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14. ABSTRACT
Prostate cancer is the second leading cause of cancer death in the United States. Although radiotherapy (RT) is one of the two curative treatments for prostate cancer patients, approximately 10% of low-risk cancer patients and 30-60% of high-risk prostate cancer patients experience biochemical recurrence within five years, among them 20% die in 10 years. The proposed research is based on the hypothesis that targeting protein arginine methyltransferase 5 (PRMT5) can sensitize primary and recurrent prostate cancer cells to RT. During the second grant period, we completed a pilot study to demonstrate that the stable cell lines established from lentiviral transduction did not show efficient knockdown of PRMT5. As an alternative approach, we have re-established stable cell lines isolated from single cells. These cells will be useful for proposed in vivo experiments. We have also completed the chemosensitization experiments and found that inhibition of PRMT5 did not sensitize LNCaP and DU-145 cells as well as their radioresistant sublines to docetaxel, cisplatin and etoposide. As etoposide is also an inducer of DNA double strand breaks, this result suggests that PRMT5 may utilize a different mechanism to sensitize prostate cancer cells to ionizing radiation. We will further test whether inhibition of PRMT5 can sensitize radioresistant sublines to ionizing radiation. We have also performed immunohistochemical analysis of PRMT5 expression in a prostate cancer tissue microarray and found that PRMT5 is highly expressed in intermediate- and high-risk prostate cancer patients, suggesting that PRMT5 expression may drive the progression of prostate cancer and possibly contribute to radioresistance. In support of this, we have continued to explore the underlying mechanism by which PRMT5 knockdown inhibits prostate cancer cell proliferation. Our new results show that PRMT5 epigenetically regulates the transcription of androgen receptor. In addition, we have also cloned and characterized the PRMT5 promoter and identified two CCAAT boxes as critical regulatory elements. Because CCAAT box is the finding site for NF-Y and because NF-Y regulates many genes involved in cancer development and progression, we will determine the role of NF-Y in PRMT5 expression.

15. SUBJECT TERMS
Prostate cancer, LNCaP, DU-145, ionizing radiation, PRMT5, PC-3, CREB, NF-Y

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1. Introduction

Prostate cancer remains the number one cancer diagnosed in men (except skin cancer), and 238,590 new patients were diagnosed and 29,720 died in the US in 2013 according to the American Cancer Society report. Radiotherapy (RT) is an important primary treatment for old patients with low-risk prostate cancer, the standard primary treatment for high-risk prostate cancer when combined with androgen deprivation therapy (ADT), and the major salvage therapy for local recurrence after surgery [1-5]. In addition, surgery plus adjuvant RT also demonstrates survival benefits when compared with surgery alone [1, 6, 7]. Despite that the majority of patients can be cured by RT, approximately 10% of patients with low-risk cancer and up to 30-60% of patients with high-risk cancer experienced biochemical recurrence within five years after RT, and among them 20% of patients died in 10 years [8-11]. Similar rate of recurrence was observed after surgery [12, 13]. Given that 96% of prostate cancer patients are present as localized disease in the US [14] and that most recurrent tumors are local recurrence [15], failure in controlling these localized primary and recurrent prostate cancers eventually leads to disease progression and contributes to the majority of prostate cancer deaths. Thus, developing effective primary and salvage RT for prostate cancer patients will have a huge impact on reducing prostate cancer mortality.

Protein arginine methyltransferases (PRMTs) are a family of proteins involved in post-translational modifications of histones and non-histone proteins [16, 17], mRNA splicing, nuclear-cytoplasmic shuttling, DNA damage response, and signal transduction [18]. Recent studies have further demonstrated that PRMT5 is involved in the DNA damage response by epigenetically modulating target gene expression or by regulating the function of proteins that are involved in the DNA damage response [19-21]. However, it remains uninvestigated how PRMT5 is involved in prostate cancer development, progression, and therapeutic responses. Based on the findings in the literature and the preliminary studies, it is hypothesized that radiation-induced or pre-existing PRMT5 overexpression contributes to the resistance of prostate cancer cells to RT in both primary and recurrent prostate cancer. The objective of the proposed research is to determine whether targeting PRMT5 can sensitize primary prostate cancer to RT, and can reprogram therapy-resistant recurrent prostate cancer to therapy-sensitive prostate cancer. Three specific aims are proposed in this project. **Aim 1** will determine that targeting PRMT5 can sensitize prostate cancer cells and prostate cancer xenograft tumors to fractionated ionizing radiation (IR) *in vitro* and in nude mice; **Aim 2** will determine that targeting PRMT5 can sensitize radiation-resistant prostate cancer cell sublines and recurrent xenograft tumors to radiation and chemotherapy *in vitro* and in nude mice; and **Aim 3** is to establish the clinical correlation between the expression level of PRMT5 and radioresistance and tumor recurrence in human prostate cancer patients. Under the support of this award, we have made the following progress during the second grant period (Aug 1, 2013 – July 30, 2014).

2. Keywords

Prostate cancer, LNCaP, DU-145, PC-3, PRMT5, CREB, ionizing radiation, NF-Y

3. Overall Project Summary

Task 1. Aim 1: To determine that targeting PRMT5 can sensitize prostate cancer cells and prostate cancer xenograft tumors to radiation *in vitro* and in nude mice (Months 1-18)

1a. Generate lentivirus for making doxycycline-inducible cell lines using LNCP, DU-145 and PC-3 cells (Months 1-6). **Completed!**

One major experimental approach is to establish lentiviurs-based knockdown of PRMT5. As reported in the last progress report, we successfully identified two potent shRNA constructs that can knock down PRMT5. Transduction of lentivirus into LNCaP cells also enabled the establishment of several cell lines. To know whether doxycycline induction works or not *in vivo*, we injected cells into five mice and started the induction after tumors grew to 200 mm^3 with drinking water containing 1 mg/ml of doxycycline, and measured the tumor volume twice a week. Compared with non-induced group (5 mice), we found that doxycycline-treated group showed initial response (suppression of tumor growth). However, comparable tumor volumes were observed at the end of 4-week treatment, though Dox-treated group showed slow growth initially (Fig. 1A). This observation suggests that some non-integrated cells may overgrow eventually. To confirm this, we performed immunohistochemistry (IHC) analysis of resected tumors and found that tissues from both treated and untreated group expressed comparable level of PRMT5 (Fig. 1B). Thus, it is likely that the stable cell line does contain a significant fraction of non-integrated cells.

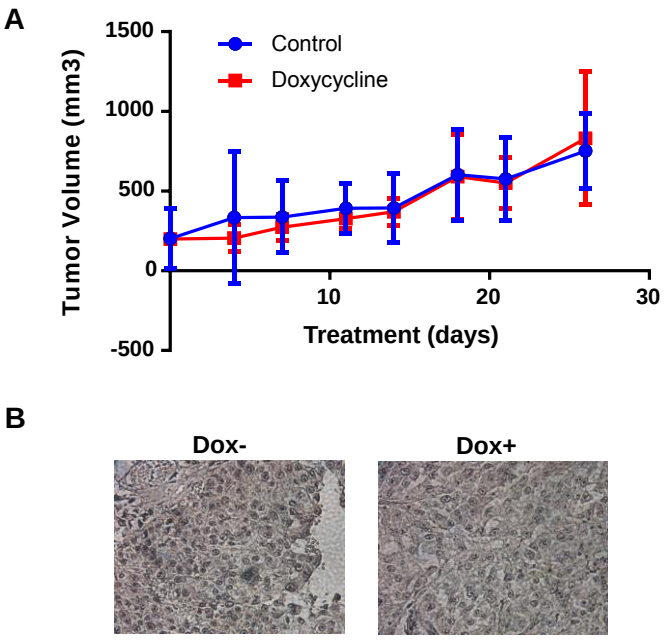

Figure 1. Effect of doxycycline induction of PRMT5 knockdown in LNCaP xenograft tumors. A. Shown are tumor growth of an established LNCaP-PRMT5 shRNA cell line using lentivirus transduction. The infected cells were selected for one week and then expanded for innoculation of $3x10^6$ cells into the hind legs of 10 male NSG mice (weeks 6-8) from the Jackson Laborary. After tumors grew to 200 mm^3, mice were randomly divided into two groups. The mice in the treated group were fed with drinking water containing doxycycline (1 mg/ml), and the mice in the control group were fed with drinking water without doxycycline. Tumor size were masured twice a week and tumor volumes were determined. **B**. Shown is a represent image for the immunohistochemical staining of PRMT5 in resected tumor tissues from untreated (Dox-) and doxycycline-treated group (Dox+).

To overcome this problem, we have isolated individual clones (starting from single cells), and doxycycline induction confirmed that clone #511 showed efficient knockdown when induced by Dox (Fig. 2). We will use this isolated clone as stable cell lines to perform proposed experiments.

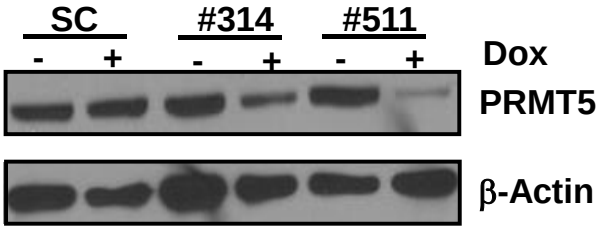

Figure 2: Effect of doxycycline induction on PRMT5 expression in isolated and stably-integrated clones. LNCaP cells infected with the lentivirus encoding PRMT5 shRNA were diluted and reseeded for isolation of individual clones. The indicated clones that can inducibly express scrambled control (SC) or the PRMT5 shRNA were treated with doxycycline (1 μg/ml) (Dox) for 4 days or without Dox treatment (Dox-). Cell lysate was prepared and the expression level of PRMT5 was determined by immunoblotting analysis. The clone #511 shows a very good knockdown.

1b. Perform radiosensitization experiments by using the knockdown cell lines and by using PRMT5 small molecule inhibitor BLL3.3 (months 7-12). **Completed !**

We completed this task and demonstrated that knockdown of PRMT5 or inhibition of PRMT5 by BLL3.3 sensitized prostate cancer cells to ionizing radiation. This was reported in the 2013 annual report

1c. Submit animal protocols for approval from Purdue University and USAMRMC. **Completed!**

We have completed the submission and approval of the animal protocols.

1d. Perform in vivo radiosensitization experiments using prostate cancer cell xenograft tumors (LNCaP and DU-145) and analyze data (months 7-12). **Ongoing**.

As discussed in Task 1a, we have isolated individual clones. If doxycycline induction works with newly established cell lines, we will move to the proposed *in vivo* experiments.

e. Analyze tumor tissues by immunohistochemistry (months 13-18). **Ongoing.**

As discussed in Task 1a, our pilot experiment suggests that doxycycline-induced PRMT5 knockdown did not work. Thus, these stable cell lines cannot be used for *in vivo* experiments. We will use the isolated clones to perform proposed *in vivo* experiments.

Task 2. Aim 2: To determine that targeting PRMT5 can sensitize recurrent (regrown) xenograft tumors to radiation and chemotherapy (Months 19-36)

2a. Isolate radiation-resistant prostate cancer sublines from DU-145 and PC-3 cells (months 19-24) **Completed and reported in the 2013 Progress Report.**

2b. Perform radiosensitization and chemosensitizatio experiments using radiation-resistant sublines (Months 25-36). **Partially completed.**

Because PRMT5 is involved in regulation of DNA damage response, we hypothesized that targeting PRMT5 may also sensitize prostate cancer cells to chemotherapeutic agents. If so, chemotherapy plus PRMT5 targeting will be more effective for treating patients with recurrence and metastasis. For this purpose, we have completed the chemosensitization experiments using parental LNCaP and DU-145 and the respective radioresistant clones by treating cells with the

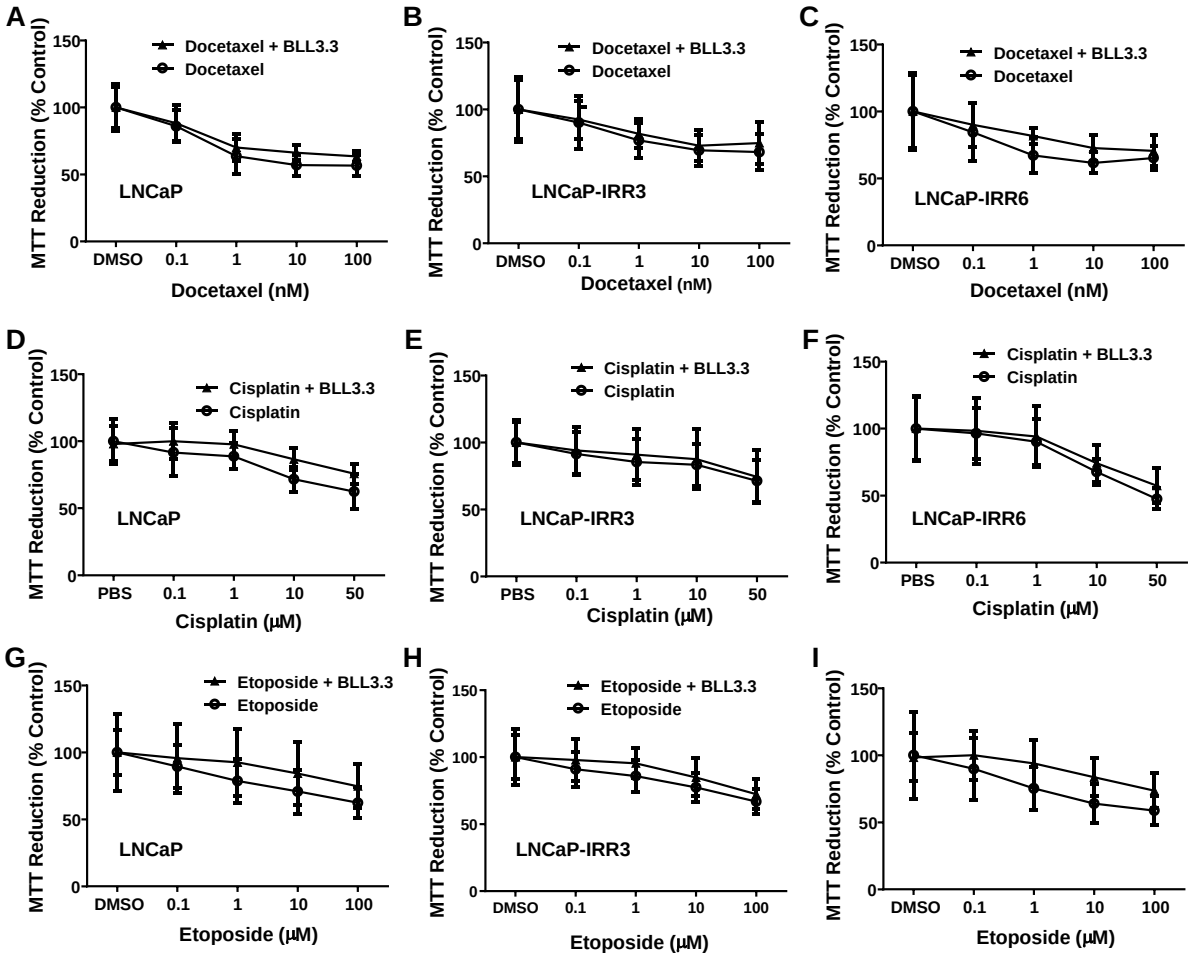

Figure 3. Effect of PRMT5 inhibition on the chemosensitivity of LNCaP and its radiation-resistant subclines LNCaP-IRR3 and LNCaP-IRR6. LNCaP or the isolated radiation-resistant sublines LNCaP-IRR3 and LNCaP-IRR6 after 40 Gy of fractionated ionizing radiation (FIR) were seeded in 48-well plate in 200 μl medium at a density of $1x10^4$ for 24 hours, and then treated with various concentrations of the indicated chemotheraputic agents with or without a PRMT5 inhibitor BLL3.3 (10 μM). Fourty-eight hours after the treatment, 70 μl of MTT working solution (0.5 mg/ml) was added into each well. After incubation at 37°C for 4 hours, 200 μl of DMSO was addded into each well and incubated for 10 min, followed by reading of the absorbtion at 560 nm with Take 3 plate reader (BioTek). Shown are mean ± SD from three independent experiments. Statistical analysis was performed with Two-way ANOVA, and there was no statistical significance between BLL3.3 treated and untraeted groups with all three chemothreapeutic agents tested.

PRMT5 inhibitor BLL3.3. Interestingly, we have found that inhibition of PRMT5 did not sensitize both parental and radioresistant sublines to three chemotherapeutic agents (Figs. 3 and 4). As etoposide also induces double strand breaks, these results suggest that PRMT5 may utilize different mechanisms to sensitize prostate cancer cells to radiation. We will test whether inhibition of PRMT5 can sensitize these resistant cells to radiation or not.

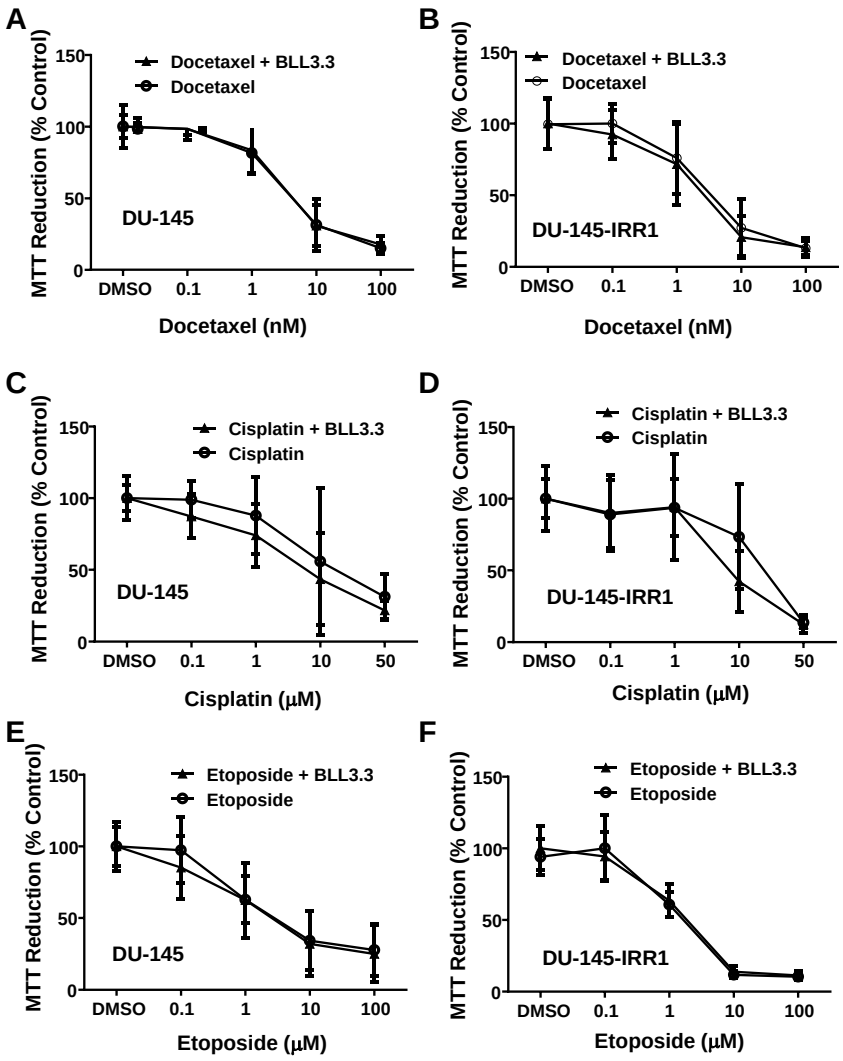

Figure 4. Effect of PRMT5 inhibition on the chemosensitivity of DU-145 and its radiation-resistant cells. DU-145 or the isolated radiation-resistant subline DU-145-IRR1 after 40 Gy of fractionated ionizing radiation (FIR) were seeded in 48-well plate in 200 μl medium at a density of $1x10^4$ for 24 hours, and then treated with various concentrations of the indicated chemotheraputic agents with or without a PRMT5 inhibitor BLL3.3 (10 μM). Fourty-eight hours after the treatment, 70 ml of MTT working solution (0.5 mg/ml) was added into each well. After incubation at 37°C for 4 hours, MTT solution and the medium were aspirated, and 200 μl of DMSO was addded into each well and incubated for 10 min, followed by reading of the absorbtion at 560 nm with Take 3 plate reader (BioTek). Shown are mean ± SD from three independent experiments. Statistical analysis was performed with Two-way ANOVA, and there was no statistical significance between BLL3.3 treated and untraeted groups with all three chemothreapeutic agents tested.

2c. Perform in vivo radiosensitization of recurrent xenograft tumors (Months 19-30). **Not started.**

We will determine whether PRMT5 targeting can sensitize recurrent cell lines to radiation. If so, we will test parental LNCaP and DU-145 as well as radioresistant cells using our *in vivo* radiosensitization approach.

2d. Analyze tumor tissues by immunohistochemistry (Months 31-36). **Not started.**

This subaim will be completed when subaim 2c is completed.

Task 3. Aim 3: To establish the clinical correlation between the expression level of PRMT5 and radioresistance and tumor recurrence (Months 1-36)

a. Submit IRB protocols to Purdue University, London Health Science Centre of the University of Ontario and USAMRMC (Months 1-6). **Completed.**

We have completed the submission of IRB protocols and we have received approvals.

b. Retrieve and review specimens for the proposed research (Months 7-12) **Ongoing.**

As reported in the 2013 Progress Report, Dr. Chin and Dr. Moussa at the University of Western Ontario have encountered some difficulties to retrieve recurrent prostate cancer specimens archived many years ago. They have also found that many primary specimens were not available in their hospital. To solve this problem, I have contacted Dr. Richard Cho at Mayo Clinic Department of Radiation Oncology based on a published paper from Mayo Clinic [22]. Dr. Cho and Dr. Herrera Hernandex (pathologist) are now working on the IRB protocol and try to retrieve some specimens for proposed experiments. Meanwhile, I have also asked Dr. Chin and Dr. Moussa at the University of Western Ontario to continue identifying specimens for the proposed experiments.

3c. Prepare two slides from each specimens for IHC analysis (Months 13-18). ***See details in 3e.***

3d. perform IHC analysis and analyze data to establish the clinical correlation between PRMT5 expression and radioresistance and tumor recurrence (Months 19-36). ***See details in 3e.***

3d. Perform IHC analysis and analyze data to establish the clinical correlation between PRMT5 expression and radioresistance and tumor recurrence (Months 19-36) ***Ongoing***

As we may have difficulty to obtain the number of proposed matched specimens from patients who were treated with radiotherapy, we have started to examine the expression level of PRMT5 in prostate cancer patients. Because high recurrence rate (30-50%) was reported in high-risk prostate cancer patients and because our data suggest that high expression level of PRMT5 confers radioresistance in prostate cancer cells (Task 1b, reported in the 2013 Progress Report), we collaborated with Dr. Jiaoti Huang at UCLA to determine the PRMT5 expression in a tissue microarray. We found that 60% of intermediate- and high-risk prostate cancer patients show moderate-to-strong expression whereas 40% of low-risk and 20% of normal control show similar extent of expression (Fig. 5). These results strongly suggest that high expression of PRMT5 may indeed contribute to radioresistance. Currently, we are trying to perform IHC analysis of PRMT5 expression in a large cohort (~1000 patients), in which clinical follow-up is available. We hope

this will eventually help us establish the clinical correlation between PRMT5 expression and the clinical outcomes.

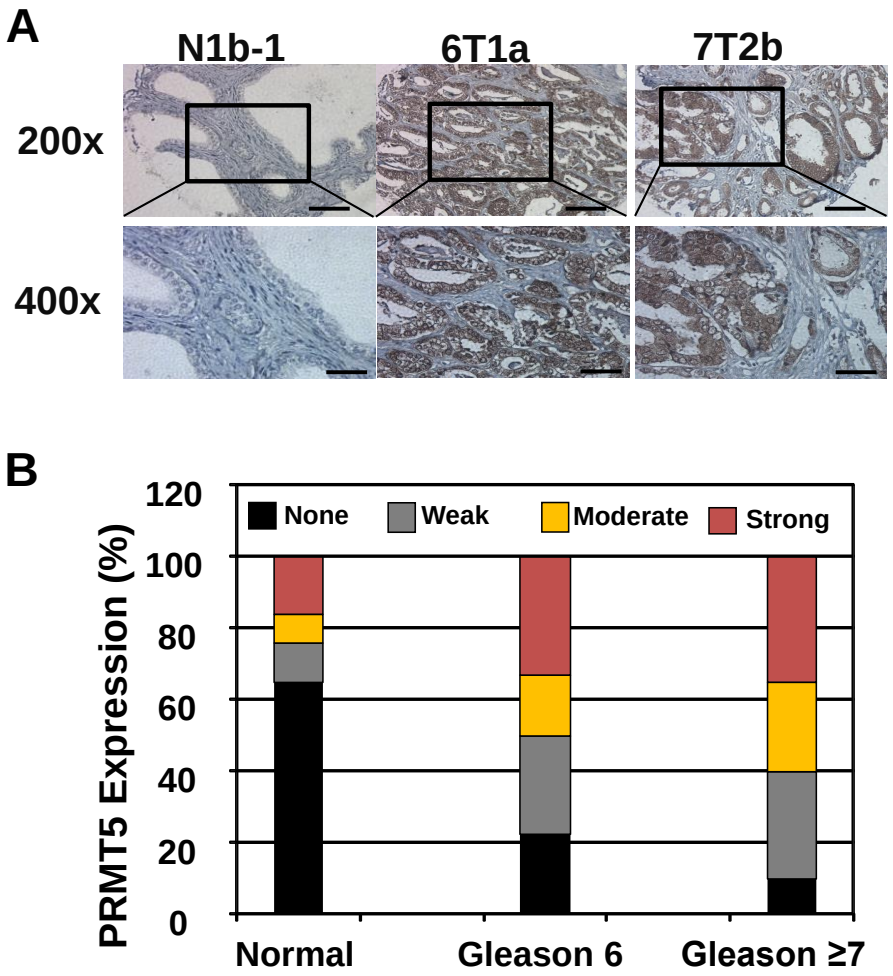

Figure 5. PRMT5 is overexpressed in high-risk prostate cancer tissues. A tissue microarray consisting of 32 cases of normal tissues, 20 cases of low-risk prostate cancer tissues (Gleason score 6), and 20 cases of intermediate- to high-risk prostate cancer tissues (Gleason score>7) was used to perform immunohistochemical analysis of PRMT5 expression with an anti-PRMT5 antibody. The expression level of PRMT5 was scored for both cytoplasm and nucleus. The intensity scores are 0 (no expression), 1 (low expression), 2 (moderate expression), and 3 (high expression), and the percentage of cells showing the expression are 0-10 with 10 as the highest percentage (100%). The expression score for cytoplasmic and nuclear PRMT5 was determined by the intensity score times the percentage (0-30), and the total expression score is the sum of cytoplasmic expression score plus nuclear expression score (0-60). **A**. shown are representative images for a normal tissues (N1b-1), low-risk prostate cancer tissue (6T1a), and a high-risk prostate cancer tissue (7T2b). **B**. The distribution of PRMT5 expression in prostate cancer tissues and normal tissues. The expression level of the sum score (cytoplasm + nucleus) is defined as: None for 0-14, Weak for 15-30, Moderate for 31-44, and Strong for 45-60. Note that PRMT5 is almost evenly distributed in both cytoplasm and nucleus in all specimen examined.

Additional accomplishments relevant to proposed research (Tasks 1 and 3)

PRMT5 epigenetically regulates AR transcription. As reported in the 2013 Progress Report, we accidently found that knockdown of PRMT5 without radiation also slowed down cell growth in LNCaP cells, and confirmed that PRMT5 regulates androgen receptor (AR) expression. This is a very exciting and important discovery relevant to the proposed research (Tasks 1 and 3). As the current gold standard treatment for high-risk prostate cancer patients is radiotherapy plus adjuvant ADT, our novel finding further led us to believe that targeting PRMT5 is a double edged sword for high-risk prostate cancer patients by suppressing expression of AR and by radiosensitizing prostate cancer cells. Thus, it is important to understand how PRMT5 regulates AR expression. Because PRMT5 is an emerging epigenetic enzyme that can epigenetically regulate expression of target genes, down-regulation of AR transcription could be due to epigenetic control of AR transcription (direct mechanism) or due to down-regulation of other

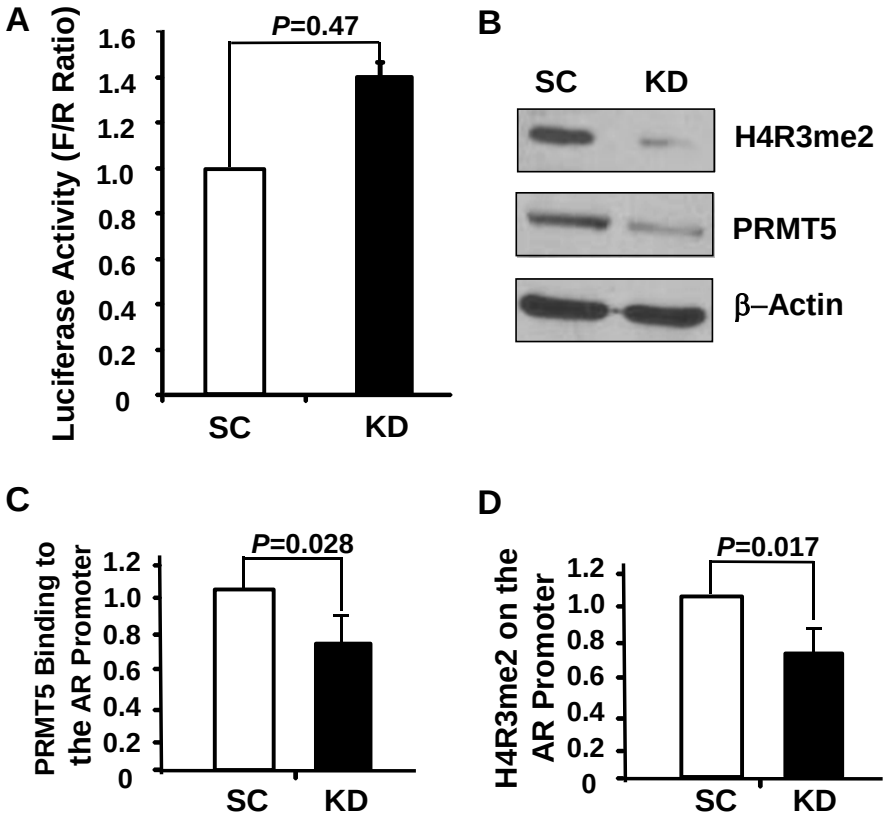

Figure 6. PRMT5 epigenetically regulates AR expression. **A**. LNCaP cells were transiently transfected with plasmids encoding scrambled control (SC) or PRMT5 shRNA (KD) along with an AR-Luc reporter gene and the Renilla control plasmid. Cell lysate was prepared 72 hours after transfection and the luciferase activity was determined using Dual-Luciferase Reporter Assay System (Promega). Results are from three independent experiments. **B**. LNCaP cells were transiently transfected with plasmids encoding SC or a PRMT5 shRNA for 72 hours and cell lysate was prepared for immunoblotting analysis of PRMT5 and the symmetric dimethylation of H4R3 (H4R3me2). **C**. Similar experiments were performed as in B and the binding of PRMT5 to the proximal end of the AR promoter was determined using ChIP analysis with anti-PRMT5 antibody. **D**. Similar experiments were performed with anti-H4R3me2 to determine the enrichment of H4R3me2 on the AR promoter. Results presented are from three independent experiments and Student's *t*-test was used for statistical analysis.

transcription activators of AR transcription (indirect mechanism). To distinguish these two possibilities, we performed an AR-luciferase reporter gene assay. We reasoned that we could see a similar inhibition of AR-Luc activity if the indirect mechanism is involved. Interestingly, knockdown of PRMT5 did not inhibit the AR-Luc activity (Fig. 6A). Instead, a slight increase was observed though not statistically significant. This suggests that PRMT5 may epigenetically regulate AR transcription. To confirm this, we examined the methylation status of H4R3 and found that knockdown of PRMT5 indeed reduced symmetric dimethylation of H4R3 (Fig. 6C). Immunoprecipitation (ChIP) analysis confirmed that PRMT5 indeed bound the AR promoter and knockdown of PRMT5 significantly decreased the binding (Fig. 6D). Consistent with this, knockdown of PRMT5 also decreased the level of H4R3me2 associated with the AR promoter (Fig. 6D). These results suggest that PRMT5 is an epigenetic regulator of AR. We will continue to explore the underlying molecular mechanism by which AR transcription is regulated by PRMT5.

Regulation of PRMT5 expression. Since we observed that IR increases PRMT5 expression and that PRMT5 is overexpressed in high-risk prostate cancer patients, it is important to determine how PRMT5 expression is regulated. This is particularly important given that recent reports

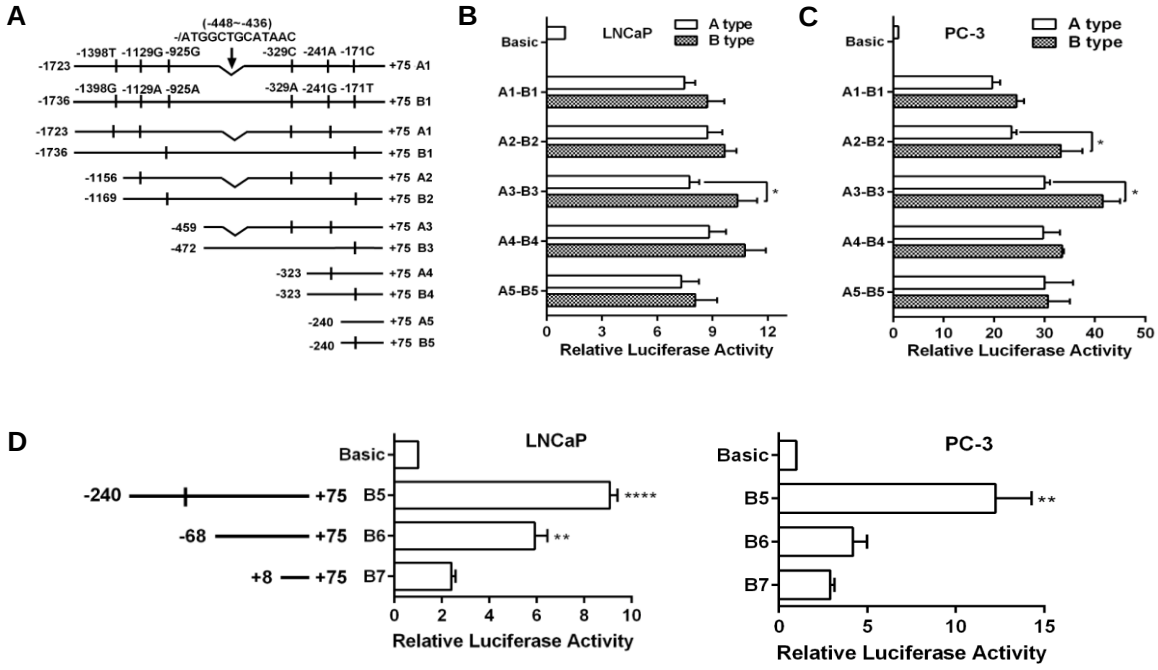

Figure 7. Identification of the proximal promoter of PRMT5. (A) Two types of PRMT5 promoters cloned from LNCaP genomic DNA with indicated SNPs and an indel polymorphism, as well as a series of 5'-truncated promoters were used to construct luciferase reporter genes. (B and C) The indicated reporter genes in A were co-transfected with pRL-TK into LNCaP and PC-3 cells for 24 hours for measurement of the luciferase activities. Results were obtained from at least three independent experiments in triplicate, and were normalized to the vector control (Basic). (*, $p<0.05$; Student's *t* test). (D) Luciferase activities of 5'-truncated reporter genes (B6 and B7) in LNCaP and PC-3 cells. Results from 4-6 independent experiments are presented as mean ± SEM. Statistical significance (**, $p<0.01$ and ****, $p<0.0001$) was determined when compared with B7 by one-way ANOVA followed by Dunnett's test.

show that PRMT5 is overexpressed in multiple human cancer tissues. However, how PRMT5 transcription is regulated has not yet been studied. As a starting point, we have cloned the PRMT5 promoter (3.5 kb) from LNCaP cells and found that PRMT5 possesses two distinct types of promoters with 7 single nucleotide polymorphisms (SNPs) and one insertion-deletion (intel) that are located in the 1.8 kb promoter region (Fig. 7A). To know whether these SNPs and the intel contribute to the promoter activity, we have made a series deletion mutants fused to the Firefly Luciferase reporter gene. Transfection of these reporter genes into LNCaP cells resulted in at least 7-fold increase in the luciferase activity when compared with the vector control (Fig. 7B). Similar results were obtained from PC-3 cells (Fig. 7C). To further identify the critical region responsible the promoter activity, we made additional deletions and found that deletions up to +8 dramatically decreased the reporter gene activity (Fig. 7D). These results suggest that the -240 region is the important part of the promoter activity. To identify potential transcription factors that may regulate PRMT5 transcription, we searched putative transcription factor binding sites. As shown in Figure 8A, there are one SP1 binding site, one GATA binding site, and three NF-Y binding sites (inverted CCAAT boxes) between -240 and +75. To assess the impact of these putative binding sites on the PRMT5 promoter activity, we introduced reported mutations into these sites that are known to abolish the binding to these transcription factors (Fig. 8B). We found that mutation of the first or the second NF-Y binding site significantly decreased the promoter activity, and mutation of both NF-Y binding sites resulted in almost 70% reduction in the reporter gene activity. However, mutation of either SP1, GATA or both increased the reporter gene activity in LNCaP cells (Fig. 8C), but had no significant effect in PC-3 cells (Fig. 8D). Interestingly, mutations of all these binding sites showed similar extent of reduction in the reporter gene activity to the mutant that has mutations in the two NF-Y binding sites (Fig. 8C and D). Note that there is a third NF-Y binding site located in +42, and mutagenesis analysis suggests that this third NF-Y binding site has a minimal effect on the overall promoter activity in LNCaP cells (Fig. 8E). Taken together, these deletion and mutagenesis analyses have demonstrated that the two CCAAT boxes in the proximal promoter region play a key role in PRMT5 transcription. Because CCAAT box is the binding site for NF-Y [23] and because NF-Y often activates transcription of many genes that are involved in cancer development and progression [24], elucidating the role of NF-Y in regulation of PRMT5 expression and prostate cancer cell growth will provide novel insights into the cellular signaling that may lead to PRMT5 overexpression in prostate cancer cells. We will determine whether NF-Y activates transcription of PRMT5 in prostate cancer cells.

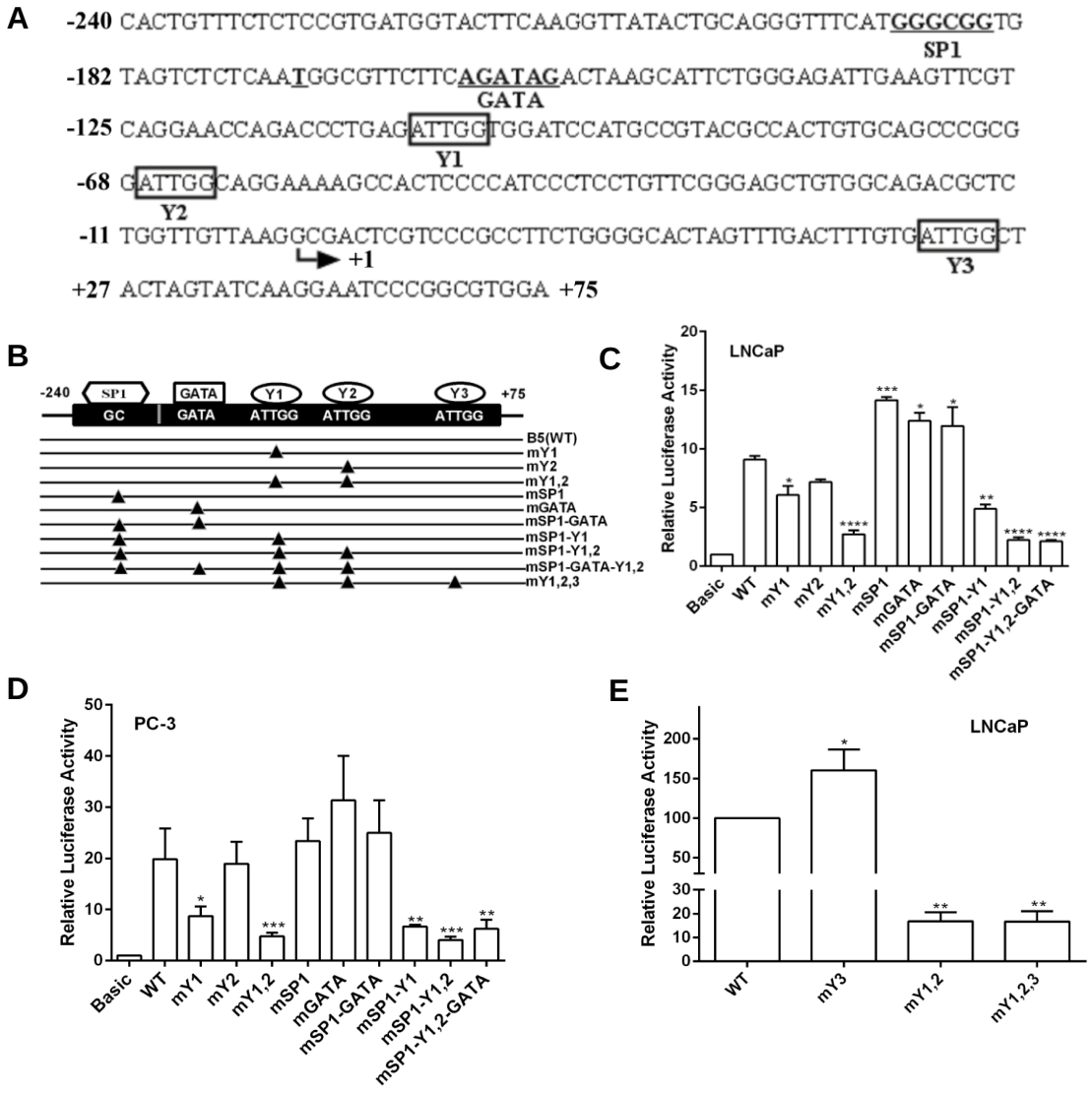

Figure 8. The two CCAAT boxes are critical for the proximal promoter activity of PRMT5. (A) Sequences of the proximal promoter region from -240 to +75 with predicted *cis*-regulatory elements. The transcription start site was indicated by arrow. Y1, Y2, or Y3 indicates the first, second or third NF-Y binding site. (B) Illustration of a series of B5-based luciferase reporter gene constructs. Triangle indicates the corresponding *cis*-regulatory element was mutated. (C-D) CCAAT boxes are critical for luciferase activity driven by the PRMT5 promoter. The luciferase activity of the indicated reporter gene constructs in B was determined in the indicated cancer cell lines. (E) The third NF-Y binding site has little effect on the PRMT5 promoter activity. The indicated luciferase reporter gene plasmid was co-transfected with pRL-TK into LNCaP cells for 24 hours, and the relative luciferase activity was determined. Results in C-E were from at least three independent experiments, and were normalized to the vector control and are presented as Mean ± SEM. Statistical significance (*, p<0.05, **, p<0.01, ***, p<0.001 and ****, p<0.0001) was determined when compared with WT by one-way ANOVA followed by Dunnett's test.

4. Key Research Accomplishments

- Confirmed that lentiviral transduction of LNCaP cells is not an appropriate approach to use for *in vivo* study.
- Isolated individual clones from single cells as stable cell lines to knock down PRMT5.
- Demonstrated that targeting PRMT5 by using BLL3.3 does not sensitize isolated radio-resistant cells and their parental LNCaP and DU-145 cells to chemotherapeutic agents (docetaxel, cisplatin, etoposide).
- Demonstrated that PRMT5 is highly expressed in intermediate- and high-risk prostate cancer tissues than normal tissues.
- Demonstrated that PRMT5 epigenetically regulates AR transcription.
- Cloned and characterized PRMT5 promoter from LNCaP cells.
- Demonstrated that the two CCAAT boxes are essential regulatory elements of PRMT5 transcription.

5. Conclusion

Under the support of this prostate cancer idea development award, we have used identified shRNA plasmids to establish stable cell lines using lentiviral transduction. However, we have found that this system only produced stable cell lines with mixed subpopulations (with or without integration). The cells with integration eventually overpopulated *in vivo* from a pilot experiment, preventing us from assessing the effect of PRMT5 targeting on radiosensitivity. Thus, we have established additional stable cell lines isolated from individual cells. With the successful isolation of radioresistant sublines from LNCaP and DU-145, we have examined the effect of PRMT5 targeting on chemosensitivity, and found that inhibition of PRMT5 by BLL3.3 does not sensitize both radioresistant sublines and their parental LNCaP and DU-145 cells to three chemotherapeutic agents (docetaxel, cisplatin and etoposide). As etoposide is one of the double-stranded break inducing agents, our results suggest that PRMT5 may utilize a different mechanism to sensitize prostate cancer cells to radiation. Nonetheless, we will test whether PRMT5 inhibition can sensitize these radioresistant cells to ionizing radiation. Since our collaborators at the University of Western Ontario have encountered some difficulties to retrieve specimens from patients who had recurrence after radiotherapy, we have started to examine PRMT5 expression in a prostate cancer tissue microarray at UCLA and found that PRMT5 is highly expressed in intermediate- and high-risk prostate cancer tissues when compared with low-risk prostate cancer tissues and normal tissues. This finding is consistent with the clinical observation that high recurrence rate was reported in intermediate- and high-risk patients. We have also approached Mayo Clinic in a hope that we will retrieve enough number of prostate cancer specimens for our analysis while our collaborators at the University of Western Ontario continue to search for appropriate specimens.

During the course of performing PRMT5 knockdown and radiosensitization experiments, we additionally found that PRMT5 regulates prostate cancer cell growth in an AR-dependent manner. Interestingly, this effect appears to be regulated by the transcription of AR. As PRMT5 is an epigenetic regulator, our novel finding suggests that PRMT5 may epigenetically regulate AR expression. We have now confirmed that PRMT5 indeed epigenetically regulates AR transcription. Given that radiotherapy combined with androgen deprivation therapy is the standard treatment for high-risk prostate cancer, our new findings collectively raise an interesting hypothesis that targeting PRMT5 is a double-egged sword for prostate cancer radiosesitization by suppressing AR expression and by sensitizing cells to radiation. We will continue to test this hypothesis.

Because PRMT5 is highly expressed in intermediate- and high-risk cancer patients and because radiation appears to induce PRMT5 expression, we have started to investigate how PRMT5 expression is regulated. We have cloned and characterized the PRMT5 promoter and identified two CCAAT boxes as critical regulatory elements of PRMT5 transcription within the proximal promoter region. Since the transcription factor NF-Y binds to CCAAT boxes and regulates expression of many genes that promote cancer cell proliferation, our results also suggest that NF-Y may regulate PRMT5 expression and promote cancer cell growth.

6. Publications, Abstracts, and Presentations

(1) Manuscripts

We are in the process of preparing several manuscripts.

(2) Presentations

a. Development of radiosensitizers: An urgent need for prostate cancer radiotherapy in the 2013 Hefei Prostate Cancer Translational Medicine and Personalized Medicine Symposium (Co-organizer, Program Committee Member, Session Chair and Speaker)

Place: Cancer Hospital, Hefei Institutes of Physical Science Chinese Academy of Sciences

Date: October 9, 2013

b. Targeting neuroendocrine differentiation as a novel radiosensitization approach for prostate cancer treatment

Place: UCLA, Departments of Pathology and Laboratory Medicine

Date: February 27, 2014

c. Advances in prostate cancer diagnosis and treatment

Place: Tongling 4th Hospital, Wannan Medical College

Date: March 25, 2014

d. Mechanism and targeting of radiotherapy-induced neuroendocrine differentiation for prostate cancer treatment

Place: Mayo Clinic Department of Radiation Oncology

Date: May 18, 2014

7. Inventions, Patents and Licenses

None

8. Reportable Outcomes

None

9. Other Achievements

We have established stable cell lines that inducibly express PRMT5 shRNA from individual cells. These cell lines will be used for proposed *in vivo* experiments.

10. References

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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, Faculty 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

Teaching Experience:

5/1988-6/1987:	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
4/1994-8/2000:	Physiology and Molecular Biology labs (medical students) in the Department of Physiology II, Kobe University
8/2003-present:	Biochemistry (MCMP304, MCMP305), Pathophysiology (MCMP440), Molecular Targets of Cancer (MCMP618), Molecular Targets of Neurological Disorders (MCMP617); Biomolecular Interactions-Theory and Practice (MCMP514), Principles of Pathophysiology and Drug Action (PHRM824); Drug Discovery and Development I (PHRM460); Integrated Lab (PHRM302); Molecular Cell Biology (LCME504, guest lecture of Molecular Biology of Cancer to Medical Students)

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 medicine 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). Study on the mutagenicity of trichloromethane
(2). Epidemiological investigation of drinking water and cancer incidence in Wuhan, China.

9/1991-3/1994: ***Guest 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). Regulation of Raf-1 kinase activity by Ha-Ras and Rap1A.
(2). Activation mechanism of Ras effectors.

4/1997-8/2000: ***Assistant Professor*** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan.
(1). Regulation of Raf kinase activity by Ha-Ras and Rap1A.
(2). Identification and characterization of novel Ras effectors and regulators.
(3). Activation mechanism 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). Establishment of bimolecular fluorescence complementation (BiFC) and multicolor bimolecular fluorescence complementation (MuFC) assays for the study of protein-protein interaction in living cells.
(2). Functional analysis of cross-family transcription factor interactions among bZIP, Rel, Smad and Myc/Max families.
(3). BiFC analysis of protein-protein interactions in *C. elegans.*

7/2003-2009: ***Assistant Professor*** in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University School of Pharmacy.
(1) Development and improvement of BiFC-based technologies
(2) AP-1 in *C. elegans* development
(3) AP-1 in prostate cancer development and therapeutic responses

8/2009- ***Associate Professor*** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University School of Pharmacy.
(1) Development and improvement of BiFC-based technologies
(2) Mechanisms and targeting of therapy-resistant prostate cancer

(3) Development of high throughput screening for discovery of inhibitors of protein-protein interactions

Award:

09/91-09/92:	Fellowship of JSPS Source: **J**apan **S**ociety for the **P**romotion of **S**cience
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
07/03-06/06	Walther Assistant Professor

Current and Past Grant Support:

Past Grant Support

04/98-03/99	Regulation of Rap1A activity by phosphorylation Source: Kobe University
04/98-03/99	Effect of phosporylation on the regulation of Rap1A activity Source: Japan Society for the Promotion of Science
04/00-03/01	Activation mechanism of phospholipase C (PLC-ε) by Ras Source: Hyogo Prefecture Science and Technology Association
04/00-03/01	Regulation of a novel phospholipase C (PLC-ε) by Ras Source: Japan Society for the Promotion of Science
08/04-07/08	Visualization of temporal and spatial interaction patterns of bZIP proteins in living *C. elegans* Source: National Science Foundation
07/06-06/08	Regulation of *c-jun* transcription by ATF2 in cardiomyocyte in response to stress Source: American Heart Association
03/08-02/09	Mass spectrometric identification of phospho-CREB in prostate cancer cells Source: Purdue University Center for Cancer Research
06/08-05/12	Interplay of ATF2 and pCREB in radiation-induced neuroendocrine differentiation in prostate cancer cells Source: Department of Defense (PCRP)
01/09-12/11	Targeting of prostate cancer transdifferentiation and proliferation via a novel DNA nanotube-based nucleic acid delivery Source: Lilly Seed Grant

01/09-12/11	Ionizing radiation induces neuroendocrine differentiation in nude mice prostate cancer xenograft models: Implication in disease progression Source: Purdue University Center for Cancer Research
06/10-05/12	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
06/10-12/11	Generation of cytoplasmic-localized ATF2 transgenic mice for prostate cancer research Source: Purdue University Center for Cancer Research
01/12-12/13	Improvement of BiFC technology and its application in the TLR signal transduction pathway (International collaborative project) Source: Natural Science Foundation of China
04/12-03/14	RO1: D2 receptor-induced sensitization of adenylate cyclase Source: NIH (Co-PI with Val Watts)

Current Grant Support

08/12-07/15	Targeting PRMT5 as a novel radiosensitization approach for primary and recurrent prostate cancer radiotherapy Source: DOD (Prostate Cancer Research Program)
09/13-09/16	Targeting neuroendocrine differentiation for prostate cancer radiotherapy Source: DOD (Prostate Cancer Research Program)
04/13-03/15	R21: Identification of Ac5 sensitization interactome using BiFC Source: NIH (Multi-PI with Val Watts)

Professional Services:

Professional Memberships

2001-	American Association for Cancer Research
2001-	American Society for Biochemistry and Molecular Biology
2003-	American Society of Cell Biology
2004-	Genetics Society of America
2009-	Society for Basic Urological Research
2010-	American Urological Association

Reviewer for Grant Applications

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

2008-present	Pennsylvania Department of Health (PADOH)
2008	UK Cancer Research, UK Diabetes
2009	Welcome Trust
2010-present	Department of Defense, Prostate Cancer Research Program (Immunology, Endocrine, Experimental Therapeutics panels)

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

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)

Members/Organizers/Session Chair of Meetings

Organizer, Program Member, and Session Chair of the 2013 Hefei Prostate Cancer Translational Research and Personalized Medicine Symposium, Hefei, China
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 of Bimolecular Fluorescence Complementation Workshop (Purdue University), 2010
Session Chair of Optical Molecular Imaging, 2008 PIBM
Session Chair of Imaging Technology Symposium, 2008 4th Modern Drug Discovery and Development Summit
Member of 2009 PIBM Program Committee
Organizer of Tristate Worm Meeting at Purdue (2005)
Organizer and invited speaker, 2013 Hefei Prostate Cancer Translational Medicine and Personal Medicine Symposium (Oct 8-9)

Administrative/Professional Services

2009-	**Member** of Purdue University Bindley Imaging Committee
2010-2013	**Seminar Coordinator** of Purdue University Center for Cancer Research
2010-	**Co-leader**, Prostate Cancer Discovery Group of Purdue University Center for Cancer Research

2011-	**Director** of Pharmacy Live Cell Imaging Facility (PLCIF) **Chair** of PLCIF Committee
2013-	**Co-leader**, CIS Program of Purdue University Center for Cancer Research
2012-	**Executive Committee Member** of Obesity and Cancer, Purdue University Center for Cancer Research
2013-	**Executive Committee Member** of Purdue University Center for Cancer Research
2013-	**Member** of Big Ten Cancer Research Consortium (BTRC) GU Clinical Trial Working Group

Invited Seminars/Meeting Presentation:

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
10/8-9//13	Place: Cancer Hospital, Hefei Institutes of Physical Science Chinese Academy of Sciences Title: Development of radiosensitizers: An urgent need for prostate cancer radiotherapy Member of Scientific Program Committee, Organizer, and Session Chair of 2013 Hefei Prostate Cancer Translational Research and Personalized Medicine
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.
Member of Scientific Program Committee
Moderator of Breakout Discussion: Imaging-based HTS for PPIs

02/05/13 Place: Tongji Hospital, HUST
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
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 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
Organizer and Speaker: BiFC Workshop

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, 15/10/08-17/10/08); Chair of Imaging Technology Symposium
Title: Multicolor fluorescence complementation in drug discovery

11/29/07 Place: UMDNJ-SOM Stratford

Title: Bimolecular fluorescence complementation 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 at HHMI Janelia Farm
Title: BiFC-FRET, a novel assay for visualization of ternary complexes in living cells (Invited for oral presentation)

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

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 interaction 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)

Publications:

1. **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) (review in Chinese).
2. **Hu, C.D.** and Zhang, X.-H. Influence of EM on specific immune responses in normal Swiss mice. *Chinese Journal of Immunology*, **4**, 176-178 (1988) (in Chinese).
3. **Hu, C.D.** and Zhang, X.-H. Influence of EM on spleen cells NK activity and its mechanisms. *Chinese Journal of Microbiology and Immunol*ogy, **8**, 11-14 (1989) (in Chinese).
4. **Hu, C.D.**, Zhan, Z.-L., and He, S.-P. Study on the mutagenicity of trichloromethane. *Chinese J. Public Health*, **5**, 220-222 (1990) (in Chinese).
5. **Hu, C.D.**, Zhan, Z.-L. and He, S.-P. Study on the influential factors and the sensitivity of microtitre fluctuation test. *Journal of Healthy and Toxicology*, **4**, 115-118 (1990) (in Chinese).
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Invited Book Chapters and Review Articles

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