/MEP50 may epigenetically repress gene transcription. This will offer a unique opportunity to understand basic mechanisms of epigenetic regulation in general. This is also supported by the finding that MEP50, an obligate PRMT5 cofactor, did not participate in epigenetic regulation of AR transcription by PRMT5. Consistent with this, we also observed that PRMT5 may cooperate with pICln to epigenetically activate transcription of genes in DNA damage response. Future identification of additional PRMT5/MEP50/pICln targets will further strengthen this hypothesis and warrant additional in depth studies. Furthermore, biochemical and structural studies will reveal how they may function as an activator vs a repressor.

4C. What was the impact on technology transfer?

Nothing to Report.

4D. What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

Nothing to Report.

6. Products

6A. Publications, conference papers, and presentations

Journal Publications: A manuscript is under preparation.

None

Presentations by Chang-Deng Hu (PI) not reported above: See students' presentations

7. Participants & Other Collaborating Organizations

7A. What individuals have worked on the project?

Name:	Chang-Deng Hu
Project Role:	Hu
Perner ID:	90024721
Nearest person month worked:	1.2
Contribution to Project	Dr. Hu has supervised students and the technician to conduct the proposed research.
Funding Support	Purdue University and PC120512

Name:	Elena Beketova
Project Role:	Graduate Student
Perner ID:	119730
Nearest person month worked:	8
Contribution to Project	Miss Beketova has generated most of the data presented in this progress report
Funding Support	Purdue Research Foundation Fellowship and PC150697

Name:	Jake Owens
Project Role:	Graduate Student
Perner ID:	147536
Nearest person month worked:	3
Contribution to Project	Mr. Owens has helped with qRT-PCR and ChIP analysis

Funding Support	CTSI Fellowship and PC150697
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Name:	Xuehong Deng
Project Role:	Technician
Perner ID:	90025073
Nearest person month worked:	3
Contribution to Project	Ms. Deng has generated stable cell lines and provided technical assistance
Funding Support	PC150697

Name:	Jonathan Malola
Project Role:	Pharmacy Student
Perner ID:	79715
Nearest person month worked:	3
Contribution to Project	Mr. Malola has helped with some plasmid constructions
Funding Support	Purdue College of Pharmacy and PC150697

7B. Has there be a change in the active other support of the PI/PI(s) or senior/key personnel since the last reporting period?

Current Active Grants

Title: Role and targeting of PRMT5 in prostate cancer

Source: NCI RO1

Role: Contact PI (**Multi-PI** with Chenglong Li and Jiaoti Huang)

Total Cost Requested: \$2,590,428

Grant Period: 06/09/2017-05/31/2022

Goal: The goal of this proposal is to elucidate the molecular mechanisms by which PRMT5 promotes prostate cancer cell growth, improve the potency of BLL3.3, and conduct a preclinical evaluation of PRMT5 inhibition for castration resistant prostate cancer treatment.

Title: Co-targeting of androgen synthesis and androgen receptor expression as a novel treatment for castration resistant prostate cancer

Source: DoD (2015 PCRP)

Role: PI

Grant Period: 08/01/16-07/30/20

Total Cost: \$557,000

Goal: The goal of this project is to evaluate whether co-targeting of androgen synthesis by abiraterone and androgen receptor expression via PRMT5 inhibition is an effective treatment for CRPC.

Title: Targeted RO1: Molecular and genetic analysis of PRMT5 in neuroendocrine prostate cancer

Source: EVPRP Targeted RO1

Period: 12/01/15-10/31/19

Total amount awarded: \$30,000

Role: PI

Goals: The goal of this project is to generate preliminary data for a RO1 proposal to determine the role of PRMT5 and its cofactor MEP50 in neuroendocrine differentiation of prostate cancer cells and validate whether targeting PRMT5/MEP50 is an effective therapeutic approach for neuroendocrine prostate cancer

Title: Discovery of inhibitors to disrupt the interaction of PRMT5 with its cofactor pICln for prostate cancer treatment

Source: Purdue University Center for Cancer Research

Period: 08/01/18-07/30/19

Total amount awarded: \$15,000

Role: PI

Goals: This support is to develop a BiFC-based high throughput screen assays for identification of inhibitors to disrupt the PRMT5/pICln interaction.

Title: Deep neural network-assisted protein structure modeling for drug development from low resolution 3D cryo-electron microscopy maps

Source: Purdue Institute for Drug Discovery

Period: 12/01/18-11/30/20

Total amount awarded: \$150,000

Role: Co-PI with Dr. Daisuke Kihara (computational biologist) and Dr. Wen Jiang (cryo-EM expert)

Goal: This support is to develop a deep learning method to predict cryo-EM structures using PRMT5/MEP50 and PRMT5/pICln interactions as a model and to identify novel interfaces for drug discovery.

7C. What other organizations were involved as partners?

Nothing to report.

8. Special Reporting Requirements

N/A

9. References

1. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer Statistics, 2017. *CA Cancer J Clin*, 2017. **67**:7-30.
2. Antonarakis, E.S. and M.A. Carducci, Future directions in castrate-resistant prostate cancer therapy. *Clin Genitourin Cancer*, 2010. **8**:37-46.
3. Chandrasekar, T., J.C. Yang, A.C. Gao, and C.P. Evans, Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Transl Androl Urol*, 2015. **4**:365-80.
4. Vlachostergios, P.J., L. Puca, and H. Beltran, Emerging Variants of Castration-Resistant Prostate Cancer. *Curr Oncol Rep*, 2017. **19**:32.
5. Grist, E. and G. Attard, The development of abiraterone acetate for castration-resistant prostate cancer. *Urol Oncol*, 2015. **33**:289-94.
6. Karkhanis, V., Y.J. Hu, R.A. Baiocchi, A.N. Imbalzano, and S. Sif, Versatility of PRMT5-induced methylation in growth control and development. *Trends Biochem Sci*, 2011. **36**:633-41.
7. Krause, C.D., Z.H. Yang, Y.S. Kim, J.H. Lee, J.R. Cook, and S. Pestka, Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol Ther*, 2007. **113**:50-87.
8. Stopa, N., J.E. Krebs, and D. Shechter, The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond. *Cell Mol Life Sci*, 2015. **72**:2041-59.
9. Deng, X., G. Shao, H.T. Zhang, C. Li, D. Zhang, L. Cheng, B.D. Elzey, R. Pili, T.L. Ratliff, J. Huang, and C.D. Hu, Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene*, 2017. **36**:1223-1231.

10. Appendices

PI's CV

Curriculum Vitae

Chang-Deng Hu

Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University College of Pharmacy
Purdue University Center for Cancer Research
201. S. University St, HANS 401A
West Lafayette, IN 47907-1333
Tel: 765-496-1971, Fax: 765-494-1414, E-mail: hu1@purdue.edu
Department URL: <http://www.mcmp.purdue.edu/faculty/?uid=cdhu>
Lab URL: <http://people.pharmacy.purdue.edu/~hu1/>

Education / Degrees Awarded:

- 9/1979-7/1984: Bachelor in Medical Science (Equivalent to *M.D.*)
Faculty of Medicine, Bengbu Medical College, Bengbu, China
- 9/1984-7/1987: *M.S.* (Cancer Immunology)
Department of Microbiology and Immunology, College of Medicine,
Tongji Medical University, Wuhan, China
- 4/1994-3/1997: *Ph. D.* (Molecular Biology)
Department of Physiology II, Kobe University School of Medicine, Japan

Research/Working Experience:

- 9/1984-7/1987: *Graduate Student (M.S.)* in the Department of Microbiology & Immunology, Tongji Medical University, Wuhan, China.
Study of anti-tumor mechanisms of a new Chinese herb in cell culture and animal models.
- 7/1987-9/1991: *Lecturer* in the Department of Epidemiology, School of Public Health, Tongji Medical University, Wuhan, China.
(1). Mutagenicity of trichloromethane in drinking water
(2). Epidemiological investigation of drinking water and cancer incidence in Wuhan, China.
- 9/1991-3/1994: *Visiting Research Associate* in the Department of Molecular Oncology, Kyoto University School of Medicine, Kyoto, Japan.
(1). Spontaneous and induced acquisition of tumorigenicity in nude mice by lymphoblastoid cell line derived from patients with xeroderma pigmentosum group A.
(2). Subtractive isolation of genes contributing to the acquisition of tumorigenicity by lymphoblastoid cell line derived from xeroderma pigmentosum group A patient.
- 4/1994-3/1997: *Graduate Student (Ph.D.)* in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan
(1). Identification of cysteine-rich domain in Raf-1 as a novel Ras binding domain for activation by Ha-Ras and Rap1A.

- (2). Activation mechanisms of Ras effectors (Raf-1, B-Raf, adenylyl cyclase).
- 4/1997-8/2000: **Assistant Professor** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan.
- (1). Differential regulation of Raf kinase activity by Ha-Ras and Rap1A.
 - (2). Identification and characterization of novel Ras effectors, (RalGDS, AF-6, PLC- ϵ) and regulators (RA-GEF-1, RA-GEF-2).
 - (3). Activation mechanisms of Ras effectors.
- 9/2000-6/2003: **Research Investigator/Specialist** in the Department of Biological Chemistry and Howard Hughes Medical Institute, University of Michigan School of Medicine.
- (1). Development of bimolecular fluorescence complementation (BiFC) and multicolor BiFC assays for visualization of protein-protein interactions in living cells.
 - (2). Functional analysis of cross-family transcription factor interactions among bZIP, Rel, Smad and Myc/Max families.
- 7/2003-6/2009: **Assistant Professor** in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.
- (1) Development and improvement of BiFC-based technologies
 - (2) BiFC analysis of AP-1 dimers in living cells and *C. elegans*
 - (3) AP-1 in prostate cancer development and therapeutic responses
- 7/2009- 7/2015: **Associate Professor** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.
- (1) Development and improvement of BiFC-based technologies
 - (2) AP-1 in prostate cancer development and progression
 - (3) Mechanisms and targeting of radiation-induced neuroendocrine differentiation in prostate cancer
 - (4) Protein arginine methyltransferase 5 (PRMT5) in prostate cancer development, progression and therapeutic response
- 8/2015- present: **Professor** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.
- (1) Mechanisms and targeting of radiation-induced neuroendocrine differentiation (NED) in prostate cancer
 - (2) Role and targeting of protein arginine methyltransferase 5 (PRMT5) in castration resistant prostate cancer (CRPC) and neuroendocrine prostate cancer (NEPC)
 - (3) Development of high throughput screens for small molecule inhibitors targeting protein-protein interactions
 - (4) Development of BiFC-based cDNA library screens for interacting proteins
- 08/2013-present: Program Co-Leader of the Cell Identity and Signaling (CIS) program of the Purdue University Center for Cancer Research (PCCR)
- 08/2013-present: Executive Committee Member of PCCR

08/2010-present: Co-Leader of the Prostate Cancer Discovery Group of PCCR
 2011-2018: Director of Pharmacy Live Cell Imaging Facility (PLCIF)
 2016-present: Director of Small Animal Radiation Facility (PCCR)
 7/2016-present: Showalter Faculty Scholar of Purdue University

Current Professional Memberships

2001- Present American Association for Cancer Research
 2009- Present Society for Basic Urological Research
 2010- Present American Urological Association
 2015-present Radiation Research Society

Awards:

09/91-09/92: Fellowship of JSPS
 Source: **Japan Society for the Promotion of Science (JSPS)**
 09/92-09/93: Kyoto University Alumni Fellowship
 Source: Kyoto University
 04/94-03/97 Senshukai Scholarship (Ph.D. student)
 Source: Kobe Senshukai Scholarship Foundation
 04/98-03/99 President Young Investigator Award
 Source: Kobe University
 04/98-03/99 Young Investigator Award
 Source: JSPS
 04/99-03/01 Young Investigator Award
 Source: Hyogo Prefecture Science and Technology Association
 07/03-08/06 Walther Assistant Professor
 07/16-06/21 University Showalter Faculty Scholar Award of Purdue University
 04/17 Pharmaceutical Sciences Teacher of the Year in the College of
 Pharmacy (completely nominated and voted by all students)
 10/17 Seed for Success Award (EVPRP)
 5/18 Lafayette Lions Club Award for Outstanding Achievements in
 Cancer Research (State Award)
 5/19 2019 Chaney Faculty Scholar Award (Research Award in the
 Purdue University College of Pharmacy)

Professional Services:

Reviewer for Grant Applications

2004 Reviewer of MAES (The Maryland Agricultural
 Experiment Station at the University of Maryland)
 2005 Reviewer for NSF Advisory Panel for Molecular and
 Cell Biology
 2006-2008 American Heart Association (MCB Panel)
 2007-2011 Qatar National Research Fund (QNRF)

2008-present Pennsylvania Department of Health (PADOH)
 2008 UK Cancer Research
 2008 UK Diabetes
 2009 Wellcome Trust
 2010-2014 Department of Defense, Prostate Cancer Research
 Program (Immunology, Endocrine, Experimental
 Therapeutics panels)
 2015-present Florida Department of Health
 2015 NIH, RTB study section (IAR)
 2016 NCI (DP5)
 2019 NIH, RTB study section (March and July)

Reviewer for Professional Journals

Combinatory Chemistry and HTS, Zebrafish, Journal of Biological
 Chemistry, Molecular and Cellular Biology, Nature Biotechnology
 Nature Methods, Molecular Cell, Molecular Biology of the Cell,
 PNAS, BMC Biotechnology, BMC Biology, Biotechniques,
 Biochemistry, ACS Chemical Biology, Chemistry & Biology, Journal
 of Innovative Optical Health Sciences, TIBS, TIBT, Current Cancer
 Drug Targets, Journal of Cell Science, PLoS One, Ontarget,
 Oncogene, Redox Biology, Cancer Letters, and etc

Editorial Board Member:

2007- Perspective in Medicinal Chemistry
 2011- American Journal of Cancer Research
 2013- Journal of Biological Methods (Founding Editorial Member)
 2014- Frontier in Surgical Oncology (review editor)
 2015- Journal of Drug Research and Development

***Organizer/Program Committee Member/Session Chair of Conferences,
 Symposiums, and Workshops***

- Organizer of Tristate Worm Meeting at Purdue (2006)
- Session Chair of Optical Molecular Imaging of the 2008 PIBM
- Session Chair of Imaging Technology Symposium of the 2008 4th
 Modern Drug Discovery and Development Summit
- Program Member of the 2009 PIBM Program Committee
- Organizer of 2010 Bimolecular Fluorescence Complementation
 Workshop (Purdue University)
- Member of the Scientific Program Committee and Moderator of
 Breakout Panel Discussion of the 2013 Drug Discovery
 Chemistry-Sixth Annual Protein-Protein Interactions, San Diego
- Organizer, Program Committee Member and Session Chair of the
 2013 Hefei Prostate Cancer Translational Medicine and
 Personalized Medicine Symposium
- Session Co-chair of the 2016 Spring SBUR Symposium

Member of Big Ten Cancer Research Consortium (BTRC) GU Clinical Trial Working Group (2013-present)

Consultation on BiFC technology

Since 2003, we have been providing BiFC plasmids, letters of support and consultations to many BiFC users worldwide. The lab provided BiFC plasmids to more than 200 labs prior to 2007. To facilitate the request process, we deposited 11 BiFC plasmids to Addgene in 2007, and 2282 samples have been distributed via Addgene as of August 1, 2019.

Invited Seminars/Presentations

- | | |
|----------|---|
| 07/08/19 | Place: Purdue-SEU Biotechnology and Data Science Symposium
Title: Bimolecular fluorescence complementation (BiFC): From single molecular visualization to genome-wide investigation |
| 06/07/18 | Place: Department of Radiation Oncology, Chinese University of Sciences and Technology First Affiliated Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 05/31/18 | Place: Jinan University School of Medicine
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 05/30/18 | Place: Sun Yat-sen University Cancer Center
Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance |
| 05/24/18 | Place: Department of Urology, Wannan Medical College Yiji Shan Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 03/28/18 | Place: Utsunomiya University Center for Biosciences Research and Education
Title: Neuroendocrine differentiation of prostate cancer: From basic research to clinical translation |
| 03/19/18 | Place: Xuhui Hospital of Fudan University Zhongshan Hospital
Title: Neuroendocrine differentiation of prostate cancer: From basic research to drug discovery |
| 03/12/18 | Place: Bengbu College of Medicine
Title: Neuroendocrine differentiation of prostate cancer: Translational medicine research and training of physician scientists |
| 09/14/17 | Place: University of Colorado Denver Cancer Center
Title: Neuroendocrine differentiation: An emerging mechanism of therapy resistance and tumor recurrence |
| 07/04/17 | Place: China Jiliang University School of Pharmacy |

Title: Title: Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery
 06/16/17 Place: Hong Kong University School of Chinese Medicine
 Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery
 06/12/17 Place: Jinan University School of Medicine
 Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer
 05/15/17 Place: Northwestern University School of Medicine, Department of Pathology
 Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance
 10/11/2016 Place: Chromatin and Epigenetics Symposium (Purdue)
 Title: PRMT5 is a master epigenetic activator of DNA damage response and a therapeutic target for prostate cancer radiosensitization (presented by Jake Owens)
 05/10/16 Place: 2016 American Urological Association (AUA) meeting
 Title: Protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic regulator of androgen receptor in prostate cancer
 01/07/16: Place: Jinan University the first affiliated hospital
 Title: How to conduct scientific research
 12/27/15: Place: Northwest University of Agriculture and Forestry
 Title: Bimolecular fluorescence complementation (BiFC): Current status and future perspectives
 01/05/15: Place: Tongling First People's Hospital
 Title: Advances in prostate cancer diagnosis and treatment- A comparative analysis between China and America
 12/29/14 Place: Jinan University the first affiliated hospital
 Title: Targeting PRMT5 for prostate cancer radiosensitization
 05/18/14 Place: Mayo Clinic, Departments of Radiation Oncology
 Title: Mechanism and targeting of radiotherapy-induced neuroendocrine differentiation for prostate cancer treatment
 03/25/14 Place: Tongling 4th Hospital, Wannan Medical College
 Title: Advances in prostate cancer diagnosis and treatment
 02/27/14 Place: UCLA, Departments of Pathology and Laboratory Medicine
 Title: Targeting neuroendocrine differentiation as a novel radiosensitization approach for prostate cancer treatment
 10/9//13 Place: Cancer Hospital, Hefei Institutes of Physical Science Chinese Academy of Sciences
 Title: Development of radiosensitizers: An urgent need for prostate cancer radiotherapy
 05/24/13 Place: Hefei Chinese Academy of Sciences Cancer Hospital
 Title: Impact of neuroendocrine differentiation in prostate cancer radiotherapy
 05/20/13 Place: Huazhong University of Science and Technology Union Hospital Cancer Institute

- 05/17/13 Title: Radiation-induced neuroendocrine differentiation in prostate cancer: From bench to bedside
Place: Jinan University School of Medicine
- 05/14/13 Title: Neuroendocrine differentiation (NED) in prostate cancer cells: From basic science to clinical practice
Place: Northwestern Agriculture and Forestry University (NWAUFU): 2013 Purdue-NWAUFU Center Symposium
- 04/17/13 Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives
Place: 2013 Drug Discovery Chemistry in San Diego: Sixth Annual Protein-Protein Interactions (Targeting PPI for Therapeutic Interventions)
- 02/05/13 Title: Bimolecular fluorescence complementation (BiFC) as a novel imaging-based screening for inhibitors of protein-protein interactions.
Place: Tongji Hospital, Huazhong University of Science and Technology
- 10/25/12 Title: Neuroendocrine differentiation (NED): A therapeutic challenge in prostate cancer management
Place: Wright State University Department of Biochemistry and Molecular Biology
- 06/06/12 Title: Bimolecular fluorescence complementation (BiFC): An imaging tool for visualization of molecular events
Place: Jiangsu University School of Medical Technology and Laboratory Medicine
- 06/4/12 Title 1: Mechanisms and targeting of radiation-induced neuroendocrine differentiation
Title 2: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
Place: Chinese Academy of Sciences (Hefei)
- 05/31/12 Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
Place: Tongling Traditional Chinese Medicine Hospital
- 05/18/12 Title: Recent advances in prostate cancer diagnosis and treatment
Place: Shanghai Center for Plant Stress Biology of Chinese Academy of Sciences
- 04/25/12 Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
Place: University of Western Ontario
- 03/13/12 Title: Radiotherapy-induced neuroendocrine differentiation: Implications in prostate cancer progression and treatment
Place: Mayo Clinic Department of Urology
- 07/11/11 Title: Mechanisms and targeting of therapy-induced neuroendocrine differentiation for prostate cancer treatment
Place: Jinan University Medical School

- 07/10/11 Title: Bimolecular fluorescence complementation: An emerging technology for biological research
Place: Sun-Yat-sun University Medical School
Title: Mechanisms and targeting of therapy-resistant prostate cancer
- 02/09/11 Place: Tulane University Medical School
Title: Mechanisms and targeting of therapy-resistant prostate cancer
- 01/17/11 Place: Penn State University College of Medicine
Title: Bimolecular fluorescence complementation (BiFC): Current Challenges and Future Developments
- 12/07/10 Place: Purdue University BiFC Workshop
Title: Bimolecular fluorescence complementation: principle, experimental design and data analysis
- 11/18/10 Place: UT Austin College of Pharmacy
Title: Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimerization in living cells and *C. elegans*
- 09/28/10 Place: Nanjing University Medical School
Title: Multicolor bimolecular fluorescence complementation (BiFC): A novel high throughput screening method for protein-protein interactions
- 09/25/10 Place: Wannan Medical College
Title: Mechanisms and targeting of therapy-resistant prostate cancer
- 09/16/10 Place: Wuhan Institute of Virology
Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives
- 09/13/10 Place: Beijing University Cancer Hospital
Title: Mechanisms and targeting of therapy resistant prostate cancer
- 09/08/10 Place: Purdue University BIG Symposium
Title: Fluorescence complementation: An emerging tool for visualization of molecular events in living cells and animals
- 10/16/09 Place: Southern China Agriculture University
Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
- 10/19/09 Place: Sun Yat-sen University Zhongshan Medical School
Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
- 10/26/09 Place: Bengbu Medical College
Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
- 10/28/09 Place: Nanjing University Medical School
Title: Seeing is believing: visualization of protein-protein interactions using bimolecular fluorescence complementation (BiFC),

05/07/09 Place: University of Chicago Graduate Program of Physiology
Title: Bimolecular fluorescence complementation (BiFC) analysis in living cells and living animals,

02/02/09 Place: Indiana University Medical School, Department of Biochemistry
Title: Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy

12/08/08 Place: University of Virginia Cancer Center
Title: Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy

11/25/08 Place: 7th International Conference on Photonics and Imaging in Biology and Medicine (Wuhan, China), Nov 24-27, 2008
Title: Fluorescence complementation: an emerging technology in biomedical research (presentation and panel discussion)

10/15/08 Place: 4th Modern Drug Discovery & Development Summit (San Diego, 10/15/08-10/17/08)
Title: Multicolor bimolecular fluorescence complementation in drug discovery

11/29/07 Place: UMDNJ-SOM Stratford
Title: Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimerization in living cells and living animals

11/28/07 Place: The Children's Hospital of Philadelphia and the University of Pennsylvania
Title: Molecular regulation and targeting of ATF2 nucleocytoplasmic shuttling

11/13/07 Place: Department of Biochemistry, Purdue University
Title: AP-1 biology, pathology, and technology

10/30/07 Place: Fluorescent proteins and Biosensors Symposium at HHMI Janelia Farm
Title: BiFC-FRET, a novel assay for visualization of ternary complexes in living cells

08/07/07 Place: International Microscopy & Microanalysis 2007 at Ft. Lauderdale
Title: Bimolecular fluorescence complementation (BiFC) and beyond

02/09/07 Place: Montana State University Department of Microbiology
Title: Functional analysis of AP-1 dimerization by bimolecular fluorescence complementation

11/01/06 Place: Vanderbilt University Institute of Chemical Biology
Title: Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system

10/04/06 Place: University of Illinois at Chicago School of Medicine
Title: Bimolecular fluorescence complementation: principle and applications

07/17/06 Place: Huazhong University of Science and Technology Tongji Medical College

Title: Bimolecular fluorescence complementation: principle and applications
 03/14/06 Place: University of Toronto Western Research Institute
 Title: Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system
 09/30/05 Place: Eli Lilly, Indianapolis
 Title: Identification of new fluorescent protein fragments for BiFC analysis under physiological conditions
 03/10/05 Place: Purdue University, School of Health Science, Purdue University
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
 09/02/04 Place: Illinois State University, Department of Biology
 Title: Role of *C. elegans* Fos and Jun homologs in development.
 08/13/04 Place: Cold Spring Harbor (Cold Spring Harbor Image Course)
 Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals using a novel using bimolecular fluorescence complementation (BiFC) approach
 05/07/04 Place: Purdue University, Department of Chemistry
 Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals
 01/14/04 Place: Purdue University, Department of Biological Science
 Title: Seeing is believing: visualization of transcription factor interactions in living cells and in living animals
 12/04/03 Place: Indiana University at Bloomington, Department of Biology
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
 11/07/03 Place: Purdue Cancer Center (Purdue Cancer Center Director's Advisory council)
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions in cancer research
 09/04/03 Place: Purdue Cancer Center (Annual Scientific Retreat)
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
 03/11/03 Place: Cincinnati Children's Hospital, Division of Experimental Hematology
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
 03/04/03 Place: Harvard Medical School, MGH, Laboratories of Photomedicine
 Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
 02/24/03 Place: Medical University of South Carolina, School of Pharmacy

	Department of Pharmaceutical Science
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
02/19/03	Place: University of Texas M.D. Anderson Cancer Center, Department of Molecular Therapeutics
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
02/06/03	Place: Ohio State University, School of Medicine Department of Physiology and Cell biology
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
12/28/02	Place: Purdue University Cancer Center
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
07/20/00	Place: Bengbu Medical College, Bengbu, China
	Title: Recent progress in the activation mechanisms of Raf by Ras
07/15/00	Place: Tongji Medical University, Wuhan, China
	Title: Cloning and functional characterization of a novel type phospholipase C (PLC- ϵ)

Development of Intellectual Property

- A novel fluorescent protein for protein-protein interaction studies, 65557.P1.US Patent filed on July 16, 2010
- Methods for identifying protein-protein interactions, 66261-01-2013 US Patent filed on June 13, 2013
- Methods for identifying protein-protein interactions, 66261-02-2014 US Patent filed on June 14, 2014
- Bimolecular fluorescence complementation (BiFC)-based screen for discovery of PRMT5 inhibitors. Provisional Patent Application No 62/121,627 filed on February 27, 2015

Publications

a. Peer-reviewed Research Articles

Vickman, R.E., Yang, J., Atallah, N., Cresswell, G.M., Zheng, F., Zhang, C., Doerge, R.W., Crist, S.A., Mesecar, A.D., Hu, C.D., and Ratliff, T. L. Cholesterol sulfotransferase SULT2B1b modulates sensitivity to death receptor ligand TNF alpha in castration resistant prostate cancer. *Molecular Cancer Research* (2019), 17:1253-1263.

Zeng, L., Wang, W.H., Arrington, J., Shao, G., Geahlen, R.L., Hu, C.D. and Tao, W.A. Identification of upstream kinases by fluorescence complementation mass spectrometry. *ACS Central Sci*, 3:1078-1085 (2017).

Deng, X., Shao, G., Zhang, H.T., Li, C., Zhang, D., Cheng, L., Elzey, B.D., Pili, R., Ratliff, T.L., Huang, J., Hu, C.D. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene*, 36:1223-1231 (2017)

Vickman, R.E., Christ, S.A., Kerian, K., Eberlin, L., Coos, R.G., Burcham, G.N., Buhman, K.K., Hu, C.D., Mesecar, A.D., Cheng, L., Ratliff, T.L. Cholesterol sulfonation enzyme, SULT2B1b, modulates AR and cell growth properties in prostate cancer. *Mol Cancer Res*, 14:776-786 (2016)

Zhang, H., Zeng, L., Tao, A.W., Zha, Z., and Hu, C.D. The E3 ubiquitin ligase CHIP mediates ubiquitination and proteasomal degradation of PRMT5. *Biochem Biophys Acta*, 1863:336-346 (2016)

Xu, D., Zhan, Y., Qi, Y., Cao, B., Bai, S., Xu, W., Gambhir, S.S., Lee, P., Sartor, O., Flemington, E.K., Zhang, H., Hu, C.D., and Dong, Y. Androgen receptor splice variants dimerize to transactivate target genes. *Cancer Res*, 75:3663-3671 (2015)

Suarez, C.D., Deng, X., and Hu, C.D. Targeting CREB inhibits radiation-induced neuroendocrine differentiation and increases radiation-induced cell death in prostate cancer cells. *Am J Cancer Res*, 4:850-861 (2014)

Zhang, H., Zha, Z. and Hu, C.D. Transcriptional activation of PRMT5 by NF- κ B is required for cell growth and negatively regulated by the PKC/c-Fos signaling in prostate cancer cells. *Biochem Biophys Acta*, 1839:1330-1340 (2014)

Hsu, C. and Hu, C.D. Transcriptional activity of c-Jun is critical for the suppression of AR function. *Mol. Cell. Endocrinol.* 372:12-22 (2013)

Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. *J. Biol. Chem.* 287:12455-12688 (2012)

Hsu, C. and Hu, C.D. Critical role of an N-terminal end nuclear export signal in regulation of ATF2 subcellular localization and transcriptional activity. *J. Biol. Chem.* 287:8621-8632 (2012)

Deng, X., Elzey, B.D, Poulson, J.M., Morrison, W.B., Ko, S.C., Hahn, N.M., Ratliff, T.L., and Hu, C.D. Ionizing radiation induces neuroendocrine differentiation in vitro, in vivo and in human prostate cancer patients. *Am. J. Cancer. Res.* 1:834:844 (2011)

Xing, J., Wang, S., Lin, F., Pan, W., Hu, C.D., and Zheng, C. A comprehensive characterization of interaction complexes of Herpes Simplex Virus type 1 ICP22, UL3, UL4 and UL20.5. *J. Virol.* 85:1881-1886 (2011)

- Kodama, Y. and Hu, C.D. An improved bimolecular fluorescence complementation assay with high signal-to-noise ratio. *Biotechniques*, 49:793-805 (2010)
- Le, T.T, Duren, H.M., Slipchenko, M.N., Hu, C.D. and Cheng, J.X. Label-free quantitative analysis of lipid metabolism in living *Caenorhabditis elegans*. *J. Lipid Res.* 51:672-677 (2010)
- Hiatt, S.M., Duren, H.M. Shyu, Y., Ellis, R.E., Hisamoto, N., Matsumoto, K., Kariya, K., Kerppola, T.K., and Hu, C.D.* *C. elegans* FOS-1 and JUN-1 regulate *plc-1* expression to control ovulation. *Mol. Biol. Cell* 20:3888-3895 (2009)
- Xu, Y., Yang W.H., Gerin, I., Hu, C.D., Hammer, G.D., and Koenig, R.J. DAX-1 and steroid receptor RNA activator (SRA) function as transcriptional coactivators for steroidogenic factor-1 in steroidogenesis. *Mol. Cell. Biol.* 29:1719-1734 (2009)
- Yuan, Z., Gong, S., Song, B., Mei, Y., Hu, C., Li, D., Thiel, G., Hu, C.D., and Li, M. Opposing role for ATF2 and c-Fos in c-Jun-mediated apoptosis induced by potassium deprivation in cerebellar granule neurons. *Mol. Cell. Biol.* 29:2431-2442 (2009)
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b. Invited Peer-reviewed Review Articles

Hu, C.D. , Choo, R., and Huang, J. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front Oncol*, Apr 14;5:90. Doi: 10.3389/fonc.2015.00090 (2015)

Kodama, Y. and Hu, C.D. Bimolecular fluorescence complementation (BiFC): A 5-year update and future perspectives. *Biotechniques*, 53:285-298 (2012)

Shyu, Y. and Hu, C.D. Recent advances in fluorescence complementation-based technologies. *Trends Biotechnol.* 26:622-630 (2008)

Hu, C.D., Zhang, X.-H., and Bi, E.-H. Role of macrophages in the modulation of NK activity. *Foreign Medicine, Part of Immunology*, 10, 16-20 (1987) (in Chinese).

c. Invited Review Article (Not peer-reviewed)

Shyu, Y., Akasaka, K., and Hu, C.D.*. Bimolecular fluorescence complementation (BiFC): A colorful future in drug discovery. *Sterling-Hoffman Life Science Journal*, July, 2007. (<http://www.sterlinglifesciences.com/newsletter/articles/article006.html>).

d. Book Chapters

Pratt, E.P.S., Owens, J.L., Hockerman, G.H., and Hu, C.D. Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interactions and assessment of subcellular localization in live cells. High resolution imaging of proteins in tissues and cells: light and electron microscopy methods and protocols (Ed, Schwartzbach, S.D., Skalli, O., and Schikorski, T.), Springer (2015).

Ejendal, K.F.K., Conley, J.M., Hu, C.D. and Watts, V.J. Bimolecular fluorescence complementation analysis of G protein-coupled receptor dimerization in living cells. *Methods Enzymol.*, 521:259-279 (2013).

Kodama, Y. and Hu, C.D.* Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interaction: How to calculate signal-to-noise ratio. *Methods Cell Biol.*, 113: 107-121 (2013).

Vidi, P.A., Przybyla, J., Hu, C.D., and Watts, V.J. Visualization of G protein-coupled receptor (GPCR) interactions in living cells using bimolecular fluorescence complementation (BiFC). *Curr. Protoc. Neurosci.*, Unit 5.29.1-5.29.15 April 2010.

Hu, C.D., Grinberg, A.V. and Kerppola, T.K. Visualization of Protein Interactions in Living Cells Using Bimolecular Fluorescence Complementation (BiFC) Analysis. (ed. Coligan JE, Dunn BM, Speicher DW, Wingfield PT) *Curr. Protoc. Protein Sci.* 41:19.10.1-19.10.21. Hoboken, John Wiley & Sons, 2005.

Hu, C.D. and Kerppola TK. Direct visualization of protein interactions in living cells using bimolecular fluorescence complementation analysis. *Protein-Protein Interactions* (ed. P. Adams and E. Golemis), Cold Spring Harbor Laboratory Press. Pp673-693, 2005.

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Kataoka, T., Kariya, K., Yamawaki-Kataoka, Y., Hu, C.D., Shirouzu, M., Yokoyama, S., Okada, T., and Shima, F. Isoprenylation-dependent and independent interaction of Ras with its effectors. In Kuzumaki, N. Cytoskeleton and G-Protein in the Regulation of Cancer. *Hokaido University Medical Library Series*, 37, 141-146 (1998).

Current and Past Grant Support at Purdue University as PI or Co-PI

Active Grant Support

Title: Role and targeting of PRMT5 in prostate cancer

Source: NCI RO1

Role: Contact PI (**Multi-PI** with Chenglong Li and Jiaoti Huang)

Total Cost Requested: \$2,590,428

Grant Period: 06/09/2017-05/31/2022

Goal: The goal of this proposal is to elucidate the molecular mechanisms by which PRMT5 promotes prostate cancer cell growth, improve the potency of BLL3.3, and

conduct a preclinical evaluation of PRMT5 inhibition for castration resistant prostate cancer treatment.

Title: Co-targeting of androgen synthesis and androgen receptor expression as a novel treatment for castration resistant prostate cancer

Source: DoD (2015 PCRP)

Role: PI

Grant Period: 08/01/16-07/30/20

Total Cost: \$557,000

Goal: The goal of this project is to evaluate whether co-targeting of androgen synthesis by abiraterone and androgen receptor expression via PRMT5 inhibition is an effective treatment for CRPC.

Title: Discovery of novel therapeutic targets for neuroendocrine prostate cancer

Source: Department of MCMP Research Enhancement Award, Purdue University

Period: 04/01/17-12/31/19

Total amount awarded: \$50,000

Role: PI

Goal: The goal of this award is to discovery altered ion channels in neuroendocrine prostate cancer as therapeutic targets

Title: Targeted RO1: Molecular and genetic analysis of PRMT5 in neuroendocrine prostate cancer

Source: EVPRP Targeted RO1

Period: 12/01/15-10/31/19

Total amount awarded: \$30,000

Role: PI

Goal: The goal of this project is to generate preliminary data for a RO1 proposal to determine the role of PRMT5 and its cofactor MEP50 in neuroendocrine differentiation of prostate cancer cells and validate whether targeting PRMT5/MEP50 is an effective therapeutic approach for neuroendocrine prostate cancer

Title: Discovery of inhibitors to disrupt the interaction of PRMT5 with its cofactor pICln for prostate cancer treatment

Source: Purdue University Center for Cancer Research

Period: 08/01/18-07/30/19

Total amount awarded: \$15,000

Role: PI (Co-I: Dr. Wen Jiang)

Goal: This support is to develop a BiFC-based high throughput screen assays for identification of inhibitors to disrupt the PRMT5/pICln interaction.

Title: Deep neural network-assisted protein structure modeling for drug development from low resolution 3D cryo-electron microscopy maps

Source: Purdue Institute for Drug Discovery

Period: 12/01/18-11/30/20

Total amount awarded: \$150,000

Role: Co-PI with Dr. Daisuke Kihara (computational biologist) and Dr. Wen Jiang (cryo-EM expert)

Goal: This support is to develop a deep learning method to predict cryo-EM structures using PRMT5/MEP50 and PRMT5/pICln interactions as a model and to identify novel interfaces for drug discovery.

Past Grant Support at Purdue University (2003-2018):

External Funding

Title: Temporal and spatial interaction patterns of bZIP proteins in living *C. elegans*

Source: National Science Foundation (MCB 0420634)

Role: PI

Grant Period: 06/04/07 – 07/30/08

Total Cost: \$4,750

Goals: The goal of this REU was to support Summer High School Student Research on the funded NSF *C. elegans* project.

Title: Regulation of *c-jun* transcription by ATF2 in cardiomyocyte in response to stress

Source: American Heart Association (AHA 0655570Z)

Role: PI

Grant Period: 07/01/06 – 06/30/08

Total Cost: \$132,000

Goals: The goal of this project was to study the role of ATF2 subcellular localization in regulating *c-jun* transcription in rat cardiomyocytes in response to hypoxia and oxidative stress.

Title: Interplay of CREB and ATF2 in radiation-induced prostate cancer transdifferentiation

Source: DoD Prostate Cancer Idea Development Award (PC073981)

Role: PI

Grant Period: 06/01/08-05/30/11

Total Cost: \$571,875

Goals: The goal of this project was to determine how CREB and ATF2 oppose each other at the transcriptional level to regulate radiation-induced neuroendocrine differentiation in prostate cancer cells.

Title: Improvement of BiFC technology and its application in the TLR signal transduction pathway (International collaborative project)

Source: Natural Science Foundation of China

Role: PI

Grant Period: 01/01/11-12/31/13

Total Cost: \$35,000

Goal: The goal of this project was to collaborate with Dr. Yayi Hou at Nanjing University to apply BiFC technologies to study the TLR signaling in immune system.

Title: D2 receptor-induced sensitization of adenylyl cyclase

Source: NIH RO1 (National Institute of Mental Health)

Role: Co-Investigator (PI: Val Watts)

Grant Period: 08/15/11-04/31/14

Total Cost: \$770,922

Goal: The goal of this RO1 grant was to investigate the molecular mechanisms underlying D2 receptor-induced sensitization of adenylyl cyclase. As a Co-Investigator, Dr. Hu provided his expertise in BiFC technology to help the analysis of D2 receptor interacting proteins.

Title: New mechanism for modulating opioid receptor mediated analgesia

Source: Showalter Trust Award

Role: Co-PI (PI: Richard van Rijn)

Total Cost: \$75,000

Grant Period: 07/01/14-06/30/16

Goal: The goal of the project is to study the mechanisms and regulation of opioid receptors and to develop agents targeting protein-protein interactions using BiFC-based technologies.

Title: Targeting PRMT5 as a novel radiosensitization approach for primary and recurrent prostate cancer radiotherapy

Source: DoD (2011 PCRP)

Role: PI

Grant Period: 08/01/12-07/30/16

Total Cost: \$559,269.91

Goal: The goal of this grant is to determine that PRMT5 is a novel therapeutic target for prostate cancer radiotherapy.

Title: Identification of the Ac5 sensitization interactome using BiFC

Source: NIH R21 (National Institute of Mental Health)

Role: Multi-PI with Val Watts

Total Cost: \$463,111

Role: Multi-PI

Grant Period: 07/19/13-06/15/17

Goal: The goal of this project is to develop BiFC-based cDNA library screening for identification of Ac5 interacting proteins.

Title: Targeting neuroendocrine differentiation for prostate cancer radiosensitization

Source: DoD (2012 PCRP)

Grant Period: 09/30/13-09/30/17

Total Cost: \$559,055

Role: PI

Goal: The goal of this grant is to use CREB targeting as a model to determine whether targeting radiation-induced NED can be explored as a novel radiosensitization approach for prostate cancer radiotherapy.

Title: Development of novel small molecule inhibitors targeting protein arginine methyltransferase 5

Source: CTSI (Indiana Drug Discovery Alliance)

Period: 12/01/14-12/30/17 (No cost extension for current year)

Total amount awarded: \$10,000

Role: PI

Goal: The goal of this project is to discover inhibitors for disruption of PRMT5/MEP50 interaction using BiFC-based screening.

Title: Developing novel therapeutic strategies for castration-resistant prostate cancer

Source: DOD (2013 PCRCP)

Total Cost: \$525,568

Role: Co-PI (PI: Kavita Shah)

Grant Period: 08/01/14-07/30/18

Goal: The goal of this project is to determine whether targeting LIMK2 can be used to treat CRPC.

Internal Funding

Title: Biochemical and cryo-EM analysis of PRMT5 in complex with its cofactor pICln

Source: Purdue University Center for Cancer Research

Period: 05/01/18-04/30/19

Total amount awarded: \$15,000

Role: PI

Goal: This support is to solve cryo-EM structure of PRMT5 in complex pICln, a novel cofactor for PRMT5.

Title: Generation of MEP50 transgenic mice for prostate cancer research

Source: Purdue University Center for Cancer Research

Period: 05/01/18-11/30/18

Total amount awarded: \$4,500

Role: PI

Goals: This support is to generate MEP50 transgenic mice for prostate cancer research.

Title: PRMT5 in prostate cancer development, progression and therapy response

Source: EVPRP Targeted RO1

Period: 12/01/15-05/30/17

Total amount awarded: \$30,000

Role: PI

Goals: The goal of this project is to generate genetically modified mouse models (PRMT5 transgenic mice and PRMT5 Floxed mice) for prostate cancer research.

Title: Discovery of PRMT5 target genes in neuroendocrine prostate cancer

Source: Purdue University Center for Cancer Research

Period: 12/01/16-06/30/17

Total amount awarded: \$10,000

Role: PI

Goals: The goal of this grant is to perform RNA-seq and ChIP-seq to identify target genes of PRMT5 contributing to the development of neuroendocrine prostate cancer.

Title: Mass spectrometric identification of pCREB interacting proteins in prostate cancer cells LNCaP

Source: Purdue Cancer Center Small Grant (Indiana Elks, Inc)

Role: PI

Grant Period: 03/01/08-02/28/09

Total Cost: \$10,000

Goals: The goal of this project was to identify cytoplasmic interacting proteins of pCREB using mass spectrometry.

Title: Identification of interacting proteins and phosphorylation of ATF2 implicated in prostate cancer transdifferentiation

Source: Purdue Research Foundation

Role: PI

Grant Period: 06/01/08-05/30/09

Total Cost: \$16,835

Goals: The goal of this PRF support was to use mass spectrometry to identify interacting proteins and phosphorylation of ATF2 in the cytoplasm in radiation-induced neuroendocrine cells and to determine how ATF2 nuclear import is impaired by ionizing radiation.

Title: Targeting of prostate cancer transdifferentiation and proliferation via a novel DNA nanotube-based nucleic acid delivery

Source: Lilly Seed Grant

Role: PI

Grant Period: 01/01/09-12/31/10

Total cost: \$100,000

Goal: The goal of this grant was to collaborate with Dr. Chengde Mao to develop DNA nanotube-based delivery of siRNAs.

Title: Targeting neuroendocrine differentiation as a novel therapeutics in prostate cancer treatment

Source: Purdue Research Foundation

Role: PI

Grant Period: 08/01/2010-07/30/2011

Total cost: \$17,000

Goal: The goal of this project was to support graduate student Chris Suarez to study the role of radiation-induced neuroendocrine differentiation in radioresistance.

Title: Ionizing radiation induces neuroendocrine differentiation in nude mice prostate cancer xenograft models: Implication in disease progression

Source: Purdue University Center for Cancer Research

Role: PI

Grant Period: 01/01/09-12/31/11

Total Cost: \$50,000

Goals: The goal of this project was to use xenograft nude mice prostate cancer cell models to investigate whether CREB and ATF2 contribute to radiation-induced neuroendocrine differentiation *in vivo* and to determine whether radiation induces changes of pCREB and ATF2 subcellular localization.

Title: Generation of cytoplasmic-localized ATF2 transgenic mice for prostate cancer research

Source: Purdue University Center for Cancer Research

Role: PI

Grant Period: 06/01/10-05/30/11

Total cost: \$2,000

Goal: The goal of this support was to supplement the cost for making a transgenic mouse strain using the shared transgenic mouse facility

Title: Chromogranin A, a novel biomarker to monitor radiation-induced neuroendocrine differentiation in prostate cancer patients

Source: The Indiana Clinical and Translational Science Institute (CTSI)-Purdue Project Development Program

Role: PI

Grant Period: 06/01/10-05/30/12

Total cost: \$10,000

Goal: The goal of this support was to conduct a pilot clinical study to determine the effect of radiotherapy on neuroendocrine differentiation in prostate cancer patients.

Title: Acquisition of a Nikon A1 Confocal Microscope

Source: Lilly Seed Grant, College of Pharmacy

Role: PI

Grant Period: 07/01/11-06/30/12

Total amount awarded: \$300,000

Goal: The goal of this support was to acquire Nikon A1 confocal microscope to set up a Pharmacy Live Cell Imaging Facility

Title: Ultrahigh performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry

Source: Office of the Vice President for Research (OVPR) Laboratory Equipment Program

Role: Co-PI (PI: Andy Tao)

Period: Purchased by May 31, 2014
Total amount awarded: \$100,000
Goal: The goal of this internal support was to acquire UHPLC.

Title: Generation of PRMT5 transgenic mice for prostate cancer research
Source: Purdue University Center for Cancer Research Shared Resource Grant
Period: 12/01/15-12/31/16
Total amount awarded: \$3,100
Role: PI
Goal: The goal of this project is to use the transgenic mouse facility to generate PRMT5-overexpressing mice.

Past Grant Support at Kobe University as PI (1998-2001): \$80,000

Title: Regulation of Rap1A activity by phosphorylation
Source: Kobe University, President Young Investigator Award
Role: PI
Grant Period: 04/01/98-03/30/99
Total Cost: ~\$10,000 (for supplies)
Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to antagonize the function of Ras in activating Raf-1.

Title: Effect of phosphorylation on the regulation of Rap1A activity
Source: Ministry of Education, Science, Sports, and Culture of Japan
Role: PI
Grant Period: 04/1/98 - 03/30/99
Total Cost: ~\$ 10,000 (for supplies)
Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to activate downstream effectors such as Raf-1 and B-Raf.

Title: Activation mechanism of phospholipase C (PLC- ϵ) by Ras
Source: Hyogo Science and Technology Association
Role: PI
Grant Period: 04/01/00 – 03/30/01
Total Cost: ~\$ 30,000 (for supplies)
Goals: The goal of this project was to investigate whether Ras regulates catalytic activity of PLC ϵ directly by their physical interaction. The approach was to use *in vitro* reconstitution system.

Title: Regulation of a novel phospholipase C (PLC- ϵ) by Ras
Source: Japan Society for the Promotion of Science
Role: PI

Grant Period: 04/01/00 – 03/30/01

Total Cost: ~\$ 30,000 (for supplies)

Goals: The goal of this project was to investigate how Ras regulates catalytic activity of PLC ϵ and determine whether membrane anchoring of PLC- ϵ by Ras is sufficient for the activation of PLC- ϵ . This project was primarily focused on the studies in cells.

Note: Research grants in Japan do not provide personnel support. All faculty members and staff are supported by the government. Postdoctoral fellows and graduate students can only be supported by fellowships.

Fellowships/Awards received by trainees

- Susan Fox, Ross Fellowship (08/2003-07/2005): ~\$56,000
- Susan Fox, 2nd place of graduate student presentation
2004 Walther Cancer Institute Annual Retreat (Aug. 5-7)
- John Y Shyu, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) (\$866)
- Susan Fox, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) (\$866)
- Zeina Shtaih, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program), \$4,000
- Jonathan Smith, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program), \$2,000
- Jonathan Smith, NSF, Summer Research Fellowship (REU), \$6,000 (IC \$1,000)
- Apinya Supatkul, Prepharmacy Student, 2006 Summer Research Fellowship (\$3,000)
- John Shyu, 1st Place of 2007 Purdue University Graduate Student Research Competition (\$500)
- Holli Duren, Travel Award from 16th International Worm Meeting (June 27-July 1, 2007, UCLA) (\$300)
- John Shyu, John Koo Travel Award for Fall 2007 (\$1,000)
- Holli Duren, Kienly Award for outstanding graduate student teaching assistant 2007, MCMP (\$750)
- Holli Duren, 2007 PRF Summer Fellowship (\$2,472.09)
- Holli Duren, 2008-2009 PRF Fellowship (\$16,835)
- Chris Suarez, Purdue University Doctoral Fellowship (08/2007-07/2009): ~\$56,000
- Susan Fox, Bilsland Dissertation Fellowship (07/2008-12/2008): ~\$14,000
- John Shyu, Bilsland Dissertation Fellowship (07/2008-12/2008): ~\$14,000
- Holli Duren, 2008-2009 Graduate Student Award for Outstanding Teaching at Purdue University
- Holli Duren, 2009 Charles J. Paget Travel Award: \$1,000

- Yutaka Kodama, 04/01/09-03/31/10 TOYOBO Postdoctoral Fellowship (~\$34,000)
- Akhil Shenoy (Texas AM U) , 06/01/09-07/26/09, Purdue SROP: \$5,000
- Yutaka Kodama, 04/01/10-03/31/12, JSPS Postdoctoral Fellowship (~\$80,000)
- Holli Duren, Bilsland Dissertation Fellowship (01/01/2010-06/30/2010): \$14,000
- Chih-chao Hsu, Ronald W. Dollens Graduate Scholarship in Life Sciences (08/2010-05/2011): \$5,000
- Yeo Jin Choi, Purdue University College of Pharmacy 2010 Summer Undergraduate Research Fellowship: \$3,000
- Chris Suarez, 2010 PRF Fellowship: \$17,000
- Chih-chao Hsu, Travel Award for conference attendance from PULSe, \$250 (2012)
- Chih-chso Hsu, 2011 PRF Fellowship: \$17,000
- Chris Suarez, 2011 Paget Travel Award from MCMP department, \$1,000
- Chris Suarez, 2012 AACR Minority Scholar in Cancer Research Award for participation in the Advances in Prostate Cancer Research conference (Feb 6-9, 2012), \$1,800
- Chih-chao Hsu, Bilsland Dissertation Fellowship (09/01/12-12/31/12): \$14,000
- Huantin Zhang (visiting student from Jinan University, China): Graduate Student Study Abroad Scholarship: \$9,000 (2012)
- Huantin Zhang (visiting student form Jinan University, China): China Scholarship Council (CSC): \$33,600 (awarded for two years 10/2013-9/2015, but stay for one year)
- Limin Zhang (PharmD student): 2014 Summer Undergraduate Research Fellowship (Lilly Endowment Fellowship): \$4,800
- Jake Owens, Ross Graduate Fellowship (2014-2015), \$38,000
- Athena He: 2016 LSAMP Summer Undergraduate Research Fellowship: \$4,800
- Jonathan Malola: 2017 College of Pharmacy Summer Undergraduate Research Fellowship: \$4,800
- Jake Owens, CTSI Predoctoral fellowship (07/01/17-06/30/19): \$24,500/year plus tuition remission
- Jake Owens, 2nd place of Presentation Award at the 2017 Indiana Urological Research Symposium: \$500
- Elena Beketova, 2018 Purdue Research Foundation (PRF) Graduate Fellowship: \$17,000 plus tuition remission
- Elena Beketova, 2018 Purdue University Center for Cancer Research Travel Award to 2018 AACR meeting, \$1,000
- Jake Owens, 2018 MCMP Koo Travel Award to 2018 SBUR meeting, \$1,500
- Samantha Tinsley, Purdue University Graduate School Andrew Fellowship (08/2017-07/2018): \$24,000/year plus tuition remission
- Ji Yang, China Council Scholarship (10/01/18-03/31/20): \$25,200

- Yi Liu, China Council Scholarship (01/12/19-01/11/20): \$16,8000
- Jonathan Malola (3/23/2019): Outstanding Nuclear Pharmacy Student Scholarship from the 2019 NANP: \$1,000
- Jake Owens (8/15/19-12/31/19): Bilsland Dissertation Fellowship (\$11,149)
- Elena Beketova (7/1/19-6/30/20): Purdue University Cancer Center SIRG Research Assistantship (\$30,657)
- Andrew Asberry (8/1/19-7/31/21): Purdue Institute for Drug Discovery Training Program (NIH T32): Full stipend/supplement/tuition

Teaching Experience

Lectures and labs

- 5/1985-6/1987: Microbiology and Immunology labs (medical students)
- 7/1987-8/1991: Epidemiology lectures and labs in the Department of Epidemiology, School of Public Health, Tongji Medical University, Wuhan
- 4/1997-8/2000: Physiology and Molecular Biology lab (medical students) in the Department of Physiology II, Kobe University
- 8/2003-present: As a faculty member in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy, I have been involved in the teaching of the following courses. The class size for the courses ranges from 5~15 for graduate students, 30-40 for BSPS students, and 150 ~205 for professional pharmacy students. The total number of lecture hours taught is approximately 40h/year. Teaching evaluation scores have been 4.5~4.8/5.0. In April 2017, I received the first teaching award of the Pharmaceutical Sciences Teacher of the Year, which was completely nominated and voted by BSPS graduates in the College of Pharmacy.

Courses Taught

Professional Pharmacy Students:

- MCMP 305 (Biochemistry I, 2004-2006)
- MCMP 304 (Biochemistry II, 2005-2008)
- MCMP 440 (Pathophysiology, 2006-2012)
- PHRM 824 (Principles of Pathophysiology and Drug Action, 2012-present)
- PHRM 302 (Integrated Lab, Neoplasia module, 2005-2012)
- PHRM 820 (Professional Program Laboratory, Neoplasia module, 2012-2015)

Graduate students:

MCMP 618/690G (Molecular Targets of Cancer, 2007-present)
MCMP 617/690N (Molecular Targets of Neurological Disorders,
2007-present)
MCMP 514 (Biomolecular Interactions-Theory and Practice, 2009-
present)
MCMP 696 (Seminars in Medicinal Chemistry and Molecular
Pharmacology, 2006-2008)
MCMP 599 (Cumulative written examinations, 2015-present)

Undergraduate students (BS in Pharmaceutic Sciences):

PHRM 460 (Drug Discovery and Development I, 2013-present)
MCMP 544 (Drug Classes and Mechanisms, 2015-present)

Medical students (Indiana School of Medicine):

LCME 504 (Molecular Cell Biology, guest lecture of Molecular
Biology of Cancer, 2013-2015)

Courses Served as Coordinator

PHRM 824 (Principles of Pathophysiology and Drug Action,
2013-present)
MCMP 440 (Pathophysiology, 2011-2012)
MCMP 696 (Seminars in Medicinal Chemistry and Molecular
Pharmacology, 2006-2008)
MCMP 599 (Cumulative written examinations, 2015-2017)

Supervision of graduate, professional and undergraduate student research

07/1987-08/1991 Supervised 6 undergraduate students at Tongji Medical University
04/1997-08/2000 Co-supervised 7 Ph.D. students for thesis research with Professor
Tohru Kataoka and supervised 5 undergraduate summer research at
Kobe University.
09/2000-06/2003 Supervised two undergraduate students at University of Michigan
07/2003-present (1) Served as thesis adviser of 12 Ph.D. students (10 graduated)
and 2 master students (graduated) and co-adviser of 5 Ph.D.
students (4 graduated)
(2) Served as a thesis committee member of 52 graduate students
(3) Served as a committee member of 41 oral preliminary
examination
(4) Supervised 39 graduate students for lab rotations
(5) Supervised 32 professional and undergraduate student research
(6) Supervised 4 high school students for summer research

Supervision of postdoctoral fellows, visiting scholars and technicians

07/2003-present Supervised 12 postdoctoral fellows, visiting scholars and
technicians

Current lab members: 9

The lab has 1 technician, 4 PhD students, 1 pharmacy student, 1 undergraduate student and 2 visiting scholars

Service Experience

Major Administrative Services in the Purdue University Center for Cancer Research

- 2010-2013 **Seminar Director** of Purdue University Center for Cancer Research
- 2012- 2016 **Executive Committee Member** of Obesity and Cancer Discovery Group, Purdue University Center for Cancer Research
- 2010-Present **Co-leader** of Prostate Cancer Discovery Group of Purdue University Center for Cancer Research
- 2012- Present **Co-Director** of Indian Basic Urological Research (IBUR) monthly meetings
- 2013- Present **Executive Committee Member** of Purdue University Center for Cancer Research
- 2013- Present **Co-leader**, Cell Identity and Signaling (CIS) Program of Purdue University Center for Cancer Research
- 2013-present Member of Big Ten Clinical Trial GU Working Group
- 2016- Present **Director** of Small Animal Radiation Facility

Major Administrative Services at Purdue University

- 2007-2009 PULSe Graduate Program Admission Committee
- 2007-2009 PULSe Graduate Program Recruitment Committee
- 2008-present Bindley Imaging Committee (BIG)
- 2010 Faculty Search Committee for a Cancer biology and Pharmacology position in the College of Veterinary Medicine
- 2012-present PULSe Graduate Program Curriculum Committee
- 2016-present Review Panel Member of CTSI PDT (Project Development Team)

Major Administrative Services in the College of Pharmacy

- 2009-2013 Member of Assessment Committee
- 2011-2018 **Director** of Pharmacy Live Cell Imaging Facility (PLCIF)
- 2011-2018 **Chair** of PLCIF Committee
- 2012-2014 Member of Grade Appeal Committee
- 2012-present Faculty Liaison for Core-Pharmacy Courses Taught by Other Schools (BIOL110/111)
- 2013-2014 Member of Honor Degree Policy Committee
- 2013-2016 Member of Curriculum committee

2014-present	Member of Pharm.D. Academic Standards and Readmissions Committee
2017-2019	Member of Area Promotion Committee
2017-2019	Member of Nomination and Awards Committee
2017-present	Member of Strategic Plan Research and Innovation Task Force

Major Administrative Services in the Department of Medicinal Chemistry and Molecular Pharmacology

2005-2011	Member of Facility and Instrumentation Committee
2008-2009	Member of Strategy Plan Task Force
2009	Member of Biochemistry Task Force
2010	Member of Business Manger Search Committee
2011	Member of Faculty Search Committee (Pharmacology)
2012	Member of Faculty Search Committee (Pharmacology)
2012	Member of Faculty Search Committee (Epigenetics)
2010-2015	Member of Graduate Admissions and Recruiting Committee
2012-2017	Member of Graduate Assessment Committee
2015-2017	Chair of Graduate Assessment Committee
2016	Chair of faculty search committee (Cancer Biology)
2017	Chair of faculty search committee (Cancer Biology)
2018	Chair of faculty search committee (Cancer Biology)
2017-present	Member of Heads Advisory Committee
2018	Member of Curriculum Committee