AWARD NUMBER: W81XWH-16-1-0394

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

PRINCIPAL INVESTIGATOR: Chang-Deng Hu

CONTRACTING ORGANIZATION: Purdue University

REPORT DATE: August 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
August 2020	Annual Report	1 Aug 2019 – 30 July 2020

4. TITLE AND SUBTITLE
Cotargeting of Androgen Synthesis and Androgen Receptor Expression as a Novel Treatment for Castration-Resistant Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0394

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Purdue University
West Lafayette, IN 47907-2040

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer is the second leading cause of cancer death among American men in 2019. 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 further validated pICln as a cofactor of PRMT5 to regulate AR expression. We have also characterized a novelPRMT5 inhibitor from Johnson & Johnson as a potential pharmacological tool to evaluate the role of PRMT5 in prostate cancer. We will extend current findings and complete all planned experiments.

15. SUBJECT TERMS
CRPC, PRMT5, AR, AR-V7, epigenetics, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	U	46	**19b. TELEPHONE NUMBER** *(include area code)*

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1. Introduction

Prostate cancer is the second leading cause of cancer death among American men in 2019 (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-E: As reported in previous annual reports, we have 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 H4R3 and both Sp1 and Brg1 are involved. In summary, we have 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). (5) The expression of nuclear PRMT5 and pICln correlates with the expression of AR and AR-V7 in HNPC and CRPC tissues at both mRNA and protein level.

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. However, we have confirmed that inhibition of PRMT5 by either BLL3.3 or JNJ-64619178 showed similar down-regulation of AR expression and suppression of cell growth as presented below in Figure 1.

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. Since it is unclear whether the potent inhibitor will be available or not, we are beginning to evaluate other PRMT5 inhibitors that may be useful for combination treatment.

We have recently confirmed that JNJ-64619178, a SAM competitive PRMT5 inhibitor from Johnson & Johnson with higher potency, can similarly down-regulate the expression of AR and AR-V7 in 22Rv1 cells and suppressed cell growth (Fig. 1A-D), recapitulating the effect of PRMT5 knockdown and PRMT5 inhibition by BLL3.3. To determine whether JNJ-64619178 can similarly down-regulate AR expression and suppress cell growth in another CRPC cell line, we treated LNCaP95 cells with 10 μM of JNJ-64619178 or BLL3.3 and found that JNJ-64619178 even showed a better suppression of cell growth when compared with BLL3.3 (Fig. 1E). Consistent with this, JNJ-64619178 also showed better down-regulation of AR expression and inhibition of PRMT5 activity as indicated by H4R3me2s (Fig. 1F and G). Thus, we have confirmed that JNJ-64619178 is a better PRMT5 inhibitor than BLL3.3 to suppress CRPC cell growth and AR expression.

We have also found that JNJ-64619178 in combination with abiraterone or enzalutamide have some additive effect on suppression of cell growth (See Figure 4 for detail).

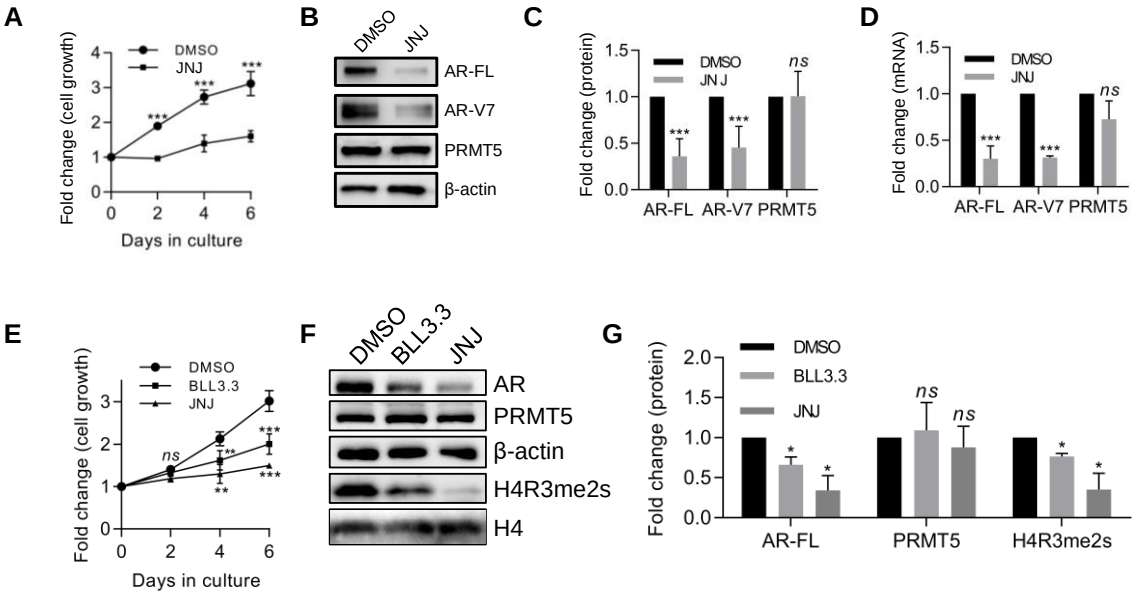

Figure 1. JJN-64619178 inhibits PRMT5 activity, down-regulates AR expression and suppresses CRPC cell growth. A. 22Rv1 cells were treated with 10 μM of JNJ-646-19178 (JNJ) or DMSO for the indicated days and cell growth was measured by MTT assays. **B**. Cells were harvested from A at day 6 and total cellular lysate was probed for expression of PRMT5, full-length AR (AR-FL) and AR-V7. **C**. Quantified protein expression from B. **D**. Similar experiments were performed as described in A and total RNA was isolated at day 6 for quantification of mRNA expression of AR-FL, AR-V7 and PRMT5 by qRT-PCR. **E**. LNCaP95 cells were treated with 10 μM of JNJ, BLL3.3 or DMSO for the indicated days and cell growth was measured by MTT assays. **F**. Cells were harvested from E at day 6 and total cellular lysate was probed for expression of PRMT5, AR, H4 and H4R3me2s. **G**. Quantified protein expression from F. For all experiments, results are mean ± SD from 3 independent experiments. Student *t*-test with Welch's correction was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Other Achievements

Differential regulatory roles of PRMT5 and pICln in cell cycle and cell survival. Since we have identified pICln as a cofactor to cooperate with PRMT5 to epigenetically regulate expression of AR and suppress cell growth in CRPC cells, we wanted to determine how cell growth was suppressed. We performed flow cytometry analysis in 22Rv1-shPRMT5#1 cell line and observed that knockdown of PRMT5 by doxycycline mainly increased G1 population, suggesting that PRMT5 mainly regulates G1 progression (Fig. 2A). However, knockdown of PRMT5 did not appear to induce cell death (Fig. 2B). We also performed similar flow cytometry analysis in 22Rv1-shpICln cell line and observed that knockdown of pICln mainly increased G2 population, suggesting that pICln may promote G2 progression (Fig. 2C). Interestingly, knockdown of pICln appeared to decrease the number of viable cells and increased the number of dead cells, suggesting that pICln may be involved in regulation of cell survival. The differential effect of PRMT5 and

pICln on cell cycle progression and cell survival suggests that PRMT5 and pICln may also have distinct cellular roles even they cooperate to regulate AR expression.

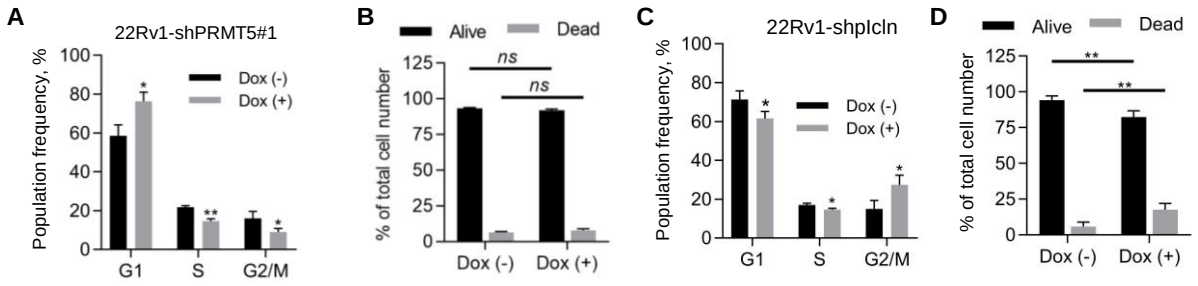

Figure 2. Effect of PRMT5 or pICln knockdown on cell cycle progression and cell death in 22Rv1 cells. **A**. Flow cytometry analysis of cells following PI staining at Day 6 of 22Rv1-shPRMT5#1 sublines in the presence (Dox(+)) or absence (Dox(-)) of doxycycline. The sub-G_1 cells were gated out. **B**. 22Rv1-shPRMT5#1 cells were similarly treated as A and viable and dead cells were determined by Trypan blue staining. **C-D.** Similar experiments were performed as described in A and B in 22Rv1-shpICln cells in which pICln knockdown was induced by Dox.

Knockdown of PRMT5 and pICln inhibits tumor cell proliferation in xenograft tumors. The distinct regulatory roles of PRMT5 and pICln *in vitro* is intriguing and prompted us to evaluate whether similar regulatory roles can be observed *in vivo*. We presented in previous annual reports that knockdown of PRMT5 or pICln suppressed the growth of 22Rv1 xenograft tumors when compared with scramble control (SC). We resected tumors and processed for formalin fixation and paraffin embedding at the end of terminating experiments. We have performed IHC analysis for the expression of PRMT5, pICln and AR and confirmed that knockdown of

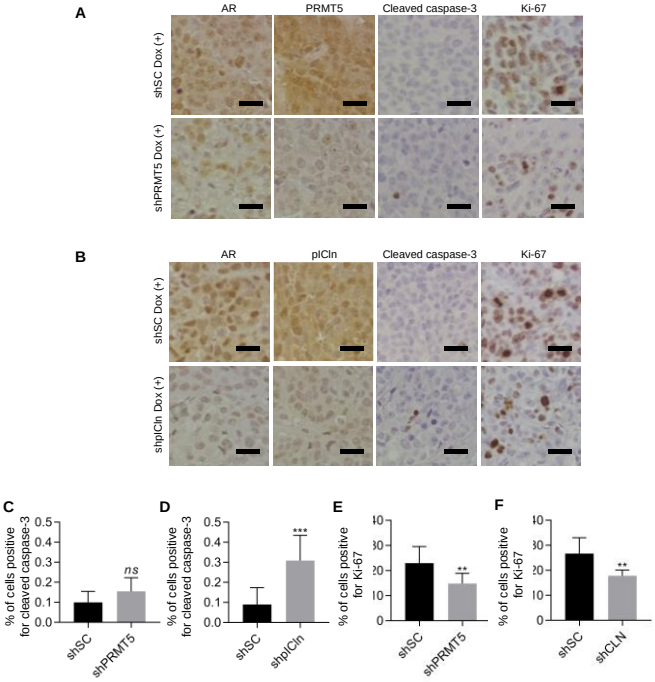

Figure 3. Knockdown of PRMT5 or pICln suppresses CRPC tumor cell prolifeartion in mice. A-B. 22Rv1 cells with Dox-inducible knockdown of PRMT5 (shPRMT5), pICln (shpICln) or scramble control (shSC) were injected subcutaneously in right flanks of surgically castrated male NRG mice. Tumor-bearing mice were treated with doxycycline in drinking water once tumors reached ~100 mm^3. At the end of treatment tumors were resected and probed for PRMT5, pICln, AR, cleaved caspase-3 and Ki-67 using IHC. Presented is the representative images. **C-F**. The percentage of positively stained cells out of total cells counted were detrmined. Scale bar indicates 40 μm. Results are mean ± standard deviation (n = 10 per group). Student *t*-test was performed to determine statistical significance. *ns* $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$.

PRMT5 or pICln indeed down-regulated the expression of AR (Fig. 3A, B). Consistent with our *in vitro* observations, knockdown of PRMT5 or pICln also suppressed tumor cell proliferation as indicated by Ki67 staining (Fig. 3E, F). However, knockdown of pICln, but not PRMT5, induced apoptosis (Fig. 3C, D). These results together indicate that PRMT5 and pICln do regulate cell growth, possibly via regulation of G1 or G2 progression but confirm that pICln may regulate apoptosis independently of PRMT5.

Co-targeting of PRMT5 by its inhibitors and AR signaling by abiraterone or enzalutamide show additive cell killing effect in 22Rv1 cells. As ASI treatment is a mainstay of CRPC treatment, we determined whether targeting PRMT5 is an effective approach to suppress the growth of CRPC cells and sensitize CRPC cells to ASI. First, we performed MTT assay with 22Rv1 cells treated with either PRMT5 enzymatic inhibitors (BLL3.3 or JNJ-64619178) or ASI (abiraterone or enzalutamide) alone, in combination, or vehicle (DMSO). Notably, the combinational treatment decreased cell growth more effectively than either of drugs alone (Fig. 4A, B). However, using the Chou-Talalay method and software CompuSyn (http://www.combosyn.com/) for the analysis of drug interaction, the combinational indexes for BLL3.3/abiraterone and BLL3.3/enzalutamide pair were 0.91 and 0.92, and for JNJ-64619178/abiraterone and JNJ-64619178/enzalutamide were 0.94 and 0.91 (Fig. 4C), respectively, indicating that PRMT5 inhibition in combination with ASI can achieve additive effect.

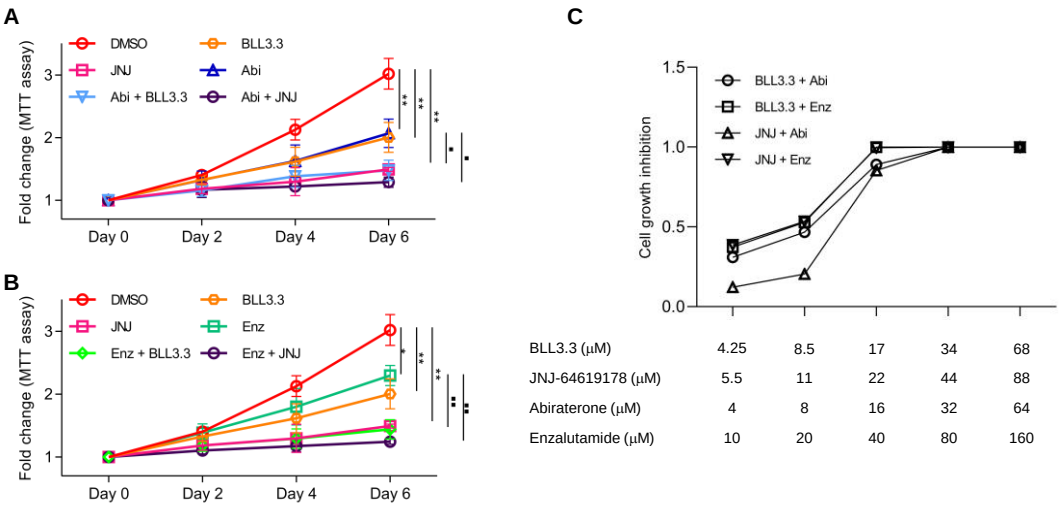

Figure 4 Targeting PRMT5 in combination with ASI significantly reduces CRPC growth. A-B, Growth curve (MTT assay) of 22Rv1 cells incubated with 10 μM PRMT5 inhibitor (BLL3.3 or JNJ-64619178, referred to as JNJ) or 10 μM of either abiraterone acetate (Abi) or enzalutamide (Enz), or equal volume of vehicle (DMSO) for 6 days. Cell proliferation assay was performed at indicated time points, and OD550 values were normalized to values from Day 0 for each cell line. ANOVA test with Welch's correction was performed to determine statistical significance. Stars represent significant difference with DMSO group, squares represent significant difference of "Abi" vs "Abi + BLL3.3", "Abi vs Abi + JNJ", "Enz" vs "Enz + BLL3.3", or "Enz vs Enz + JNJ" groups. Results are mean ± SD from 3 independent experiments. **C**. MTT assay of 22Rv1 cells incubated with indicated concentrations of PRMT5 inhibitor (BLL3.3 or JNJ-64619178, referred to as JNJ) or either abiraterone acetate (Abi) or enzalutamide (Enz) for 72 hours.

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 fifth-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 three oral presentations. She received a travel award from the 2019 Society for Basic Urological Research (SBUR) and was invited to do an oral presentation titled "Protein arginine methyltransferase 5 promotes prostate cancer growth via interaction with pICln to epigenetically activate androgen receptor expression" at the 2019 SBUR annual conference (11/2019). She also received Jenkins-Knevel Award for Outstanding Graduate Research from Purdue University College of Pharmacy and gave an oral presentation titled "Role of PRMT5 in regulation of AR expression and prostate cancer cell growth." She also gave an invited presentation to the PULSe graduate students and title of her presentation was "Role and function of protein arginine methyltransferase 5 in prostate cancer" (1/2020). In addition, she received Purdue University Center for Cancer Research SIRG Graduate Assistantship for partial support. As usual, 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 two manuscripts for submission.

Jake Owens, a sixth-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 data analysis. Jake attended 2019 SBUR (Society of Basic Urological Research) conference and presented a poster on the role of PRMT5 in epigenetic regulation of DNA damage response genes. In addition, Jake presented in the lab meetings (6 timed 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 and published his work in *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 with some IHC analysis.

Jogendra Pawar, Ph.D. Dr. Pawar was recruited to the lab as a postdoc in February, 2020. Unfortunately, the lab lockdown due COVID-19 pandemic was announced soon after he just completed paperwork. However, he has been reviewing literature of the field and presented two talks during our lab virtual journal club. In addition, he attended the virtual lab meetings on a weekly basis. I also met with him once a week to discuss experimental design and planning. Since the lab reopening in the end of June, he has been involved in evaluating the effect of JNJ-64619178 as a PRMT5 inhibitor in regulation of other target genes including AR and DNA damage response genes.

3C-2. Conference presentations

Beketaova E. Role and function of protein arginine methyltransferase 5 in prostate cancer cell growth. PULSe Graduate Program Seminar Series (1/2020)

Beketaova E., Owens, J.L., and Hu, C.D. Protein arginine methyltransferase 5 promotes prostate cancer growth via interaction with pICln to epigenetically activate androgen receptor expression. 2019 SBUR (Nov, 2019)

Beketaova E. Role of PRMT5 in regulation of AR expression and prostate cancer cell growth. Jenkins-Knevel Award for Outstanding Graduate Research Symposium (Purdue University College of Pharmacy, Nov, 2019)

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 2019 SBUR Annual Conference (Nov 2019)

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. We have also made additional accomplishments by establishing the positive correlation of PRMT5/pICln expression with AR expression in metastatic prostate cancer tissues and hormone naïve prostate cancer tissues at the mRNA level and protein level. Mechanistic analyses have suggested that PRMT5 mainly regulates G1 cell cycle progression whereas pICln regulates G2 cell cycle progression. Interestingly, PRMT5 does not seem to regulate cell survival whereas pICln is required for cell survival. To further understand the distinct roles of PRMT5 and pICln in regulation of cell cycle, survival and death, we have performed RNA-seq for knockdown of PRMT5, MEP50 and pICln. We will complete analysis of RNA-seq data to understand how PRMT5, MEP50 and pICln regulate the growth, survival and death of CRPC cells at genome-wide level and to confirm that PRMT5 and pICln do regulate AR signaling. In addition, we will also retrieve data from existing databases to see whether their expression has any impact on patient survival.

Major Goal 2: We have completed all proposed experiments in Major Goal 2. We have also analyzed the role of JNJ-64619178, a potent PRMT5 inhibitor, in regulation of CRPC cell growth. Combination of JNJ-64619178 with either abiraterone or enzalutamide seems to show additive effect in suppression of cell growth. We will perform similar experiments if our collaborator Dr. Chenglong Li develops a better PRMT5 inhibitor.

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 if available. If not available, we will perform PRMT5 knockdown in combination with abiraterone or enzalutamide to determine their effect on the growth of 22Rv1 xenograft tumors. Alternatively, the PRMT5 inhibitor JNJ-64619178 from Johnson & Johnson could be used in combination 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 valid 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 this support 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 *in vitro*. 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 have also identified pICln as a novel cofactor of PRMT5 to activate 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 novle 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 demonstrated that pICln cooperates with PRMT5 to epigenetically activate transcription of genes in DNA damage response (10). 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. We are working to solve the structure of PRMT5 in complex with pICln to further understand how

they work together to activate transcription of AR and DNA damage response gens and to help future development of novel inhibitors targeting PRMT5/pICln interaction.

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 revision.

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:	3
Contribution to Project	Dr. Hu has supervised students, postdoc and the technician to conduct the proposed research.
Funding Support	Purdue University, R01 CA212403 and PC150697

Name:	Jogendra Pawar

Project Role:	Postdoc
Perner ID:	10020769
Nearest person month worked:	6
Contribution to Project	Dr. Pawar has been involved in evaluation of JNJ-64619178 as a novel PRMT5 inhibitor
Funding Support	PC150697

Name:	Jake Owens
Project Role:	Graduate Student
Perner ID:	00147536
Nearest person month worked:	7.5
Contribution to Project	Mr. Owens has helped with qRT-PCR, ChIP analysis and RNA-seq data analysis
Funding Support	Bisland Fellowship and PC150697

Name:	Xuehong Deng
Project Role:	Technician
Perner ID:	90025073
Nearest person month worked:	10
Contribution to Project	Ms. Deng has generated stable cell lines and provided technical assistance
Funding Support	PC150697 and R01 CA212403

Name:	Elena Beketova
Project Role:	Graduate Student
Perner ID:	00119730
Nearest person month worked:	10

Contribution to Project	Elena has been mainly working on the project to elucidate the role of PRMT5 in regulation of AR expression
Funding Support	She has been supported by Purdue University Center for Cancer Research SIRG Graduate Research Fellowship

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?

<u>Current Active Grants</u>
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: 08/01/16-07/30/21
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 inhibitors to disrupt the interaction of PRMT5 with its cofactor pICln for prostate cancer treatment
Source: Purdue University Center for Cancer Research
Period: 08/01/18-12/30/20 (NCE due to COVID-19 lockdown)
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. R. L. Siegel, K. D. Miller, A. Jemal, Cancer statistics, 2019. *CA Cancer J Clin* **69**, 7-34 (2019).
2. E. S. Antonarakis, M. A. Carducci, Future directions in castrate-resistant prostate cancer therapy. *Clin Genitourin Cancer* **8**, 37-46 (2010).
3. T. Chandrasekar, J. C. Yang, A. C. Gao, C. P. Evans, Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Translational andrology and urology* **4**, 365-380 (2015).
4. P. J. Vlachostergios, L. Puca, H. Beltran, Emerging Variants of Castration-Resistant Prostate Cancer. *Current oncology reports* **19**, 32 (2017).
5. E. Grist, G. Attard, The development of abiraterone acetate for castration-resistant prostate cancer. *Urol Oncol* **33**, 289-294 (2015).
6. V. Karkhanis, Y. J. Hu, R. A. Baiocchi, A. N. Imbalzano, S. Sif, Versatility of PRMT5-induced methylation in growth control and development. *Trends Biochem Sci* **36**, 633-641 (2011).
7. C. D. Krause *et al.*, Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol Ther* **113**, 50-87 (2007).
8. N. Stopa, J. E. Krebs, D. Shechter, The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond. *Cell Mol Life Sci* **72**, 2041-2059 (2015).
9. X. Deng *et al.*, Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene* **36**, 1223-1231 (2017).
10. J. L. Owens *et al.*, PRMT5 Cooperates with pICln to Function as a Master Epigenetic Activator of DNA Double-Strand Break Repair Genes. *iScience* **23**, 100750 (2020).

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
7/2020- Steve and Lee Ann Taglienti Chair in Pharmacy

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: **J**apan **S**ociety for the **P**romotion of **S**cience (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
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	Welcome Trust
2010-2019	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
- Session Co-chair of the 2019 Fall 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

09/15-18/20	Place: 2020 5th International Conference on Pharmacy and Pharmaceutical Science (Tokyo) Title: Treatment-induced neuroendocrine differentiation in prostate cancer: Therapeutic challenges and opportunities **Invited Keynote Speaker** (http://www.icpps.org/keynote.html)
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 Hostpital 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: Am 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: Northwesten 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 Title: Radiation-induced neuroendocrine differentiation in prostate cancer: From bench to bedside
05/17/13	Place: Jinan University School of Medicine Title: Neuroendocrine differentiation (NED) in prostate cancer cells: From basic science to clinical practice
05/14/13	Place: Northwestern Agriculture and Forestry University (NWAFU): 2013 Purdue-NWAFU Center Symposium Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives
04/17/13	Place: 2013 Drug Discovery Chemistry in San Diego: Sixth Annual Protein-Protein Interactions (Targeting PPI for Therapeutic Interventions) Title: Bimolecular fluorescence complementation (BiFC) as a novel imaging-based screening for inhibitors of protein-protein interactions.
02/05/13	Place: Tongji Hospital, Huazhong University of Science and Technology Title: Neuroendocrine differentiation (NED): A therapeutic challenge in prostate cancer management
10/25/12	Place: Wright State University Department of Biochemistry and Molecular Biology Title: Bimolecular fluorescence complementation (BiFC): An imaging tool for visualization of molecular events
06/06/12	Place: Jiangshu University School of Medical Technology and Laboratory Medicine Title 1: Mechanisms and targeting of radiation-induced neuroendocrine differentiation Title 2: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
06/4/12	Place: Chinese Academy of Sciences (Hefei) Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
05/31/12	Place: Tongling Traditional Chinese Medicine Hospital Title: Recent advances in prostate cancer diagnosis and treatment
05/18/12	Place: Shanghai Center for Plant Stress Biology of Chinese Academy of Sciences Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
04/25/12	Place: University of Western Ontario

	Title: Radiotherapy-induced neuroendocrine differentiation: Implications in prostate cancer progression and treatment
03/13/12	Place: Mayo Clinic Department of Urology Title: Mechanisms and targeting of therapy-induced neuroendocrine differentiation for prostate cancer treatment
07/11/11	Place: Jinan University Medical School Title: Bimolecular fluorescence complementation: An emerging technology for biological research
07/10/11	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 dimierzation 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

Beketova, E., Owens, J.L., Asberry, A.M. and Hu, C.D. PRMT5: A putative oncogene and therapeutic target in prostate cancer. *Oncogene (invited review)*

Beketova, E., Fang, S., Owens, J.L., Liu, S., Chen, X., Zhang, Q., Asberry, A.M., Deng, X., Maloa, J., Huang, J., Li, C., Pili, R., Elzey, B.D., Ratliff, T.L., Wan, J. and Hu, C.D. Protein arginine methyltransferase 5 promotes androgen receptor transcription in a pICln-dependent manner in castration-resistant prostate cancer. *Cancer Res* (*revision submitted*)

Li, Y.H., Tong, K.L., Lu, J.L., Lin, J.B., Li, Z.Y., Yang, J., Sang, Y., Ghodbane, A., Liu, N., Gao, X.J., Tam, M.S., Hu, C.D. Zhang, H.T., and Zha, Z.G. PRMT5-TRIM21 interaction regulates the senescence of osteosarcoma cells by targeting the TXNIP/p21 axis. *Aging* (2020), 12:2507

Owens, J.L., Beketova, E., Liu, S., Tinsley, S.L., Asberry, A.M., Deng, X., Huang, J., Li, C., Wan, J., and Hu, C.D. PRMT5 cooperates with pICln to function as a master epigenetic acgivator of DNA double-strand break repair genes. *iScience* (2020), 23:100750

Doyle T.B., Muntean, B.S., Ejendal, K.F., Hayes, M.P., Soto-Velasquez, M. Martemyanov, K.A., Dessauer, C.W., Hu, C.D., and Watts, V.J. Identification of novel adenylyl cyclase 5 (AC50 signaling networks in D1 and D2 medium spiny neurons using bimolecular fluorescence complementation screening. *Cells* (2019), 8:1468.

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 protesomal 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-Y 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)

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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 proeins 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).

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Hu, C.D., Grinberg A., and Kerppola TK. Visualization of protein interaction in living cells using bimolecular fluorescence complementation (BiFC) analysis. In *Curr. Protoc. Cell Biol.* (ed. Bonifacino JS, Dasso M, Harford JB, Lippincott-Schwartz J, Yamada KM) pp. 21.3.1-21.3.21. Hoboken, John Willey & Sons, 2005

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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: Request of a Nikon A1RSI confocal microscope
Source: NIH (S10 OD027043-01)
Period: 09/20/19-09/19/20
Role: Co-Investigator (PI: Robert Stahelin)
Total Cost: $538,359
Goal: The goal of this support is to acquire a new Nikon confocal microscope for the Pharmacy Live Cell Imaging Facility (PLCIF), which was established and run by Dr. Hu (2011-2018)

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: 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: 08/01/18-03/30/20
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 adenylate 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 adenylate 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: DOoD (2013 PCRP)
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: 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: 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 an 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 phosporylation 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