Targeting Neuroendocrine Differentiation for Prostate Cancer Radiosensitization

4. TITLE AND SUBTITLE

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

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Radiotherapy (RT) is an important primary treatment for low-risk prostate cancer and the standard treatment for high-risk prostate cancer when combined with hormone therapy. Despite that many patients can be cured by RT, several studies suggest that approximately 10% of patients with low-risk cancer and up to 30-60% of patients with high-risk cancer experience biochemical recurrence within five years after RT, among them 20% of patients die in 10 years.

Neuroendocrine differentiation (NED) is a process by which prostate cancer cells transdifferentiate into neuroendocrine-like (NE-like) cells. NED is associated with disease progression and treatment failure. Based on our finding that the transcription factor cAMP response element (CREB) is responsible for fractionated ionizing radiation (FIR) induced NED, we hypothesized that targeting neuroendocrine differentiation can sensitize prostate cancer cells to radiation. We proposed two CREB targeting strategies as a model system to test our hypothesis. During the first year of grant support, we have established multiple stable and doxycycline/tetracycline-inducible cell lines that express short hairpin RNAs (shRNAs) to knock down CREB or express ACREB, a dominant negative mutant of CREB. We have examined the effect of ACREB expression on FIR-induced cell death in LNCaP cells, and found that induction of ACREB during the first two weeks (weeks 1-2), the second two weeks (weeks 2-4), or the entire four weeks (weeks 1-4) efficiently increased FIR-induced cell death and inhibited the extent of NED in survival cells. Further, clonogenic assays have also showed that ACREB expression sensitized LNCaP cells to radiation in a dose-dependent manner. In support of this notion, CREB knockdown also sensitized LNCaP cells to radiation in clonogenic assays. We will continue to extend these findings to two other cancer cell lines DU-145 and PC-3 cells using established cell lines, and we will determine the effect of CREB targeting on NED and on FIR-induced tumor killing as planned. However, CREB knockdown did not appear to increase FIR-induced cell death during the first two weeks. We will evaluate the effect of CREB knockdown on FIR-induced NED during 40 Gy of FIR. Collectively, our results so far demonstrate the critical role of CREB in FIR-induced NED, and suggest that targeting NED is an effective approach to radiosensitizing prostate cancer cells.

15. SUBJECT TERMS

Prostate cancer, LNCaP, DU-145, PC-3, neuroendocrine differentiation, NED PRMT5, CREB, PKA, CaMKII, fractionated ionizing radiation, FIR
Table of Contents

1. Introduction ................................................................. 4
2. Keywords ................................................................. 5
3. Overall Project Summary ............................................... 6-11
4. Key Research Accomplishments ..................................... 12
5. Conclusion ............................................................... 13
7. Inventions, Patents and Licenses ..................................... 14
8. Reportable Outcomes .................................................... 14
9. Other Achievements ..................................................... 14
10. References ............................................................. 15-17
11. Appendices .............................................................. 18-34
1. Introduction

Prostate cancer remains the number one non-cutaneous cancer diagnosed and the second leading cause of cancer deaths among American men. In 2010, 217,730 new patients were diagnosed and 32,050 died [1]. Radiotherapy (RT) is a first-line treatment for low-risk prostate cancer and, when combined with neoadjuvant hormonal therapy, is a standard treatment for high-risk prostate cancer (PSA >20 ng/mL and/or clinical stage cT ≥ 3 and/or biopsy Gleason score ≥8) [2-4]. Importantly, RT is the most common treatment for patients who are 65-74 years old when compared with surgery and active surveillance [5]. Although a majority of prostate cancer patients are 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 20-30% of those relapsed died within 10 years [6-9]. Given that 96% of US patients presented with localized cancer, including 25% of patients with high-risk cancer [1, 10], failure to control these localized high-risk prostate cancers eventually leads to disease progression and contributes to the majority of prostate cancer deaths. Because RT and surgery are the only curative treatments for prostate cancer, enhancing the efficacy of prostate cancer cells to RT will have an enormous impact on reducing prostate cancer mortality.

Neuroendocrine (NE) cells represent a minor portion (<1%) of the epithelial cells in normal human prostate. Interestingly, NE-like cells, which also express NE markers such as chromogranin A (CgA) and neuron specific enolase (NSE), are present in almost all cases of prostatic adenocarcinoma and an increase in the number of NE-like cells is implicated in prostate cancer progression and is an indicator of poor prognosis [11-15]. A number of agents can induce prostate cancer cells to transdifferentiate into NE-like cells, a process known as neuroendocrine differentiation (NED), via multiple pathways [16-27]. Because NE-like cells produce peptide hormones and growth factors that facilitate the growth of surrounding tumor cells in a paracrine manner and because NE-like cells are highly resistant to apoptosis [27-29], many studies have focused on establishing a clinical correlation between the extent of pre-existing NE-like cells and the therapeutic responses to RT and hormonal therapy and disease progression [11, 12, 25, 30, 31]. Because NED is reversible [32], these cells may be a dormant population under conditions of cellular stress and contribute to prostate cancer recurrence [15, 32]. The fact that hormonal therapy induces NED [33-38] suggests that therapy-induced NED may represent a novel pathway by which cancer cells survive treatment and contribute to tumor recurrence. This hypothesis is further supported by our recent findings that fractionated ionizing radiation (FIR) treatment also induces NED in vitro, in vivo and in prostate cancer patients [39, 40]. Based on the findings in literature and the preliminary studies, it is hypothesized that targeting the CREB signaling can inhibit RT-induced NED and enhance RT-induced cell killing. To test this hypothesis, three specific aims are proposed. Aim 1 will determine that targeting CREB can inhibit radiation-induced NED and increase radiation-induced cell killing in vitro. Aim 2 will determine that targeting critical upstream regulators of CREB can inhibit radiation-induced NED and increase radiation-induced cell killing in vitro. And Aim 3 will determine that targeting CREB signaling can inhibit radiation-induced NED and increase radiation-induced tumor killing in vivo. Under the support of this award, we have made the following progress.
2. Keywords

Prostate cancer, neuroendocrine differentiation, LNCaP, DU-145, PC-3, PRMT5, CREB, fractionated ionizing radiation, FIR
3. Overall Project Summary

Task 1. Aim 1: To determine that CREB targeting can inhibit radiation-induced NED and increase radiation-induced cell killing in vitro (months 1-18)

1a. Establish tetracyclin-inducible stable cell lines using LNCaP, DU-145 and PC-3 cells. Completed!

Establishment of stable cell lines for CREB targeting is the major reagent we need to generate for the proposed work. We have made two different types of shRNA expressing plasmids using the pRNATinH1.2 (Genescript) and pLKO-Tet-On (Addgene). The former relies on the availability of a stable cell line expressing Tet repressor whereas the later has the repressor encoding sequence in the same vector. We used pRNATinH1.2 to generate some shRNA constructs before. However, we recently switched to pLKO-Tet-On because of convenience to make stable cell lines with one transfection. We selected four validated targeting sequences from the Sigma Aldrich and used the last three digits corresponding to the Sigma TRCN sequence number (TRCN0000007308, TRCN0000226467, TRCN0000226468, and TRCn0000226469). We generated lentiviruses using these shRNA expressing plasmids and transduced the viruses into LNCaP cells for selection of cells that have stable integration of the plasmids. Western blotting analysis confirmed that induction of #468 shRNA by doxycycline (Dox+) showed 85% down-regulation of CREB when compared with non-induced control (Dox-).

![Figure 1. Establishment of prostate cancer stable cell lines expressing CREB shRNAs. A. Screening of CREB targeting sequences for establishment of CREB knockdown stable and doxycycline-inducible cell lines. Lentiviruses were generated for each the shRNA plasmids and transduced into LNCaP cells for selection of stable integration of the plasmids for one week. Cells were induced with doxycycline (Dox+) at 1 μg/ml or without the induction (Dox-) for three days and harvested for Western blotting analysis of CREB expression. The numbers below the blot show the relative expression level when compared with Dox- for each stable cell line. B-D. Knockdown efficiency of CREB in established stable cell lines using LNCaP (B), DU-145 (C), and PC-3 (D). The #468 lentiviruses were used to establish independent stable cell lines using LNCaP, DU-145 and PC-3 cells, and efficient knockdown of CREB (KD) was observed when compared with Dox- or the scrambled control (SC).](image-url)
and that #467 and #469 showed approximately 50% down-regulation of CREB (Fig. 1A). We then used #468 lentiviruses to establish stable cell lines in LNCaP (Fig. 1B), DU-145 (Fig. 1C) and PC-3 (Fig. 1D). Induction of shRNA expression by doxycycline resulted in efficient knockdown of CREB in all three stable cell lines.

We previously used pcDNA4-TO system (Invitrogen) to establish stable cell lines expressing the dominant negative ACREB and observed that ACREB expression increased fractionated ionizing radiation (FIR)-induced cell killing (Fig. 4 in the proposal). However, we observed that non-induced cells also died when we performed long-term FIR. This is likely due to radiation-induced damage to the Tet repressor binding element in the promoter region. To circumvent this problem, we switched to the pLVX expression system (Clontech) that does not rely on the dissociation of the Tet repressor protein form the tetracycline-resistant operon, and established three stable cell lines using LNCaP cells. Doxycycline induction resulted in similar level of ACREB expression (Fig. 2). As CREB transcription is auto-regulated, it is evident that the expression level of CREB was also down-regulated, indicating that ACREB does act as a dominant negative mutant. We also tried to establish stable cell lines expressing ACREB in DU-145 and PC-3 cells. Unfortunately, we were unable to obtain any stable clones after three tries for the reason unknown. We decided not to pursue this as LNCaP is the best cell line that can be induced to undergo NED.

1b. Perform radiation-induced cell killing experiments using the established cell lines. 
Completed!

Using the established cell lines (#468) in Figure 1, we examined the effect of CREB knockdown on radiation-induced cell death. However, induction of CREB shRNAs during the first week did not increase FIR-induced cell death (Fig. 3). Similar results were observed when cells were irradiated for two weeks (20 Gy of FIR). The inability of CREB knockdown to increase FIR-induced cell death is not due to the selection of established stable clones as transient expression of CREB shRNAs also failed to increase FIR-induced cell death after 10 Gy of FIR, and another CREB knockdown construct targeting a different region of the CREB coding sequence yielded similar results. This is surprising, given that CREB phosphorylation was induced even after 10 Gy of FIR [40]. Because there are at least 3 members in the CREB/CREM/ATF-1 family that can form dimers with CREB to regulate target gene transcription [41], we reasoned that these family members might compensate for the reduction of CREB to regulate expression of target genes essential for cell survival. Alternatively, the residual amount of CREB might be sufficient to regulate expression of these target genes. Therefore, we performed similar experiments with ACREB stable cell lines. Because ACREB retains the ability to dimerize with endogenous CREB and other CREB dimerization partners but cannot bind DNA, overexpressed ACREB can efficiently inhibit transcription of CREB target genes [42, 43]. We expected to see a potent effect with ACREB expression.
Using the pcDNA4-TO-ACREB plasmid, we established four stable cell lines. Induction by tetracycline (Tet) resulted in expression of HA-ACREB in all four cell lines with the highest induction in ACREB#1 (Fig. 4A). Consistent with this, CREB expression was also down-regulated by 90%. Similarly, CREB expression in ACREB#4 was also down-regulated by 90%. We then performed MTT assays to determine the effect of ACREB expression on FIR-induced cell killing using the ACREB#1 cell line. As shown in Figure 4B, ACREB expression significantly increased FIR-induced cell killing in a dose-dependent manner. Similar results were
observed with the ACREB#4 line. However, we did not see any significant effect of ACREB expression in ACREB#2 and ACREB#3 cell lines. Given that the CREB level in these two cell lines was only down-regulated by 60% and 13%, respectively, it is likely that efficient knockdown of CREB expression is necessary for FIR-induced cell killing.

To determine the effect of long-term expression of ACREB on FIR-induced cell death, we performed long-term FIR treatment. While attempting these experiments, using clones derived from the Invitrogen pcDNA6/TR/pcDNA4/TO expression system, there was excessive cell death under both induced and non-induced conditions, which is likely due to the effect of radiation-induced damage to the DNA encoding the tetracycline-resistance operon [44]. To overcome this problem, we utilized the Clontech pLVX-Tet-On lentiviral expression system that does not rely on the dissociation of the Tet repressor protein from the tetracycline-resistance operon [45]. Stable clones were prepared using three independent transductions and induction of ACREB sufficiently down-regulated the expression of CREB in each cell line (Fig. 2). To separate the role of CREB in both phases, we specifically induced ACREB expression during the NED phase only (weeks 3 and 4, post-20 Gy induction) and during the entire 4 weeks (pre-induction) to assess the impact of ACREB expression on the total number of viable cells at the end of 40 Gy FIR (Fig. 5A). Induction of ACREB during the entire FIR treatment period resulted in a 7.6-fold reduction in cell number, and induction of ACREB during the NED phase also resulted in a 2.5-fold reduction (Fig. 5B). Because of extensive cell death, we were unable to assess the impact of ACREB on chromogranin A (CgA) and neuron specific enolase (NSE) expression. However, some of the remaining survival cells only displayed short neurites. These results demonstrate that CREB plays a critical role in the acquisition of radioresistance and the acquisition of NED during the process of FIR-induced NED.

We will continue to evaluate the effect of CREB knockdown on FIR-induced cell death and NED during the long-term treatment of FIR.

![Figure 5](image)

**Figure 5. Effect of ACREB expression on radiation-induced cell death during the course of FIR-induced NED.** **A.** Shown are two experimental designs to determine the effect of HA-ACREB expression on cell survival shown in **B**. HA-ACREB were induced by Dox during the entire 40 Gy of FIR (Pre-induction) or during NED acquisition phase only (Post-20 Gy induction). **B.** The established three stable cell lines in Figure 2 were subjected 40 Gy of FIR (2 Gy/day, 5 days/week), and doxycycline (Dox) at 1 µg/ml was added during the entire four weeks or during the last two weeks as designed in **A**. The number of viable cells was determined by Trypan Blue Exclusion at the end of 40 Gy irradiation, and Student’s *t*-test was applied for statistical analysis.

1c. Perform radiosensitization experiments using the established cell lines (clonogenic assay) (months 9-15). **Partially Completed!**
To determine whether targeting CREB can radiosensitize prostate cancer cells, we have performed clonogenic assays using the established LNCaP stable cell line expressing ACREB (Fig. 2). We have observed significant radiosensitization in all doses when ACREB was expressed (Fig. 6A). Because clonogenic assay assesses the reproductive ability of cells after a single exposure, which is different from FIR in which DNA damages could be repaired during the interval of irradiation by functional compensation of other CREB family members, we also performed clonogenic assay with the LNCaP stable cell line expressing CREB shRNA#468 (Fig. 1B). Indeed, knockdown of CREB also sensitized LNCaP cells to radiation in a dose-dependent manner (Fig. 6B). These results collectively suggest that targeting CREB can sensitize LNCaP cells to radiation. We will use the established DU-145 and PC-3 stable cell lines (Fig. 1C and Fig. 1D) to perform similar clonogenic assays.

![Figure 6. CREB targeting sensitizes prostate cancer cells to radiation.](image)

**Figure 6. CREB targeting sensitizes prostate cancer cells to radiation.** Indicated stable and doxycycline-inducible LNCaP cell lines expressing HA-ACREB (A) or CREB shRNA#468 (KD) (B) or scrambled control (SC) were induced to express HA-ACREB for 48 hours or CREB shRNA#468 for 72 hours and then subjected to a single exposure of the indicated dose of IR, followed by seeding of various numbers of cells in 6-well plates for colony formation. Colony formation was counted 2 weeks later and survival fraction was calculated. Shown are mean from three independent experiments. *, P < 0.05; **, P < 0.01.

1d. Perform radiation-induced NED experiments (Months 15-24) **Partially completed!**

As discussed in subtask 1b, we have completed the proposed experiments with ACREB stable cell lines and demonstrated that ACREB expression during the first two weeks (radioresistance acquisition), the second two weeks (NED acquisition) or during the entire four weeks inhibited FIR-induced NED and increased radiation-induced cell killing. In fact, clonogenic assay confirmed that ACREB expression can radiosensitize LNCaP cells (Fig. 6A). We will evaluate the effect of CREB knockdown during long-term FIR to specifically evaluate whether it has any effect on FIR-induced cell death and NED.

**Task 2.** Aim 2: To determine that targeting critical upstream regulators of CREB can inhibit radiation-induced NED and increase radiation-induced cell killing *in vitro* (Months 13-36) **Ongoing.**
We will evaluate the role of PKA, CaMKII and PRMT5 in radiation-induced NED and determine whether targeting these upstream regulators can inhibit FIR-induced NED and sensitize prostate cancer cells to radiation. We have constructed a dominant negative CaMKII mutant, and we will establish stable cell lines for proposed experiments. We have also identified several shRNAs that can efficiently knockdown PRMT5, and these will be used to establish stable cell lines as well.

**Task 3.** Aim 3: To determine that targeting CREB signaling can inhibit radiation-induced NED and increase radiation-induced tumor killing *in vivo* (Months 7-30).  
**Partially completed.**

3a. *Submit animal protocols for approval from Purdue University and USAMRMC (Months 1-6).*  
**Completed!**  
We have submitted the animal protocols and received the approvals from Purdue University and USAMRMC.

3b. *Optimize tetracycline concentrations for induction of ACREB and CREB shRNAs in xenograft tumors (Months 7-12).*  
**Ongoing.**  
Since our animal facility has never done doxycycline induction in mice, we have been working with Dr. Elzey (Co-Investigator) to optimize the doxycycline induction in mice in general (animal facility). Once successful, we will test the induction conditions with our stable cell lines and perform proposed animal experiments in 3c and 3d.

3c. *Perform CREB targeting on IR-induced NED in mice (Months 13-18).*  
**Not started yet.**

3d. *Perform CREB targeting on tumor regrowth (Months 19-30).*  
**Not started yet.**
4. Key Research Accomplishments

- We have successfully demonstrated that CREB targeting by expressing a dominant negative mutant ACREB significantly increases FIR-induced cell death and sensitizes prostate cancer cells to radiation. Importantly, expression of ACREB during the first two weeks in which irradiated cells develop radioresistance, or during the second two weeks in which cells differentiate into NE-like cells, equally increases FIR-induced cell death. These observations suggest that targeting radiotherapy-induced NED is an effective approach for development of novel radiosensitizers.
5. Conclusion

Under the support of this prostate cancer idea development award, we have developed multiple stable cell lines using LNCaP, DU-145 and PC-3 to inducibly express the dominant negative CREB, ACREB, or to inducibly express CREB shRNAs. With the use of ACREB stable cell lines, we have demonstrated that ACREB induction by doxycycline can efficiently increase FIR-induced cell death during the first two weeks, during the last two weeks, or during the entire four weeks. These experimental experiments provide evidence that CREB is involved in the acquisition of radioresistance during the first two-week irradiation and in the acquisition of NED during the second two-week irradiation. These results also strongly suggest that targeting the CREB signaling could be explored to develop novel radiosensitizers for prostate cancer treatment. In fact, clonogenic assays have shown that ACREB expression or CREB knockdown does sensitize LNCaP cells to radiation. We will continue to extend this finding to DU-145 and PC-3 cells by utilizing established stable cell lines.

We have completed proposed tasks during the first year except that we failed to establish stable cell lines expressing ACREB in DU-145 and PC-3. Despite several tries with different conditions, we failed to isolate any cell line that can inducibly express ACREB. We suspect that a tiny amount of leaked expression of ACREB may be detrimental to these two cell lines. As LNCaP is the best cell line that can undergo NED induced by FIR, we believe the lack of these two cell lines will not affect our conclusion. Further, we have established stable cell lines that can inducibly knock down CREB in DU-145 and PC-3 cells. These cell lines should help us extend our findings from LNCaP cells to DU-145 and PC-3 cells.

We do not anticipate any technical challenges to complete proposed experiments at this moment, and our experiments are going smoothly and as planned. As the animal facility in the Cancer Center has never tried doxycycline induction in nude mice, we are working with Dr. Elzey to optimize the induction conditions using their own cell lines. Once completed, we will move to proposed experiments in Aims 2 and 3 while finishing the remaining experiments in Aim 1 (1c and 1d).

(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 under the support of PC111190. These cell lines will be used for proposed experiments in Aim 2.
10. References

[16] Lee SO, Chun JY, Nadiminty N, Lou W, and Gao AC. Interleukin-6 undergoes transition from growth inhibitor associated with neuroendocrine differentiation to stimulator accompanied
by androgen receptor activation during LNCaP prostate cancer cell progression. Prostate. 2007;67:764-773.


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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

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.

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: Japan Society for the Promotion of Science

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 phosphorylation 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**

**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
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:


*Equal contribution to this work


**Invited Book Chapters and Review Articles**


