

Award Number: W81XWH-13-1-0398

TITLE: Targeting Neuroendocrine Differentiation for Prostate Cancer Radiosensitization

PRINCIPAL INVESTIGATOR: Chang-Deng Hu

CONTRACTING ORGANIZATION: Purdue University
West Lafayette, IN 47907-2040

REPORT DATE: October 2014

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2014		2. REPORT TYPE Annual		3. DATES COVERED 30 Sept 2013 – 29 Sept 2014	
4. TITLE AND SUBTITLE Targeting Neuroendocrine Differentiation for Prostate Cancer Radiosensitization		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER W81XWH-13-1-0398			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S) Chang-Deng Hu, Xuehong Deng, Gyeon Oh E-Mail: hu1@purdue.edu		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Purdue University West Lafayette, IN 47907-2040		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT 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 3-4), 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					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 34	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	5
3. Overall Project Summary.....	6-11
4. Key Research Accomplishments.....	12
5. Conclusion.....	13
6. Publications, Abstracts, and Presentations.....	14
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 $\geq$ 3 and/or biopsy Gleason score $\geq$ 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 (TRCN0000007**308**, TRCN0000226**467**, TRCN0000226**468**, and TRCN0000226**469**). 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-

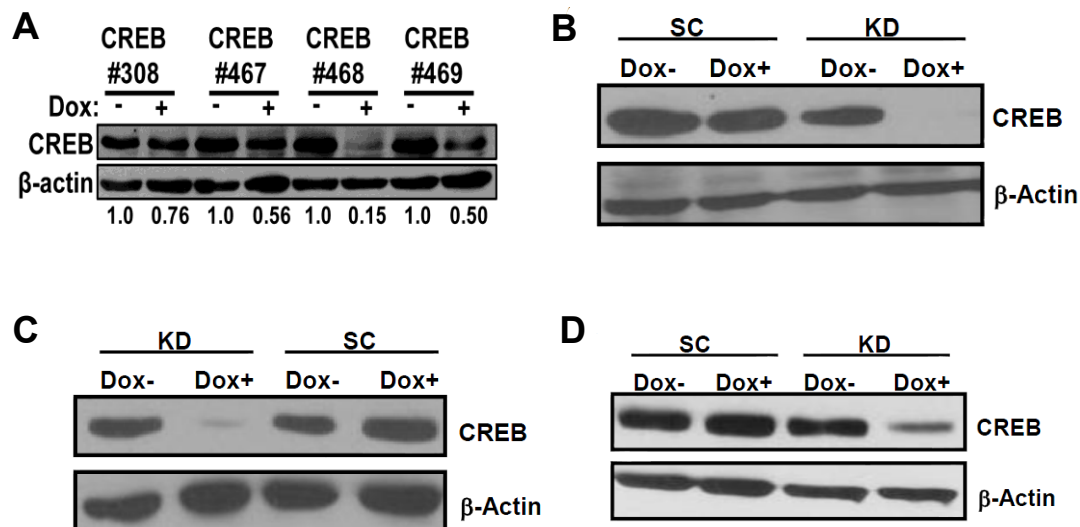


Figure 1. Establishment of prostate cancer stable cell lines expressing CREB shRNAs. A. Screening of CREB targeting sequences for establishment of CREB knockdown stable and doxycycline-inducible cell lines. Lentiviruses were generated for each the shRNA plasmids and transduced into LNCaP cells for selection 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).

), 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 from 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.

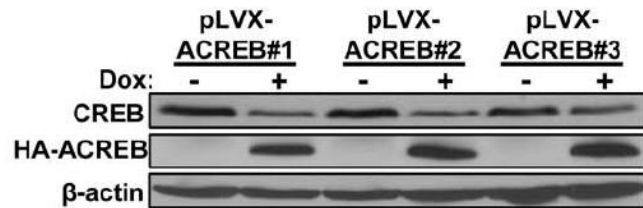


Figure 2. Establish of LNCaP stable cell lines that can inducibly express ACREB. The pLVX-Tet-On system from Clontech was used to establish three stable cell lines using LNCaP to inducibly express HA-ACREB by doxycycline (Dox). All three clones showed similar level of HA-ACREB induction and the down-regulation of CREB.

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.

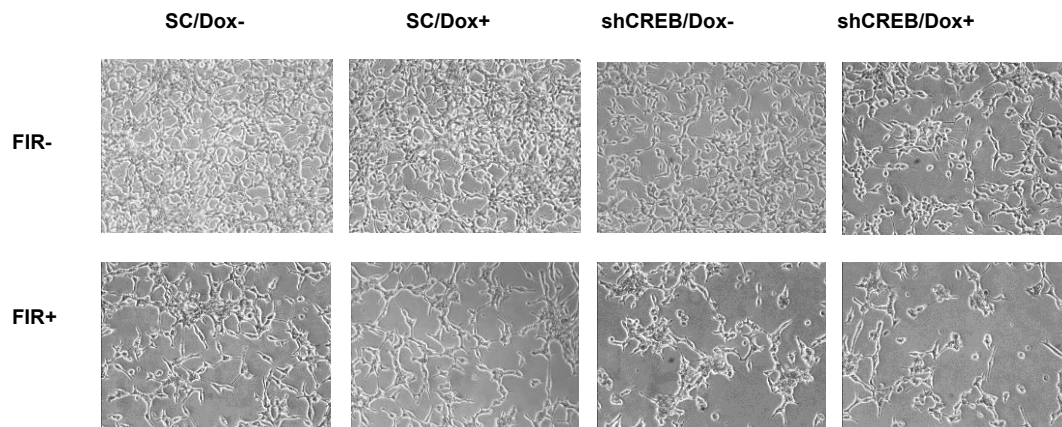


Figure 3. Effect of CREB knockdown on radiation-induced cell death. The established CREB knockdown cell line #468 (shCREB) and the stable cell line expressing scrambled control (SC) were induced with doxycycline (Dox+) at 1 μ g/ml or without induction (Dox-) for two days, and then subjected 10 Gy of fractionated ionizing radiation (FIR+) or without irradiation (FIR-). Shown are representative images acquired 24 hours after the last irradiation.

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

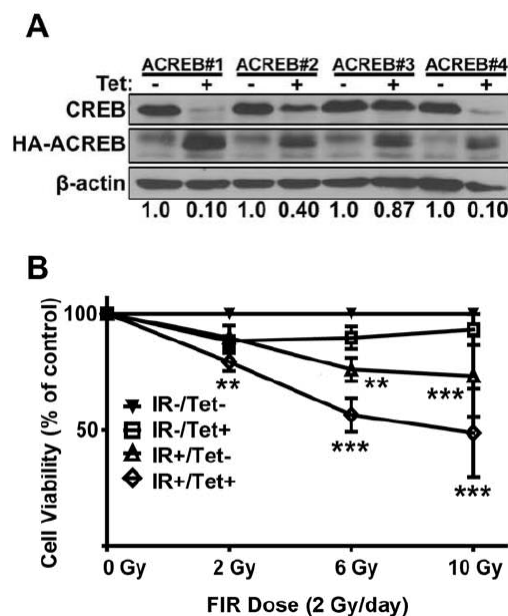


Figure 4. Effect of ACREB on radiation-induced cell death. A.

Establishment of 4 independently isolated stable and tetracycline-inducible LNCaP clones expressing HA-ACREB using the pcDNA4TO expression system (Invitrogen). Induction of HA-ACREB inhibited auto-regulation of CREB. The numbers indicate relative level of tetracycline-induced (Tet+) CREB expression when compared with non-induced (Tet-). **B.** The stable cell line ACREB#1 in A was subjected to the indicated doses of fractionated ionizing radiation (FIR) (2 Gy/day) and cell viability was analyzed using the MTT assay. **, $P < 0.01$; ***, $P < 0.001$.

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.

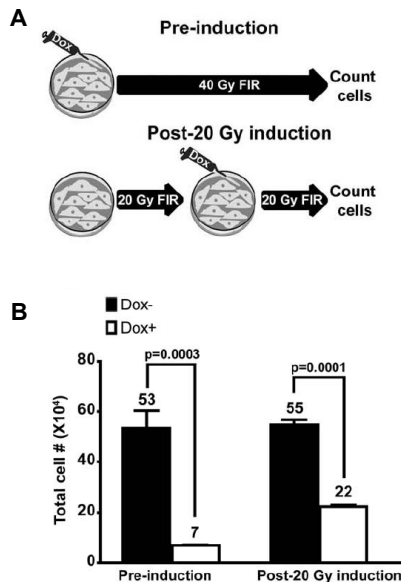


Figure 5. Effect of ACREB expression on radiation-induced cell death during the course of FIR-induced NED. A. Shown are two experimental designs to determine the effect of HA-ACREB expression on cell survival shown in B. HA-ACREB were induced by Dox during the entire 40 Gy of FIR (Pre-induction) or during 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.

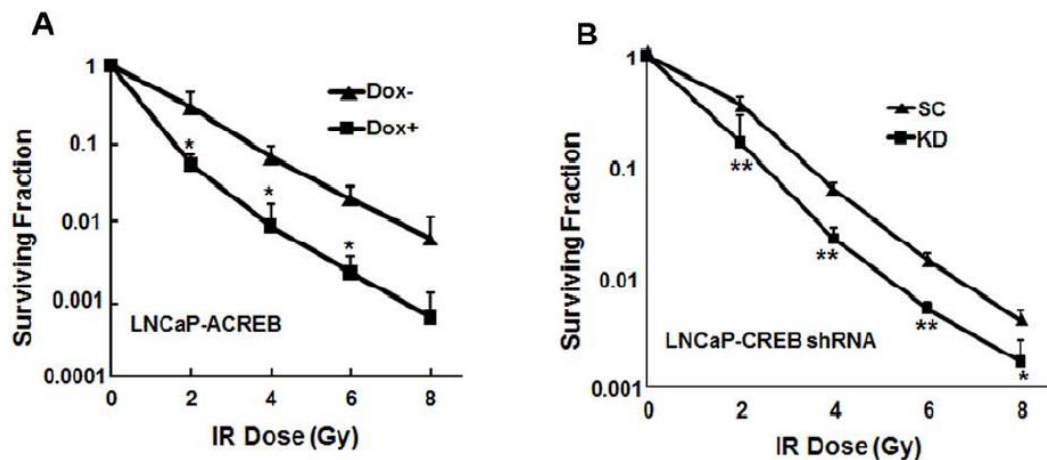


Figure 6. CREB targeting sensitizes prostate cancer cells to radiation. Indicated stable and doxycycline-inducible LNCaP cell lines expressing HA-ACREB (A) or CREB shRNA#468 (KD) (B) or scrambled control (SC) were induced to express HA-ACREB for 48 hours or CREB shRNA#468 for 72 hours and then subjected 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).

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

10. References

- [1] Jemal A, Siegel R, Xu J, and Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010;60:277-300.
- [2] Rosenthal SA, and Sandler HM. Treatment strategies for high-risk locally advanced prostate cancer. *Nat Rev Urol.* 2010;7:31-38.
- [3] Cooperberg MR, Broering JM, Litwin MS, Lubeck DP, Mehta SS, Henning JM, and Carroll PR. The contemporary management of prostate cancer in the United States: lessons from the cancer of the prostate strategic urologic research endeavor (CapSURE), a national disease registry. *J Urol.* 2004;171:1393-1401.
- [4] Choe KS, and Liauw SL. Radiotherapeutic strategies in the management of low-risk prostate cancer. *ScientificWorldJournal.* 2010;10:1854-1869.
- [5] Siegel R, Desantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, Lin C, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, and Ward E. Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin.* 2012, 62:220-241.
- [6] Kuban DA, Thames HD, Levy LB, Horwitz EM, Kupelian PA, Martinez AA, Michalski JM, Pisansky TM, Sandler HM, Shipley WU, Zelefsky MJ, and Zietman AL. Long-term multi-institutional analysis of stage T1-T2 prostate cancer treated with radiotherapy in the PSA era. *Int J Radiat Oncol Biol Phys.* 2003;57:915-928.
- [7] Zietman AL, DeSilvio ML, Slater JD, Rossi CJ, Jr., Miller DW, Adams JA, and Shipley WU. Comparison of conventional-dose vs high-dose conformal radiation therapy in clinically localized adenocarcinoma of the prostate: a randomized controlled trial. *JAMA.* 2005;294:1233-1239.
- [8] D'Amico AV, Chen MH, Renshaw AA, Loffredo B, and Kantoff PW. Risk of prostate cancer recurrence in men treated with radiation alone or in conjunction with combined or less than combined androgen suppression therapy. *J Clin Oncol.* 2008;26:2979-2983.
- [9] Agarwal PK, Sadetsky N, Konety BR, Resnick MI, and Carroll PR. Treatment failure after primary and salvage therapy for prostate cancer: likelihood, patterns of care, and outcomes. *Cancer.* 2008;112:307-314.
- [10] Ryan CJ, Elkin EP, Cowan J, and Carroll PR. Initial treatment patterns and outcome of contemporary prostate cancer patients with bone metastases at initial presentation: data from CaPSURE. *Cancer.* 2007;110:81-86.
- [11] Daneshmand S, Quek ML, and Pinski J. Neuroendocrine differentiation in prostate cancer. *Cancer Therapy.* 2005;3:383-396.
- [12] Nelson EC, Cambio AJ, Yang JC, Ok JH, Lara PN, Jr., and Evans CP. Clinical implications of neuroendocrine differentiation in prostate cancer. *Prostate Cancer Prostatic Dis.* 2007;10:6-14.
- [13] Bonkhoff H. Neuroendocrine differentiation in human prostate cancer. Morphogenesis, proliferation and androgen receptor status. *Ann Oncol.* 2001;12 Suppl 2:S141-144.
- [14] di Sant'Agnese PA. Neuroendocrine differentiation in prostatic carcinoma: an update on recent developments. *Ann Oncol.* 2001;12 Suppl 2:S135-140.
- [15] Huang J, Wu C, di Sant'Agnese PA, Yao JL, Cheng L, and Na Y. Function and molecular mechanisms of neuroendocrine cells in prostate cancer. *Anal Quant Cytol Histol.* 2007;29:128-138.
- [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.
- [17] Deebble PD, Murphy DJ, Parsons SJ, and Cox ME. Interleukin-6- and cyclic AMP-mediated signaling potentiates neuroendocrine differentiation of LNCaP prostate tumor cells. *Mol Cell Biol*. 2001;21:8471-8482.
- [18] Wang Q, Horiatis D, and Pinski J. Interleukin-6 inhibits the growth of prostate cancer xenografts in mice by the process of neuroendocrine differentiation. *Int J Cancer*. 2004;111:508-513.
- [19] Xie S, Lin HK, Ni J, Yang L, Wang L, di Sant'Agnese PA, and Chang C. Regulation of interleukin-6-mediated PI3K activation and neuroendocrine differentiation by androgen signaling in prostate cancer LNCaP cells. *Prostate*. 2004;60:61-67.
- [20] Spiotto MT, and Chung TD. STAT3 mediates IL-6-induced neuroendocrine differentiation in prostate cancer cells. *Prostate*. 2000;42:186-195.
- [21] Qiu Y, Robinson D, Pretlow TG, and Kung HJ. Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proc Natl Acad Sci U S A*. 1998;95:3644-3649.
- [22] Farini D, Puglianiello A, Mammi C, Siracusa G, and Moretti C. Dual effect of pituitary adenylate cyclase activating polypeptide on prostate tumor LNCaP cells: short- and long-term exposure affect proliferation and neuroendocrine differentiation. *Endocrinology*. 2003;144:1631-1643.
- [23] Zelivianski S, Verni M, Moore C, Kondrikov D, Taylor R, and Lin MF. Multipathways for transdifferentiation of human prostate cancer cells into neuroendocrine-like phenotype. *Biochim Biophys Acta*. 2001;1539:28-43.
- [24] Bang YJ, Pirnia F, Fang WG, Kang WK, Sartor O, Whitesell L, Ha MJ, Tsokos M, Sheahan MD, Nguyen P, and et al. Terminal neuroendocrine differentiation of human prostate carcinoma cells in response to increased intracellular cyclic AMP. *Proc Natl Acad Sci U S A*. 1994;91:5330-5334.
- [25] Amorino GP, and Parsons SJ. Neuroendocrine cells in prostate cancer. *Crit Rev Eukaryot Gene Expr*. 2004;14:287-300.
- [26] Mori R, Xiong S, Wang Q, Tarabolous C, Shimada H, Panteris E, Danenberg KD, Danenberg PV, and Pinski JK. Gene profiling and pathway analysis of neuroendocrine transdifferentiated prostate cancer cells. *Prostate*. 2009;69:12-23.
- [27] Vanoverberghe K, Vanden Abeele F, Mariot P, Lepage G, Roudbaraki M, Bonnal JL, Mauroy B, Shuba Y, Skryma R, and Prevarskaya N. Ca²⁺ homeostasis and apoptotic resistance of neuroendocrine-differentiated prostate cancer cells. *Cell Death Differ*. 2004;11:321-330.
- [28] Humez S, Monet M, Legrand G, Lepage G, Delcourt P, and Prevarskaya N. Epidermal growth factor-induced neuroendocrine differentiation and apoptotic resistance of androgen-independent human prostate cancer cells. *Endocr Relat Cancer*. 2006;13:181-195.
- [29] Slovin SF. Neuroendocrine differentiation in prostate cancer: a sheep in wolf's clothing? *Nat Clin Pract Urol*. 2006;3:138-144.
- [30] Lilleby W, Paus E, Skovlund E, and Fossa SD. Prognostic value of neuroendocrine serum markers and PSA in irradiated patients with pN0 localized prostate cancer. *Prostate*. 2001;46:126-133.
- [31] Krauss DJ, Hayek S, Amin M, Ye H, Kestin LL, Zadora S, Vicini FA, Cotant M, Brabbins DS, Ghilezan MI, Gustafson GS, and Martinez AA. Prognostic Significance of

- Neuroendocrine Differentiation in Patients with Gleason Score 8-10 Prostate Cancer Treated with Primary Radiotherapy. *Int J Radiat Oncol Biol Phys.* 2011, 81:e119-125.
- [32] Cox ME, Deeble PD, Lakhani S, and Parsons SJ. Acquisition of neuroendocrine characteristics by prostate tumor cells is reversible: implications for prostate cancer progression. *Cancer Res.* 1999;59:3821-3830.
- [33] Yuan TC, Veeramani S, Lin FF, Kondrikou D, Zelivianski S, Igawa T, Karan D, Batra SK, and Lin MF. Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. *Endocr Relat Cancer.* 2006;13:151-167.
- [34] Ismail AH, Landry F, Aprikian AG, and Chevalier S. Androgen ablation promotes neuroendocrine cell differentiation in dog and human prostate. *Prostate.* 2002;51:117-125.
- [35] Wright ME, Tsai MJ, and Aebersold R. Androgen receptor represses the neuroendocrine transdifferentiation process in prostate cancer cells. *Mol Endocrinol.* 2003;17:1726-1737.
- [36] Jin RJ, Wang Y, Masumori N, Ishii K, Tsukamoto T, Shappell SB, Hayward SW, Kasper S, and Matusik RJ. NE-10 neuroendocrine cancer promotes the LNCaP xenograft growth in castrated mice. *Cancer Res.* 2004;64:5489-5495.
- [37] Jiborn T, Bjartell A, and Abrahamsson PA. Neuroendocrine differentiation in prostatic carcinoma during hormonal treatment. *Urology.* 1998;51:585-589.
- [38] Zhang XQ, Kondrikov D, Yuan TC, Lin FF, Hansen J, and Lin MF. Receptor protein tyrosine phosphatase alpha signaling is involved in androgen depletion-induced neuroendocrine differentiation of androgen-sensitive LNCaP human prostate cancer cells. *Oncogene.* 2003;22:6704-6716.
- [39] Deng X, Elzey BD, Poulson JM, Morrison WB, Ko SC, Hahn NM, Ratliff TL, and Hu CD. Ionizing radiation induces neuroendocrine differentiation of prostate cancer cells in vitro, in vivo and in prostate cancer patients. *Am J Cancer Res.* 2011;1:834-844.
- [40] Deng X, Liu H, Huang J, Cheng L, Keller ET, Parsons SJ, and Hu CD. Ionizing radiation induces prostate cancer neuroendocrine differentiation through interplay of CREB and ATF2: Implications for disease progression. *Cancer Res.* 2008;68:9663-9670.
- [41] Shaywitz AJ, and Greenberg ME. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem.* 1999;68:821-861.
- [42] Ahn S, Olive M, Aggarwal S, Krylov D, Ginty DD, and Vinson C. A dominant-negative inhibitor of CREB reveals that it is a general mediator of stimulus-dependent transcription of c-fos. *Mol Cell Biol.* 1998;18:967-977.
- [43] Impey S, McCorkle SR, Cha-Molstad H, Dwyer JM, Yochum GS, Boss JM, McWeeney S, Dunn JJ, Mandel G, and Goodman RH. Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell.* 2004;119:1041-1054.
- [44] Dobrovolsky VN, McGarrity LJ, Morris SM, and Heflich RH. Detection of mutation in transgenic CHO cells using green fluorescent protein as a reporter. *Mutat Res.* 2002;518:55-64.
- [45] Clontech Laboratories I. Lenti-X Tet-On Advanced Inducible Expression System User Manual. 2010:1-21.

Chang-Deng Hu

Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University School of Pharmacy
575 Stadium Mall Drive, RHPH 224D
West Lafayette, IN 47907-1333
Tel: 765-496-1971, Fax: 765-494-1414, E-mail: hu1@purdue.edu
Department URL: <http://www.mcmp.purdue.edu/faculty/?uid=cdhu>
Lab URL: <http://people.pharmacy.purdue.edu/~hu1/>

Education / Degrees Awarded:

- 9/1979-7/1984: Bachelor in Medical Science (Equivalent to **M.D.**)
Faculty of Medicine, Bengbu Medical College, Bengbu, China
- 9/1984-7/1987: **M.S.** (Cancer Immunology)
Department of Microbiology and Immunology, 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: 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 <i>C. elegans</i> Source: National Science Foundation
07/06-06/08	Regulation of <i>c-jun</i> 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	<i>Ad hoc</i> 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 Wellcome 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 (NWAUFU): 2013 Purdue-NWAUFU 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: Jiangsu 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 <i>C. elegans</i> |
| 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: 7 th 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: 4 th 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 <i>C. elegans</i> 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 Immunology*, **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).
6. **Hu, C.D.**, Kariya, K., Tamada, M., Akasaka, K., Shirouzu, M., Yokoyama, S., and Kataoka, T. Cysteine-rich region of Raf-1 interacts with activator domain of post-translationally modified Ha-Ras. *J. Biol. Chem.*, **270**, 30274-30277 (1995).

7. Yanagihara, C., Shinkai, M., Kariya, K., Yamawaki-Kataoka, Y., **Hu, C.D.**, Masuda, T., and Kataoka, T. Association of elongation factor 1 α and ribosomal protein L3 with the proline-rich region of yeast adenylyl cyclase-associated protein CAP. *Biochem. Biophys. Res. Commun.*, **232**, 503-507(1997).
8. **Hu, C.D.**, Kariya, K., Kotani, G., Shirouzu, M., Yokoyama, S., and Kataoka, T. Coassociation of Rap1A and Ha-Ras with Raf-1 N-terminal region interferes with Ras-dependent activation of Raf-1. *J. Biol. Chem.*, **272**, 11702-11705 (1997).
9. Tamada, M., **Hu, C.D.**, Kariya, K., Okada, T., and Kataoka, T. Membrane recruitment of Raf-1 by association is not only the major function of Ras in Raf-1 activation, *Oncogene*, **15**, 2959-2964 (1997).
10. Shibatohe, M., Kariya, K., Liao, Y., **Hu, C.D.**, Watari, Y., Goshima, M., Shima, F., and Kataoka, T. Identification of PLC210, a *C. elegans* homolog of phospholipase C, as a putative effector of Ras, *J. Biol. Chem.*, **273**, 6218-6222 (1998).
11. Shirouzu, M., Morinaka, K., Koyama, S., **Hu, C.D.**, Hori-Tamura, N., Okada, T., Kariya, K., Kataoka, T., Kikuchi, A., and Yokoyama, S. Interactions of the amino acid residue at position 31 of the c-Ha-Ras with Raf-1 and RalGDS, *J. Biol. Chem.*, **273**, 7737-7742 (1998).
12. Ohnishi, M., Yamawaki-Kataoka, Kariya, K., Tamada, M., **Hu, C.D.**, and Kataoka, T. Selective inhibition of Ras interaction with its particular effector by synthetic peptides corresponding to the Ras effector region, *J. Biol. Chem.*, **273**, 10210-10215 (1998).
13. Kataoka, T., Kariya, K., Yamawaki-Kataoka, Y., **Hu, C.D.**, Shirouzu, M., Yokoyama, S., Okada, T., and Shima, F. Isoprenylation-dependent and independent interaction of Ras with its effectors. In Kuzumaki, N. Cytoskeleton and G-Protein in the Regulation of Cancer. *Hokaido University Medical Library Series*, **37**, 141-146 (1998).
14. Watari, Y., Kariya, K., Shibatohe, M., Liao, Y., **Hu, C.D.**, Goshima, M., Tamada, M., Kikuchi, A., and Kataoka, T. Identification of Ce-AF-6, a novel *Caenorhabditis elegans* protein, as a putative Ras effector, *Gene*, **224**, 53-58 (1998).
15. **Hu, C.D.**, Kariya, K., Okada, T., Qi, X., Song, C., and Kataoka, T. Effect of phosphorylation on activities of Rap1A to interact with Raf-1 and to suppress Ras-dependent Raf-1 activation, *J. Biol. Chem.*, **274**, 48-51 (1999).
16. Okada, T., **Hu, C.D.**, Jin T.-G., Kariya, K., Yamawaki-Katatoka, Y., and Kataoka, T. The strength of interaction at the Raf cysteine-rich region domain is a critical determinant of response of Raf to Ras family small GTPase. *Mol. Cell. Biol.* **19**:6057-6064 (1999).
17. Tanaka, Y., Minami, Y., Mine, S., Hirano, H., **Hu, C.D.**, Fujimoto, H., Fujii, K., Saito, K., Tsukada, J., van Kooyk, Y., Figdor, C. G., Kataoka, T., and Eto, S. H-Ras signals to cytoskeletal machinery in induction of integrin-mediated adhesion of T cells. *J. Immunol.*, **163**, 6209-6216 (1999).
18. Liao, Y., Kariya, K., **Hu, C.D.**, Shibatohe, M., Goshima, M., Okada, T., Watari, Y., Gao, X., Jin, T.-G., Yamawaki-Katatoka, Y., and Kataoka, T. RA-GEF, a novel Rap1A guanine nucleotide exchange factor containing a Ras/Rap1A-

- associating domain, is conserved between nematode and humans. *J. Biol. Chem.* **274**, 37815-37820 (1999).
19. Shima, F., Okada, T., Kido, M., Sen, H., Tanaka, Y., Tamada, M., **Hu, C.D.**, Yamawaki-Kataoka, Y., Kariya, K., and Kataoka, T. Association with CAP forms a second Ras-binding site of yeast adenylyl cyclase which mediates activation by posttranslationally modified Ras protein. *Mol. Cell. Biol.* **20**, 26-33 (2000).
 20. Sen, H., **Hu, C.D.**, Wu, D., Song, C., Yamawaki-Kataoka, Kotani, J., Okada, T., Shima, F., Kariya, K., and Kataoka, T. Role of Raf-1 conserved region 2 in regulation of Ras-dependent Raf-1 activation. *Biochem. Biophys. Res. Commun.*, **271**, 596-602 (2000).
 21. Song*, C., **Hu*, C.D.**, Masago, M., Kariya, K., Yamawaki-Kataoka, Y., Shibatohe, M., Sen, H., Wu, D., Satoh, T., and Kataoka, T. Regulation of a novel human phospholipase C, PLC- ϵ through differential membrane targeting by Ras and Rap1 *J. Biol. Chem.* **276**, 2752-2757 (2001).
*Equal contribution to this work
 22. Liao, Y., Satoh, T., Gao, X., Jin, T.-G., **Hu, C.D.**, and Kataoka, T. RA-GEF-1, a guanine nucleotide exchange factor for Rap1, is activated by translocation induced by association with Rap1GTP and enhances Rap1-dependent B-Raf activation. *J. Biol. Chem.* **276**, 28478-28483 (2001).
 23. Jin T.-G., Satoh T., Liao Y., Song C., Gao X., Kariya K., **Hu, C.D.**, and Kataoka T. Role of the CDC25 homology domain of phospholipase C-epsilon in amplification of Rap1-dependent signaling. *J. Biol. Chem.* **276**, 30301-30307 (2001).
 24. Gao X., Satoh T., Liao Y., Song C., **Hu, C.D.**, Kariya K., and Kataoka T. Identification and characterization of RA-GEF-2, a Rap guanine nucleotide exchange factor that serves as a downstream target of M-Ras. *J. Biol. Chem.* **276**, 42219-42225 (2001).
 25. **Hu, C.D.**, Chinenov, Y., and Kerppola, T Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell.* **9**, 789-798 (2002).
 26. **Hu, C.D.** and Kerppola, T. Simultaneous visualization of interactions between multiple proteins in living cells using multicolor bimolecular fluorescence complementation analysis. *Nat. Biotechnol.* **21**, 539-545 (2003).
 27. Grinberg A., **Hu, C.D.**, and Kerppola T. Visualization of Myc/Max/Mad family dimers and the competition for dimerization in living cells. *Mol. Cell Biol.* **24**, 4294-4308 (2004).
 28. Shyu, Y., Liu, H., Deng, X., and **Hu, C.D.** Identification of new fluorescent fragments for BiFC analysis under physiological conditions. *BioTechniques*, **40**:61-66 (2006).
 29. Liu, H., Deng, X., Shyu, Y., Li, J.J., Taparowsky, E.J., and **Hu, C.D.** Mutual regulation of c-Jun and ATF2 by transcriptional activation and subcellular localization. *EMBO J.*, **25**:1058-1069 (2006).
 30. Wang ,KZQ, Wara-Asparati, N., Boch, J.A., Yoshida, Y., **Hu, C.D.**, Galson, D.L., and Auron, P.E. TRAF6 activation of PI3 kinase-dependent cytoskeletal changes is cooperative with Ras and mediated by an interaction with cytoplasmic c-Src. *J. Cell Sci.* **119**:1579-1591 (2006).

31. Tong, E.H.Y., Guo, J.J., Haung, A., Liu, H., **Hu, C.D.**, Chung, S.S.M., and Ko, C.B. Regulation of nucleocytoplasmic trafficking of transcription factor OREBP/TonEBP/NFAT5. *J. Biol. Chem.* **281**:23870-23879 (2006).
32. Shyu, Y., Suarez, C., and **Hu, C.D.** Visualization of AP-1-NF- κ B ternary complexes in living cells by using a BiFC-based FRET. *Proc Natl Acad Sci U.S.A.*, **105**:151-156 (2008).
33. Shyu, Y., Fox, SM., Duren, HM., Ellis, R.E., Kerppola, T.K. and **Hu, C.D.** Visualization of protein interaction in living *Caenorhabditis elegans* using bimolecular fluorescence complementation (BiFC) analysis. *Nat Protocol.*, **4**:588-596 (2008).
34. Shyu, Y., Suarez C.D., and **Hu, C.D.** Visualizing ternary complexes in living cells using BiFC-FRET analysis. *Nat. Protocol.* **3**:1693-1702 (2008).
35. Vidi, P.A., Chemel, B.R., **Hu, C.D.**, Watts, V.J. Ligand-Dependant Oligomerization of Dopamine D₂ and Adenosine A_{2A} Receptors in Living Neuronal Cells. *Mol. Pharmacol.* **74**:544-551 (2008)
36. Hiatt, S.M., Shyu, Y., Duren, H.M, and **Hu, C.D.** Bimolecular fluorescence complementation (BiFC) analysis of protein interactions in living *C. elegans*. *Methods*, **45**:185-191 (2008)
37. Deng, X., Liu, H., Huang, J., Cheng, L., Keller, E.T., Parsons, S.J., and **Hu, C.D.** Ionizing radiation induces prostate cancer neuroendocrine differentiation through interplay of CREB and ATF2: Implications for disease progression. *Cancer Res.* **68**:9663-9670 (2008)
38. Yuan, Z., Gong, S., Song, B., Mei, Y., Hu, C., Li, D., Thiel, G., **Hu, C.D.**, and Li, M. Opposing role for ATF2 and c-Fos in c-Jun-mediated apoptosis induced by potassium deprivation in cerebellar granule neurons. *Mol. Cell. Biol.* **29**:2431-2442 (2009)
39. Xu, Y., Yang W.H., Gerin, I., **Hu, C.D.**, Hammer, G.D., and Koenig, R.J. DAX-1 and steroid receptor RNA activator (SRA) function as transcriptional coactivators for steroidogenic factor-1 in steroidogenesis. *Mol. Cell. Biol.* **29**:1719-1734 (2009)
40. Hiatt, S.M., Duren, H.M. Shyu, Y., Ellis, R.E., Hisamoto, N., Matsumoto, K., Kariya, K., Kerppola, T.K., and **Hu, C.D.** *C. elegans* FOS-1 and JUN-1 regulate *plc-1* expression to control ovulation. *Mol. Biol. Cell* **20**:3888-3895 (2009)
41. Le, T.T, Duren, H.M., Slipchenko, M.N., **Hu, C.D.***, and Cheng, J.X. Label-free quantitative analysis of lipid metabolism in living *Caenorhabditis elegans*. *J. Lipid Res.* **51**:672-677 (2010) *Co-Corresponding Author
42. Kodama, Y. and **Hu, C.D.** An improved bimolecular fluorescence complementation assay with high signal-to-noise ratio. *Biotechniques*, **49**:793-805 (2010)
43. Xing, J., Wang, S., Lin, F., Pan, W., **Hu, C.D.**, and Zheng, C. A comprehensive characterization of interaction complexes of Herpes Simplex Virus type 1 ICP22, UL3, UL4 and UL20.5. *J. Virol.* **85**:1881-1886 (2011)
44. Deng, X., Elzey, B.D, Poulson, J.M., Morrison, W.B., Ko, S.C., Hahn, N.M., Ratliff, T.L., and **Hu, C.D.** Ionizing radiation induces neuroendocrine differentiation in vitro, in vivo and in human prostate cancer patients. *Am. J. Cancer. Res.* **1**:834:844 (2011)

45. Hsu, C. and Hu, C.D. Critical role of an N-terminal end nuclear export signal in regulation of ATF2 subcellular localization and transcriptional activity. *J. Biol. Chem.* 287:8621-8632 (2012)
46. Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD. Zhang J, Kester M, Wang HG. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. *J. Biol. Chem.* 287:12455-12688 (2012)
47. Hsu, C. and Hu, C.D. Transcriptional activity of c-Jun is critical for the suppression of AR function. *Mol. Cell. Endocrinol.* 372:12-22 (2013)

Invited Book Chapters and Review Articles

1. Hu, C.D., Grinberg A., and Kerppola TK. Visualization of protein interaction in living cells using bimolecular fluorescence complementation (BiFC) analysis. In *Current Protocol in Cell Biology* (ed. Bonifacino JS, Dasso M, Harford JB, Lippincott-Schwartz J, Yamada KM) pp. 21.3.1-21.3.21. Hoboken, John Willey & Sons, 2005
2. Hu, C.D. and Kerppola TK. Direct visualization of protein interactions in living cells using bimolecular fluorescence complementation analysis. *Protein-Protein Interactions* (ed. P. Adams and E. Golemis), Cold Spring Harbor Laboratory Press. Pp673-693, 2005.
3. Hu, C.D., Grinberg, A.V. and Kerppola, T.K. Visualization of Protein Interactions in Living Cells Using Bimolecular Fluorescence Complementation (BiFC) Analysis. (ed. Coligan JE, Dunn BM, Speicher DW, Wingfield PT) *Curr. Protoc. Protein Sci.* 41:19.10.1-19.10.21. Hoboken, John Willey & Sons, 2005.
4. Shyu, Y., Akasaka, K., and Hu, C.D. Bimolecular fluorescence complementation (BiFC): A colorful future in drug discovery. *Sterling-Hoffman Life Science Journal*, July, 2007. (<http://www.sterlinglifesciences.com/newsletter/articles/article006.html>).
5. Shyu, Y. and Hu, C.D. Recent advances in fluorescence complementation-based technologies. *Trends Biotechnol.* 26:622-630 (2008)
6. Vidi, P.APrzybyla, J., Hu, C.D., and Watts, V.J. Visualization of G protein-couple receptor (GPCR) interactions in living cells using bimolecular fluorescence complementation (BiFC). *Current Protocol in Neuroscience*, Unit 5.29.1-5.29.15 April 2010.
7. Kodama, Y. and Hu, C.D. Bimolecular fluorescence complementation (BiFC): A 5-year update and future perspectives. *Biotechniques*, 53:285-298 (2012)
8. Ejendal, K.F.K., Conley, J.M., Hu, C.D. and Watts, V.J. Bimolecular fluorescence complementation analysis of G protein-coupled receptor dimerization in living cells. *Methods Enzymol.*, 521:259-279 (2013).
9. Kodama, Y. and Hu, C.D. Bimolecular fluorescence complementation (BiFC) analysis of *protein-protein* interaction: How to calculate signal-to-noise ratio. *Methods Navigator*, 2013 (In press).

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