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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
West Lafayette, IN 47907

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Chang-Deng Hu, Xuehong Deng, Elena Beketova, Jake Owens and Jonathan Malola

E-Mail: hu1@purdue.edu

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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 successfully demonstrated that targeting PRMT5 with BLL3.3 in combination with abiraterone or enzalutamide is more effective in killing CRPC cells. In addition, we also found that PRMT5 expression correlates with AR expression at mRNA levels and knockdown of PRMT5 suppresses 22Rv1 xenograft tumor growth.

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

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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: <u>Partially completed</u>

We previously reported in Oncogene paper (2017) that PRMT5 expression in the nucleus correlates with the expression of nuclear AR in prostate cancer tissues (HNPC) [9]. To determine whether PRMT5 expression correlates with the expression of AR-FL and AR-V7, we proposed to examine whether there is any correlation between PRMT5 and AR in CPRC tissues in Alternative Approaches. We are still working on the acquisition of CRPC samples and will complete this experiment once we have enough number of tissues from our collaborator Dr. Jiaoti Huang. As an alternative, we have retrieved expression data from cBioPortal and confirmed that PRMT5 expression indeed correlates positively with the expression of AR at mRNA level (Fig. 1).

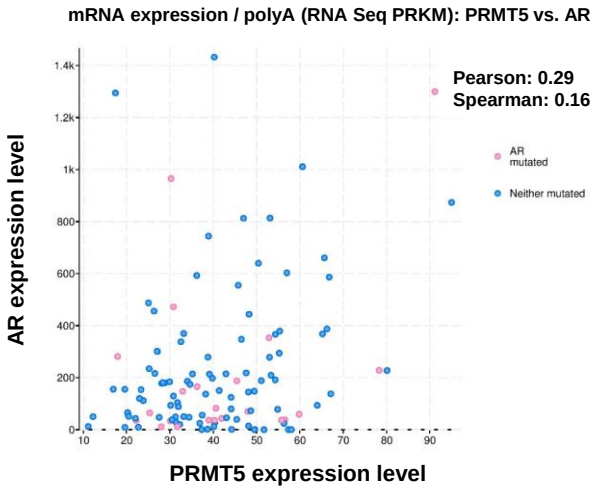

Figure 1. PRMT5 expression correlates with AR in metastatic prostate cancer. The mRNA expression data of 150 metastatic prostate cancer patients was retrieved from cBioPortal database based on the SU2CPCF Dream Team study (Robinson et al., Cell 2015), and correlation was analyzed by Pearson and Spearman.

Goal 1E. Biological evaluation of a novel PRMT5 inhibitor BLL3.3. <u>Completed</u>

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). <u>*Completed*</u>

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. During the last grant period, we evaluated whether this effect can be extended to other CRPC cells. We performed similar experiments in 22Rv1 cells and observed that co-targeting of PRMT5 with BLL3.3 and androgen synthesis with abiraterone or the potent AR inhibitor enzalutamide is also more effective in 22Rv1 cells. Similar results were obtained in 22Rv1 cells (Fig. 2). Currently, we are investigating the underlying mechanisms and find out whether the combination treatments would increase cell death (apoptosis or other types of cell death) or induce cell cycle arrest.

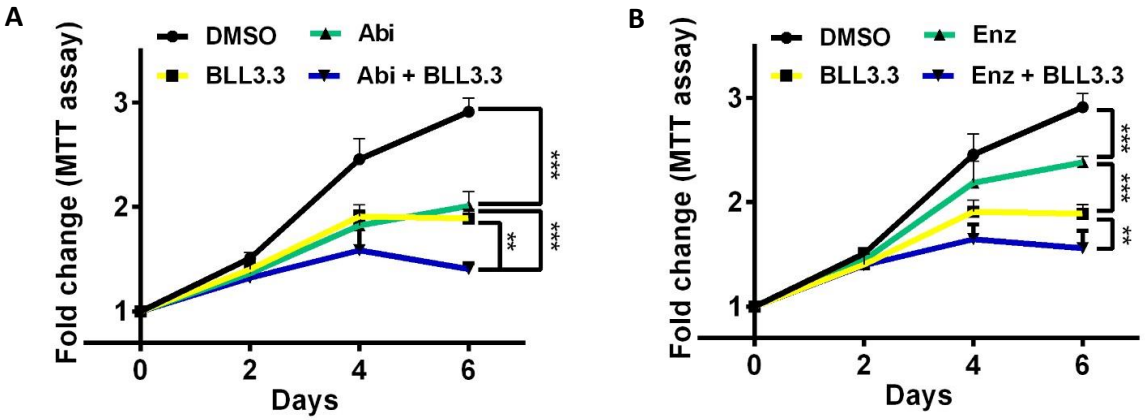

Figure 2: Co-targeting PRMT5 with BLL3.3 in combination with androgen signaling inhibitors (ASI) significantly decreases 22Rv1 proliferation. 22Rv1 cells were seeded in 48-well plates and treated with 10 μM ASI alone, 10 μM BLL3.3, vehicle (DMSO) or in combination for the indicated times and cell proliferation was measured using MTT assays. **A**. Abiraterone (Abi) co-treatment. **B**. Enzalutamide (Enz) co-treatment. Results are mean±SD**, $P < 0.01$; ***, $P < 0.001$.

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***

Our collaborator Dr. Chenglong Li has generated several potent analogs of BLL3.3. Unfortunately, their pharmacokinetic properties are not acceptable for *in vivo* study. His lab currently is making additional analogs. Once available, we will perform this experiment. However, we have already evaluated whether knockdown of PRMT5 itself can suppress xenograft tumor growth. To this end, we used our established doxycycline (Dox)-inducible knockdown cells in 22Rv1 (22Rv1-shPRMT5 and 22Rv1-sc) and injected $2x10^5$ cells into right flank of pre-castrated NRG mice (10 for each cell line, male, ages 6-8). Once tumor volumes reached to an average of 100 mm^3, mice were were fed drinking water containing Dox (1 mg/ml) and tumor growth were monitored by weekly caliber measurement (twice/week) for 4 weeks. At the end of 4-week treatment, xenograft tumors were resected for verification of knockdown of PRMT5 and down-regulation of AR by immunohistochemistry (IHC) analysis. As shown in Fig. 3, induced PRMT5 knockdown by Dox significantly suppressed xenograft tumor growth when compared with mice

in the SC control group. These results demonstrate that knockdown of PRMT5 is effective to suppress CRPC tumor growth in vivo.

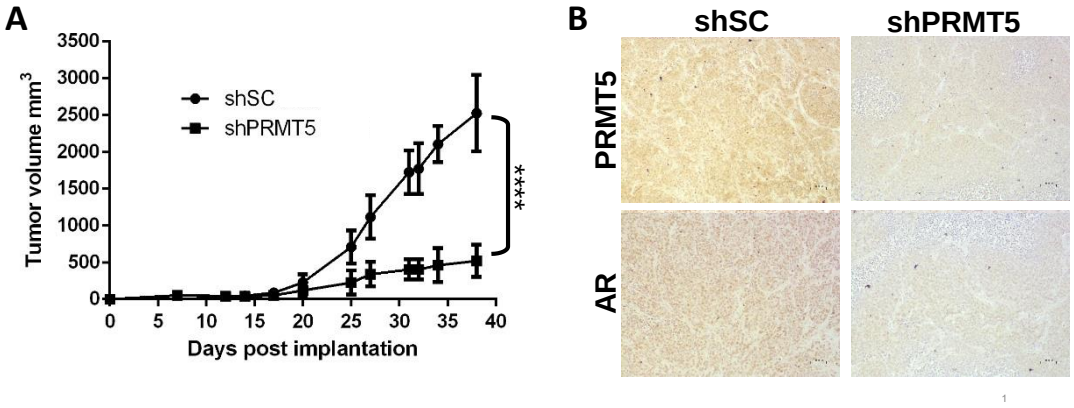

Figure 3. PRMT5 knockdown significantly suppresses 22Rv1 xenograft tumor growth. 22Rv1-shPRMT5 (shPRMT5) or 22Rv1-SC cells (shSC) (2 x 10^5) were injected subcutaneously into right flanks of castrated NRG male mice (10 mice/group). After tumor size reached 100 mm^3, mice were treated with Dox in drinking water (1 mg/mL) for 4 weeks. In the end of experiment tumors were resected, formalin fixed and embedded in paraffin for immunohistochemistry analysis of PRMT5 and AR expression. A, Tumor volume measurement. B, representative immunohistochemistry staining for PRMT5 and AR of tumor xenografts. ***, $P < 0.001$.

Other Achievements

PRMT5 functions by forming a complex with its cofactor MEP50 to regulate gene expression [10, 11]. To determine whether MEP50 also participates in the regulation of AR transcription in prostate cancer cells, we established Dox-inducible stable cell lines in LNCaP (LNCaP-shMEP50) and examined the effect of MEP50 knockdown on AR expression. Knockdown of MEP50 by Dox for 6 days did not show any significant effect on AR expression (Fig. 4A). To examine whether MEP50 binds to the AR proximal promoter region, we performed ChIP assays and found that MEP50 did not bind to the AR promoter region as PRMT5 does (Fig. 4B), though the anti-MEP50 antibody efficiently immunoprecipitated MEP50 (Fig. 4C).

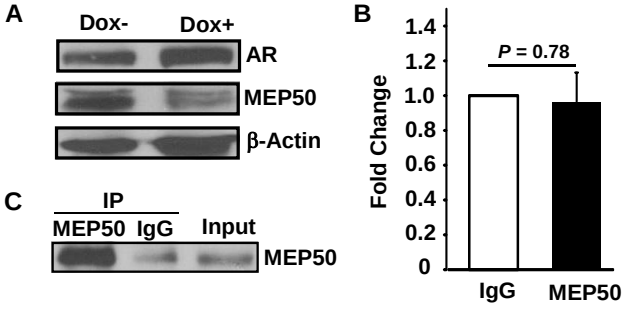

Figure 4. MEP50 knockdown does not affect expression of AR. **A**. LNCaP-shMEP50 cell line was induced by Dox for 4 days and the total cell lysate was prepared for Western blotting detection of AR and MEP50 expression. **B**. LNCaP cells cultured in 10 cm dishes were used for ChIP analysis using anti-MEP50 antibody (MEP50) or IgG control (IgG). The immunoprecipitated DNA of the AR proximal promoter region was quantified by qRT-PCR. Results are mean±SD from three independent experiments. *P* value was obtained using Student's *t* test when compared with IgG control. **C**. The ability of anti-MEP50 antibody was determined by immunoprecipitation (IP) when compared to IgG control using LNCaP total cellular lysate.

To explore this further, we next examined the effect of MEP50 on the expression of AR-FL and AR-V7 in 22Rv1 cells. Consistent with our finding in LNCaP cells, MEP50 knockdown did not show any significant effect on the expression of both AR-FL and AR-V7 in 22Rv1 cells at both protein level (Fig. 5A and 5B) and mRNA level (Fig. 5C). Consistent with these, MEP50 did not bind to the AR proximal promoter region (Fig. 5D). In contrast, MEP50 bound to the promoter region of involucrin, a PRMT5/MEP50 target gene reported previously [12], confirming again that the anti-MEP50 antibody is specific and the ChIP assay condition is optimal.

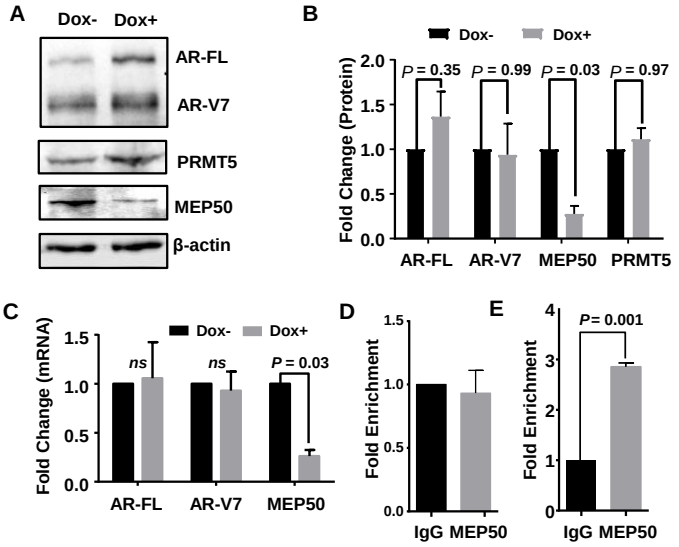

Figure 5. MEP50 knockdown does not regulate AR expression in 22Rv1 cells. **A**. 22Rv1-shMEP50 cells was induced to knock down MEP50 by Dox for 6 days and total cell lysate was prepared for Western blotting detection of full-length AR (AR-FL), AR-V7, PRMT5 and MEP50. **B**. Similar induction of MEP50 knockdown was performed as described in A and shown are quantified expressions (mean±SD) of indicated proteins in fold change after MEP50 knockdown (Dox+) when compared with those in cells without MEP50 knockdown (Dox-) from three independent experiments. **C**. Similar induction of MEP50 knockdown was performed as described in B and total mRNA was isolated for qRT-PCR measurement of mRNA expression of the indicated genes. Results are mean±SD from three independent experiments. *P* value was obtained using Student's t test when compared with Dox- group.

The lack of effect of MEP50 is surprising given that MEP50 is considered to be the only cofactor that forms a complex with PRMT5 and increase the enzyamatic activity of PRMT5. We will investigate this further to find out whether there is any new PRMT5 interacting protein(s) that can functionally substitute for MEP50 to epigenetically activate transcription of AR in prostate cancer cells.

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 third 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. It was a perfect timing for her to work on the project. I spent most of the time to train her to acquire basic lab skills, in particular molecular biological and biochemical techniques during her first year, and she is now able to work independently on her project with the help of the lab technician Xuehong Deng. Over the past year, she has generated most of the data reported in this progress report, gave two presentations, and received a Third Place Award for her poster presentation at one meeting, received a travel award from Purdue University

Center for Cancer Research, and a Graduate Research Fellowship from the College of Pharmacy (2018-19). In addition, Elena also attended a summer workshop for Big Data Sciences from which she learned basic bioinformatics tools for data mining using public database such as cBioPortal database she used in this report.

Jake Owens, a fourth year graduate student of MCMP (Medicinal Chemistry and Molecular Pharmacology) program was partially working on the project. His major roles include help for Elena to design and optimize conditions for qRT-PCR and ChIP experiments. Currently, he is teaching Elena to perform cell cycle analysis.

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. She helped to generate several stable cell lines that can inducibly express shRNAs to knockdown PRMT5 or MEP50.

Jonathan Malola, a second 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.

3C-2. Conference presentations

Beketova E., Owens J.L., Deng X., Hu C.D. (Oct 9th, 2017) Protein arginine methyltransferase 5 as an epigenetic activator of androgen receptor expression in castration resistant prostate cancer. Poster presentation at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

Beketova, E., Deng, X., Owens, J.L., Hu, C.D., Targeting PRMT5 as a novel approach for the treatment of castration-resistant prostate cancer. Midwest Chromatins and Epigenetics Meeting, Purdue University, June 10-12, 2018

Owens, J.L. and Hu, C.D. (March 2018) PRMT5: An emerging epigenetic regulator of the DNA damage response and novel therapeutic target for prostate cancer radiosensitization – Oral presentation at Purdue University, STAT598 bioinformatics seminar

Owens, J.L. and Hu, C.D. (March 2018) Basic and translational research update: PRMT5's connection to radiation therapy – Oral presentation, Indiana CTSI pre-doctoral fellowship annual meeting.

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (May 2017) 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 2017 annual meeting

Owens, J.L., Deng, X., Beketova, E., and Hu, C.D. (October 2017) PRMT5 functions as a master epigenetic activator of DNA damage response – Poster presentation at Turkey Run, Medicinal Chemistry and Molecular Pharmacology retreat

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

Dr. Chang-Deng Hu was the 2018 recipient of the Lafayette Lions Club Award for outstanding achievements in cancer research at Purdue. He presented his research to the Club, a local cancer research support community, on May 16, 2018.

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. Currently, we are collaborating with Dr. Jiaoti Huang at Duke University to acquire additional tissues from CRPC patients and perform immunohistochemistry analysis to establish their correlation at the protein level. Although these are proposed in the alternative experiments, we will pursue these and address the clinical relevance of our findings.

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. We will continue to verify this finding as some other reports suggest that abirateroen treatment can induce AR-V7 expression in LNCaP95 cells.

Major Goal 3: We have already confirmed that knockdown of PRMT5 significantly suppressed 22Rv1 xenograft tumor growth in NRG mice. However, tumors still grew though in a much slower rate. Based on in vitro results from Aim 2, we will evaluate whether knockdown of PRMT5 in combination with abiraterone or enzalutamide can completely suppressed tumor growth or even kill tumors.

Our collaborator Dr. Chenglong Li is still optimizing his PRMT5 inhibitors. Once a potent inhibitor is available for *in vivo* studies, we will evaluate co-targeting of AR expression via PRMT5 inhibition and androgen synthesis via abiraterone is an effective approach for killing CRPC using PDX models of CRPC.

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 androngen synthesis inhibition or AR inhibition.

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 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, we will investigate how PRMT5 and additional transcriptional and epigenetic proteins are assembled on the proximal AR promoter region to regulate AR 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, may not participate in epigenetic regulation of AR transcription by PRMT5. Future identification of additional PRMT5 interacting proteins on the AR promoter should provide new insight into the role of PRMT5-medicated epigenetic regulation of gene expression in general.

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:

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

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 determined the role of PRMT5 in regulation of AR and AR-V7 expression in CWR22Rv1 and LNCaP95 cells
Funding Support	Department Teaching Assistantship and PC120512

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	Department Teaching Assistantship and PC120512

Name:	Xuehong Deng
Project Role:	Technician
Perner ID:	90025073
Nearest person month worked:	3
Contribution to Project	Ms. Deng has characterized the role of PRMT5 regulation of prostate cancer cell growth and AR expression in HNPC and CRPC cells and established many stable cell lines. In addition, she has provided training and technical support to students.
Funding Support	PC120512

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 construction
Funding Support	Purdue College of Pharmacy Summer Undergraduate 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?

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: 08/01/16-07/30/19
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: 04/01/17-12/31/18
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

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

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.

7C. What other organizations were involved as partners?

Nothing to report.

8. Special Reporting Requirements

N/A

9. References

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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.
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11. Timm, D.E., V. Bowman, R. Madsen, and C. Rauch, Cryo-electron microscopy structure of a human PRMT5:MEP50 complex. *PLoS One*, 2018. **13**:e0193205.
12. Saha, K., G. Adhikary, and R.L. Eckert, MEP50/PRMT5 Reduces Gene Expression by Histone Arginine Methylation and this Is Reversed by PKCdelta/p38delta Signaling. *J Invest Dermatol*, 2016. **136**:214-24.

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-present: 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: **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 (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

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
2010-2014	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)

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 2095 samples have been distributed via Addgene as of Jun 2018.

Invited Seminars/Presentations

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 PRMT5 inhibitor for treatment of neuroendocrine tumors

  US Patent filing in process

- 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. High impact manuscripts submitted and in preparation (will be completed within two years)

Owens J.L., Beketova, E., Liu, S., Deng, X., Li, C., Wan, J. and Hu, C.D. PRMT5 is a master epigenetic regulator of radiation-induced DNA damage repair. *Submitted to Nature, Reviewed by Nature Communications. Under revision for resubmission to Molecular Cell*

Beketova, E., Owens, J.L., Deng, X., Asberry, A.M., Tinsley, S.L., Jiang, W. and Hu, C.D. PRMT5 epigenetically regulates the growth of castration resistant prostate cancer cells in a MEP50-independent but pICln-dependent manner. *In preparation for submission to Science*

Owens J.L., Beketova, E., Deng, X., and Hu, C.D. Targeting PRMT5 inhibits fractionated ionizing radiation-induced neuroendocrine differentiation and sensitizes prostate cancer cells and xenograft tumors to radiation. *In preparation for submission to Cancer Cell*

Beketova, E., Owens, J.L., Deng, X., Asberry, A.M., Tinsley, S.L., Jiang, W. and Hu, C.D. Targeting PRMT5 to suppress AR expression sensitizes castration resistant prostate cancer to abiraterone and enzalutamide. *In preparation for submission to Cancer Research*

Deng, X., xxxx, Dai, M., Jiang, W., and Hu, CD. Discovery of a novel PRMT5 inhibitors for treatment of neuroendocrine prostate cancer and small cell lung cancer. *In plan and will be submitted to Cell/Nature/Science.*

b. Peer-reviewed Research Articles

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 proerties 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)

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)

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c. 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)

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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*).

d. 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).

e. 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.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 Willey & Sons, 2005.

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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: 08/01/16-07/30/19
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: 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: 08/01/14-07/30/20
Goal: The goal of this project is to determine whether targeting LIMK2 can be used to treat CRPC.

Title: Discovery of novel therapeutic targets for neuroendocrine prostate cancer
Source: Department of MCMP Research Enhancement Award, Purdue University
Period: 04/01/17-12/31/18
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
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: 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: 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
Goals: This support is to solve cryo-EM structure of PRMT5 in complex pICln, a novel cofactor for PRMT5.

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.

Pending proposals: Several internal and external proposals submitted and to be submitted (DoD, NIH)

Past Grant Support at Purdue University (2003-2016):

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: 08/15/04 – 07/30/08
Total Cost: $ 458,000
Goals: The goal of this project was to establish *C. elegans* BiFC assay to visualize temporal and spatial interactions of *C. elegans* bZIP proteins.

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.

Internal Funding

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

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 Member** of Purdue University Center for Cancer Research
2013- 3/2018	**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-present	**Director** of Pharmacy Live Cell Imaging Facility (PLCIF)
2011-present	**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 (non-neuro)
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