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Award Number: W81XWH-06-1-0010

TITLE: Molecular Modulation of Inhibitors of Apoptosis as a Novel Approach for Radiosensitization of Human Prostate Cancer

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REPORT DATE: November 2007

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
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1. REPORT DATE 01-11-2007		2. REPORT TYPE Annual		3. DATES COVERED 15 Oct 2006 – 14 Oct 2007	
4. TITLE AND SUBTITLE Molecular Modulation of Inhibitors of Apoptosis as a Novel Approach for Radiosensitization of Human Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-06-1-0010	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Liang Xu, M.D., Ph.D. E-Mail: liangxu@umich.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Michigan Ann Arbor, MI 48109-0582				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 Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT The major goal of the project is to investigate the radiosensitization activity and mechanism of action of novel IAP-inhibitors in prostate cancer. In the second year of the project, we have investigated the in vivo radiosensitization activity of our lead IAP-inhibitors, SH-130 and Embelin, in human prostate cancer cell xenograft model. IAP-inhibitors potentially enhanced radiation-induced tumor growth inhibition. In nude mouse xenograft models, IAP-inhibitors Embelin and SH-130 potentially sensitized the DU-145 tumors to X-ray radiation. Bioluminescence imaging confirmed SH-130 plus radiation resulted in complete tumor regression in 6 out of 10 tumors, comparing 2/10 tumors with radiation alone, and 0/10 tumors with SH-130 alone. Mechanism studies show that NK-kB pathway activation was also inhibited in the combination therapy. Based on our exciting data obtained from this PCRP project, together with data from other collaborators, we are working with FDA for IND filing aiming for Phase I clinical trial with SH-130 as radiosensitizer for prostate cancer.					
15. SUBJECT TERMS apoptosis, radiation, IAP					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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I. Introduction:

In this project, we will investigate in vitro and in vivo radiosensitization activity and the mechanism of action of IAP-inhibitors in human prostate cancer with IAPs overexpression. Our basic hypothesis to be tested is that (1) The IAPs play a critical role in radiation resistance of human prostate cancer overexpressing IAPs; (2) Inhibition of the anti-apoptotic function of IAPs by small molecule IAP inhibitors will overcome radioresistance rendered by the overexpressed IAPs, that in turn will enhance tumor response and restore sensitivity of prostate cancer cells to ionizing irradiation. Our goal is to investigate and validate that IAPs are promising novel targets for radiosensitization of human prostate cancer with IAP-overexpression, with the ultimate goal to establish the molecular modulation of IAPs by potent small molecule IAP inhibitors as a novel approach for overcoming radiation resistance of human prostate cancer with high levels of IAPs.

II. Research progress and key research accomplishments:

This is the second year of the project. We have finished the tasks proposed in the *approved* Statement of Work for the corresponding period of time. Specifically, we have finished the following tasks:

A. Task 2. To investigate in vitro radiosensitization activity of potent small molecule inhibitors of IAPs in human prostate cancer with IAP overexpression. (months 13-18)

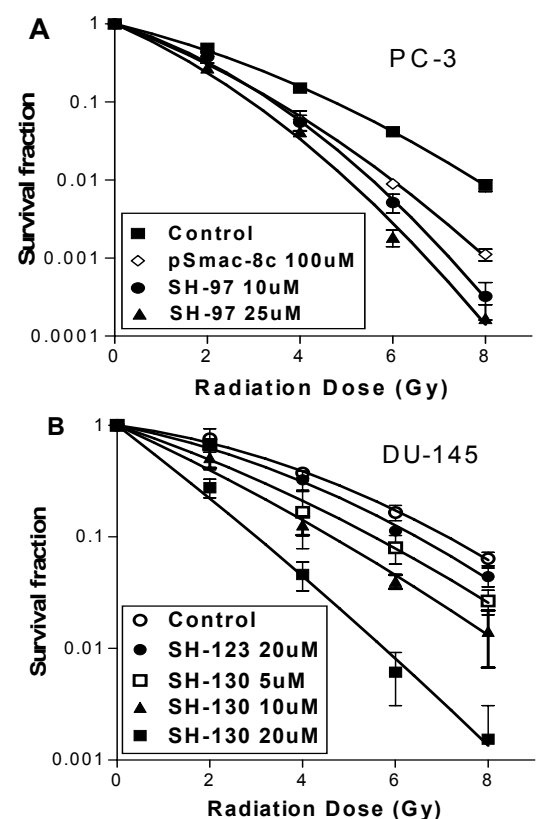
A.1. Employ clonogenic assay to investigate radiosensitization activity of potent small molecule inhibitors of IAPs in human prostate cancer in vitro. (months 13-18)

Overexpression of XIAP and/or other IAP proteins in cancer cells have been shown to inhibit apoptosis induced by not only chemotherapeutic agents but also radiation⁴⁴. Therefore, we hypothesize that treatment of cancer cells with a potent small molecule Smac-mimetic may sensitize the cancer cells to X-ray radiation by overcoming the protective effect of IAP proteins.

The clonogenic assay is considered to be the gold standard assay to assess radiation response of cells.^{4,80} We carried out the clonogenic assay to evaluate the effect of our novel IAP-inhibitors on radiation response in cancer cells. Human prostate cancer PC-3 and DU-145 cells were treated with our lead IAP-inhibitors and irradiated with X-ray within one hour. After 10-14-day's culture, the culture plates were stained with crystal violet, and the colonies with over 50 cells were counted with a ColCount colony counter. The cell-permeable Smac peptide, pSMAC-8c, was used as the positive control in this experiment. The cell survival curves were plotted with linear-quadratic curve fitting.^{1,4,80}

As shown in **Figure 1A**, pSmac-8c significantly sensitized PC-3 cells to X-ray radiation, indicating that IAP is a valid target for overcoming radiation resistance in the prostate cancer cells. Treatment of PC-3 cells with 10 and 25 μ M of SH-97 significantly increased the anti-tumor activity of radiation. At the 6 Gy dose of radiation, 10 and 25 μ M of SH-97 resulted in more than 10-fold reduction of clonogenic cell survival as compared to radiation alone. At 8 Gy, 10 and 25 μ M of SH-97 resulted in 40- and 50-fold reductions of cell survival as compared to radiation alone. 10 μ M of SH-97 appears to be more effective than 100 μ M of pSmac-8c, in terms of radiosensitizing potential, at both 6 and 8 Gy (**Figure 1A**), suggesting that SH-97 is at least 10-fold more potent than pSmac-8c in radiosensitization of PC-3 cells. In DU145 cells, IAP-inhibitor SH-130 showed dose-dependent radiosensitizing

Figure 1: IAP-inhibitors-mediated radiosensitization of human prostate cancer PC-3 (A) and DU-145 (B) cells. (Clonogenic assay survival curves)



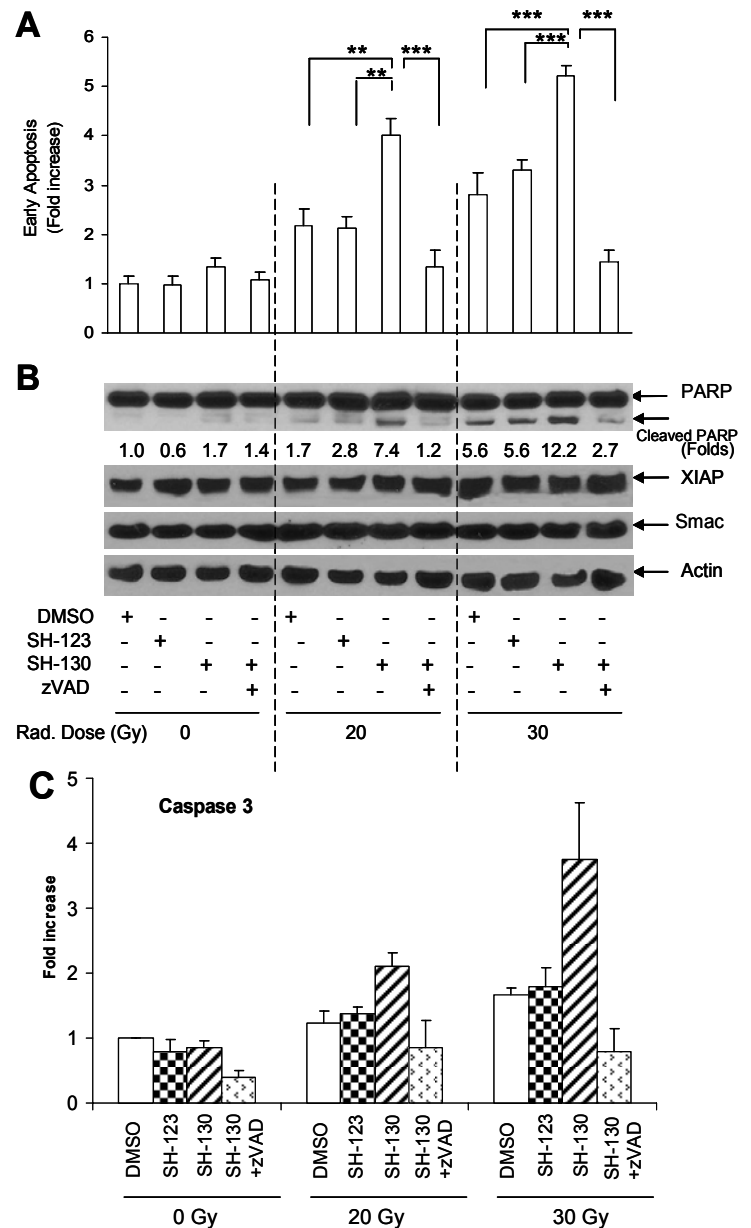
potential, while the inactive analog SH-123 showed no activity (**Figure 1B**).

These data provide strong support that small molecule inhibitors targeting IAPs have a promising therapeutic potential to improve radiation response of human prostate cancer cells, suggesting that targeting IAPs may be a promising novel approach for overcoming radiation resistance of human prostate cancer with IAP overexpression.

A.2. Evaluate the IAP inhibitor mediated potentiation of radiation-induced apoptosis in human prostate cancer cell lines in vitro. (months 13-18)

To provide evidence for the role of SH-130 in mediating radiation-induced apoptosis, we performed apoptosis assays in prostate cancer cells. DU-145 cells were treated with SH-130 or SH-123, with or without X-ray radiation, and assays for Annexin V – PI staining (by flow cytometry), Western blot and Caspase-3 activation were conducted from the same set of samples (**Fig. 2**). As shown in **Fig.2A**, induction of early apoptotic events (Annexin V positive) became evident when treated by both 20 Gy and 30 Gy radiation together with 10 μ M SH-130, about 4~5 folds increase compared with untreated control and 2 folds higher than radiation alone ($p<0.01$). Pretreatment with pan-caspase inhibitor, zVAD, abolished this increase of radiation-induced early-apoptosis by SH-130 ($p<0.001$, **Fig.2A**), indicating that the SH-130-mediated radiosensitization is Caspase-dependent. In contrast, neither SH-130 nor SH-123 alone induced significant apoptosis, suggesting a non-toxic profile of these compounds. We also calculated the total (early plus late) apoptotic events, and the combination therapy was also more potent than that of either treatment alone ($p<0.05$). The results were further confirmed by Western blot analysis (**Fig. 2B**) and Caspase-3 activation assay (**Fig.2C**). In the cells treated with radiation and SH-130, a clear PARP cleavage was observed, as compared with radiation alone. As PARP is one of the substrates of Caspase-3, our function assay also showed the same tendency of Caspase-3 activation (**Fig. 2C**),

Figure 2. SH-130 enhances X-ray radiation induced apoptosis. DU-145 were treated by 10 μ M SH-130 and X-ray radiation, with or without pan-caspase inhibitor zVAD. Twenty-four hours after incubation, cells were collected and processed to further analysis. **(A)**, Early apoptotic cell populations after treatment. Collected DU-145 cells were stained with Annexin V-FITC and PI (Trevigen) and determined by flow cytometry. Data represented the mean of three measurements $\pm$ SD, and were analyzed by Student's *t* test. **, $p<0.01$, ***, $p<0.001$. **(B)**, Western blot analysis of apoptosis related proteins. Whole-cell lysates were prepared and 10 μ g of the cell lysates were subjected to SDS-PAGE, electrotransferred to nitrocellulose membrane, and probed with antibodies against PARP, Caspase 3, XIAP and Smac. Actin was shown as loading control. Band intensity of cleaved PARP was normalized by Actin. **(C)**, Caspase-3 activation. Cells were lysed by the lysis buffer as indicated. Lysates (20 μ g) were reacted with fluorogenic substrate DEVD-AFC of Caspase-3. After incubation at 37°C for 2 h, released AFC was monitored by a microplate reader (BMG LABTECH). Data represented the mean of three measurements $\pm$ SD. Fold increase of fluorescence signal was expressed by normalizing activity to untreated control. Data represented one of the three independent experiments.



consistent with PARP cleavage. Here again, zVAD efficiently blocked the SH-130 effect on Caspase-3 activation and PARP cleavage, indicating that the radiosensitization effect of SH-130 is Caspase-dependent. For all the samples treated (**Fig. 2B**), expression level of XIAP and Smac didn't change, indicating that SH-130 worked on apoptosis pathway not through inhibiting the protein expression. The data showed here was repeated twice with similar results; confirming SH-130 enhances radiation-induced apoptosis in DU-145 cells.

B. Task 3. To investigate in vivo radiosensitization activity of small molecule inhibitors of IAP in human prostate cancer animal models with IAP overexpression. (months 13-36)

B.1. Preliminary toxicology study to evaluate the toxicity of IAP inhibitors (months 13-15)

We have evaluated the MTD of our lead IAP inhibitors. SH-130 has an MTD of 60 mg/kg, i.v., q.d. x 3 weeks. Embelin has an MTD > 100 mg/kg, p.o., q.d. x 3 weeks. Therefore, we used the doses of SH-130 50 mg/kg i.v. and Embelin 50-60 mg/kg p.o. in our animal experiments.

B.2. Employ nude mouse xenograft model of human prostate cancer to investigate radiosensitization efficacy of IAP inhibitors in vivo (months 15-24)

To evaluate whether our Smac-mimetic IAP-inhibitors can radiosensitize human prostate cancer with high levels of IAPs *in vivo*, we carried out pilot animal studies using human prostate cancer PC-3 and DU-145 xenograft models in nude mice. The PC-3 and DU-145 tumor models were established as we previously described. When the tumors reached a size of 120mm³ for PC-3 model, the mice were randomized (6-8 mice/12-16 tumors/group) and treated with our lead Smac-mimetic IAP-inhibitor, Embelin, 60mg/kg p.o. via oral gavage, q.d.5 x 3, or X-ray irradiation, 2 Gy q.d.5 x 2, or Embelin followed with radiation within 30min as combination therapy. The irradiation was performed as we recently described, with only tumor area exposed while the main body of a mouse was shielded. As shown in **Figure 3**, although Embelin alone had moderate effect on tumor growth, it significantly enhanced PC-3 tumor response to X-ray irradiation ($p<0.01$, two-way ANOVA, $n=12$).

For DU-145 tumor model, when the tumors reached a size of 60-80mm³, the mice were randomized (5-8 mice/10-16 tumors/group) and treated with our lead Smac-mimetic IAP-inhibitor, **SH-130**, 50mg/kg i.v. q.d.5 x 2, or X-ray irradiation, 2 Gy q.d.5 x 2, or SH-130 followed with radiation within 30min. As shown in **Figure 4**, the DU-145 tumor model is more resistant to radiation, consistent with our earlier reports. However, SH-130 potently sensitized the DU-145 tumors to X-ray irradiation, without increasing toxicity to the animals. The combination therapy inhibited the tumor growth, significantly more effective than either treatment alone ($p<0.001$, $n=14$). No obvious animal toxicity was observed. Notably, 5 out of 14 tumors in the combination group showed complete regression that did not grow back 5 months after the therapy, whereas radiation alone had only 2 out of 10 tumors with complete regression, no complete regression in SH-130 alone or vehicle control groups.

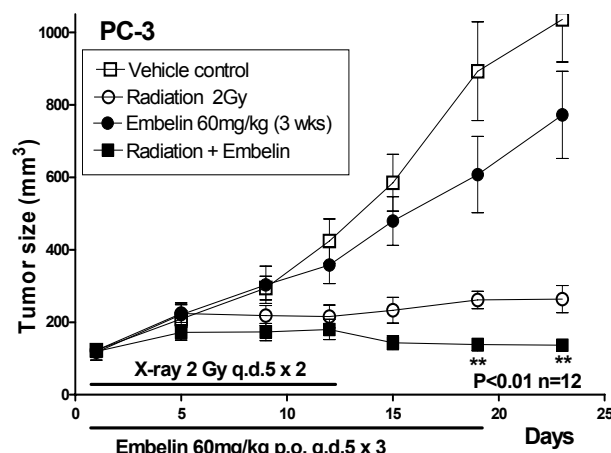


Figure 3. Embelin induced radiosensitization of human prostate cancer PC-3 in nude mouse s.q. xenograft model.

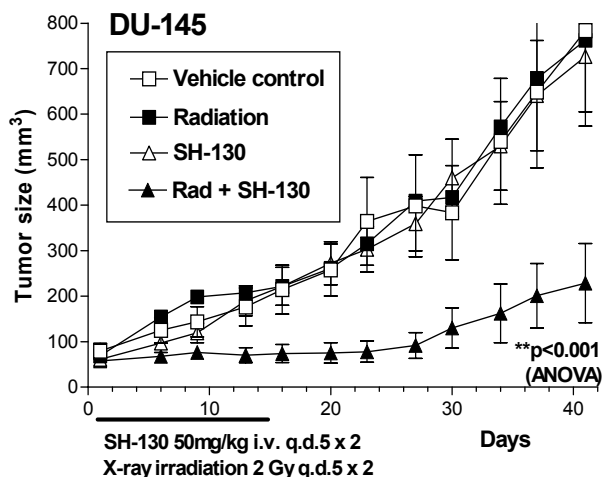


Figure 4. SH-130 enhanced the efficacy of X-ray irradiation in the radioresistant human prostate cancer DU-145 xenograft model.

B.3. *In vivo* radiosensitization potential in orthotopic PC-3^{lux} and DU-145^{lux} models using Bioluminescence Imaging and Micro-MRI (months 25-36)

In a repeat experiment for *in vivo* radiation, we used the DU-145^{lux} tumor model and employed Bio-Luminescence Imaging in University of Michigan Small Animal Imaging Core to evaluate the tumor response. In the first experiment we met with a problem that the mice tails could not be sustained for daily i.v. beyond two weeks for mice in the radiation groups. Therefore, in this second study, we changed SH-130 schedule from daily i.v. for 2 weeks to every other day for 3 weeks, and X-ray irradiation 2 Gy q.d.5 x 3 weeks. **Figure 5** shows the bioluminescence images at Day 24 (end of the 3-week treatment) of the four groups (**Fig.5A**) and the quantitative data (**Fig.5B**). **SH-130** enhanced the efficacy of X-ray irradiation; the combination therapy resulted in two-log reduction of bioluminescence signal as compared with radiation alone group, 4-5-log reduction as compared with vehicle control group. Bio-Luminescence Imaging analysis is quantitative, more sensitive and reliable than hand or caliper measurement. Another advantage of Bio-Luminescence Imaging is that we can identify directly from the images in **Fig.5A** the complete tumor regression without any trouble. SH-130 plus radiation led to complete tumor regression (undetectable in Bioluminescence Imaging in **Fig.5A**) in 6 out of 10 tumors (60%), while only 2/10 complete regression in radiation alone group (20%), none in vehicle control and SH-130 only groups (0%) (Summarized in **Fig.5B**).

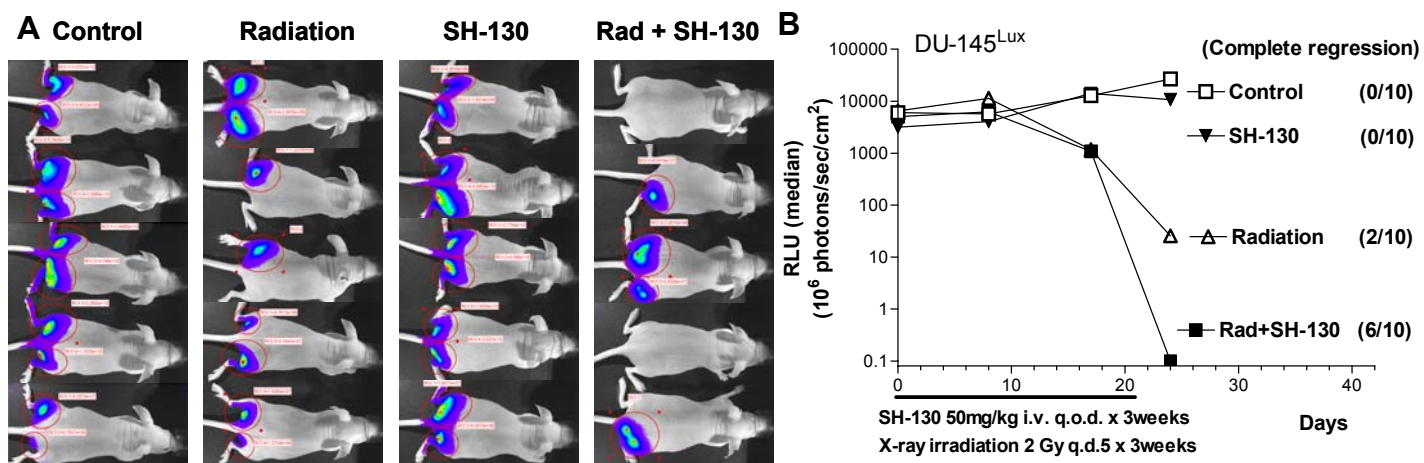


Figure 5. Bioluminescence Imaging of DU-145LUX tumor model in the 2nd *in vivo* tumor irradiation study. (A) Bioluminescence images of mice in the four treatment groups. (B) Quantitative data shown as median relative light unit (RLU) (10^6 photons/second/cm²) over the course of 3-week therapy.

These preliminary *in vivo* radiation studies provide us a strong proof-of-principle that a potent IAP-inhibitor can indeed sensitize a radioresistant tumor to radiation therapy, although the dose, schedule, and sequence of the two treatments are yet to be optimized in the combination therapy. The latter will be investigated in detail in the current proposal in multiple animal tumor models. Interestingly, in both *in vivo* studies, SH-130 alone showed no obvious effect on tumor growth; this is consistent with our *in vitro* data that SH-130 does not have single agent activity in terms of cytotoxicity. Having potent radiosensitizing activity without direct cytotoxicity to cells may be the unique feature for our lead compound SH-130 to be developed as a novel molecularly targeted radiosensitizer for cancers with IAP overexpression.

B.4. Immunohistological study of apoptosis induction and biomarkers in above animal models in response to IAP inhibitors and radiation therapy. (months 15-36)

We have collected all the samples in our efficacy studies and processed for immunohistological analysis. The data will be organized in our second manuscript to be submitted in a few months.

C. Task 4. To investigate the mechanism of action of small molecule inhibitors of IAP in overcoming radiation resistance of prostate cancer. (months 13-36)

C.1. Molecular target selectivity analysis using transfected cells.

We have established a series of clones of IAP transfected cells. The evaluations of these clones are still ongoing.

C.2 Investigation of the ability of IAP inhibitors to directly activate caspase-9 and -3 in response to radiation. (months 19-24)

Representative data are shown in **Figure 2** above and **Figure 6**.

C.3 Analysis of intra-cellular molecular targets of Smac-mimetic IAP inhibitors (months 19-36)

We have used biotin-pulldown assay with Western blot analysis or proteomics analysis to elucidate the intracellular molecular targets of the IAP inhibitors.

To further determine if our potent small molecule IAP-inhibitors can bind to the IAP family of proteins in cells, we carried out IAP pull-down assay using biotin-labeled IAP inhibitors. SH-series compounds were labeled with biotin via chemical conjugation. FP-based binding assay confirmed that biotin-labeled compounds SH-130BL have the same binding affinity to XIAP as unlabeled compounds. Cancer cell lysates were treated with SH-130BL or solvent DMSO for 1 hour on ice. Then streptavidin-agarose (Roche) was added and mixed for 2 hr at 4 °C. The agarose beads were washed 3-5 times and heated to 95 °C for 5 min in sample buffer. The released proteins were subjected to Western blot analysis probed with antibody against XIAP or cIAP-1. As shown in **Figure 6**, biotin-labeled IAP-inhibitors successfully pulled down both XIAP and cIAP-1 in DU-145 cells. Competition with unlabeled SH-130 can effectively block the pull-down of XIAP by SH-130BL. Similar results were also obtained in PC-3 cells (data not shown). The data provide strong support that the IAP-inhibitors can indeed target IAPs in prostate cancer cells, suggesting that XIAP and cIAP-1 may very likely be the molecular targets of our IAP inhibitors in these cells.

The IAP family of proteins are multi-functional proteins that bind to various binding proteins or adaptor proteins. These so-called binding proteins or adaptor proteins of IAPs play critical roles in multiple functions of IAPs in regulating cell division, cell cycle, and cell signaling, besides Caspase inhibition. Therefore, it is important to explore proteins other than IAPs that can also be pulled down by the biotin-labeled IAP inhibitors, *either directly or indirectly*. In collaboration with the Proteomics Core in University of Michigan Comprehensive Cancer Center,

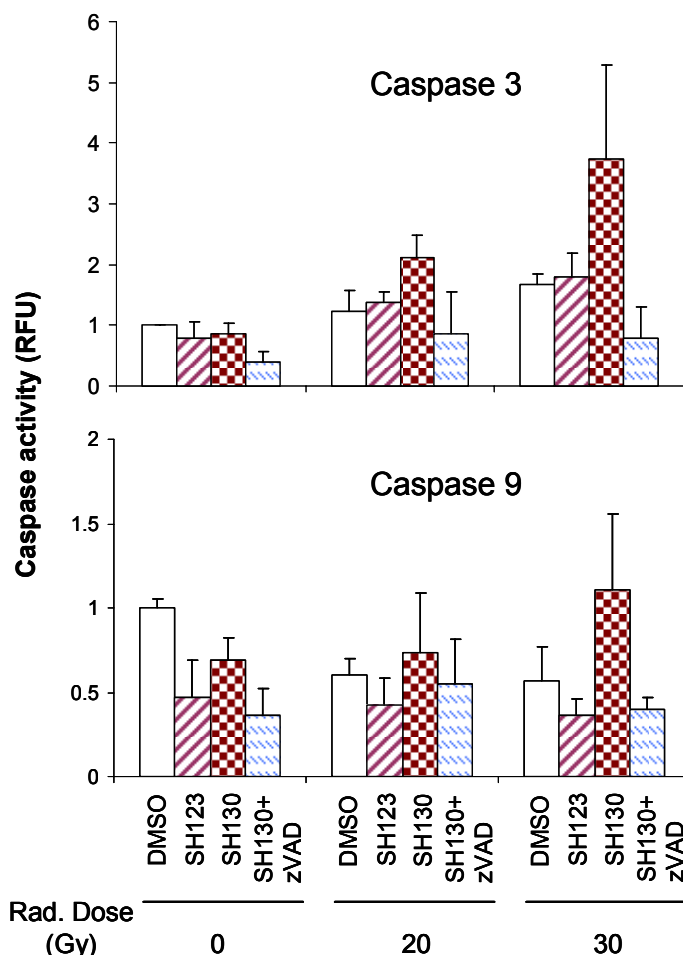


Figure 5. Analysis of Caspase 3 and 9 activation after treatment with SH-130 and radiation.

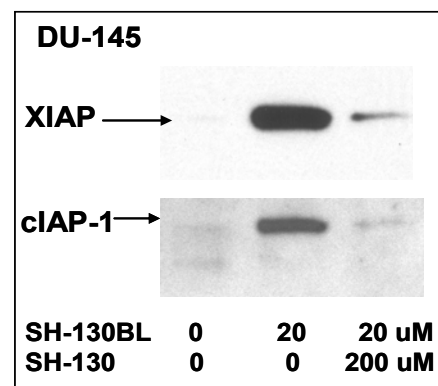


Figure 6. IAP pull-down assay. IAP pull-down assay with biotin-labeled SH-130 (SH-130BL) in DU-145 cells. Cell lysates were incubated with SH-130BL, or solvent DMSO, then add streptavidin-agarose to pull down the bound proteins. The Western blot analysis was performed. Both XIAP and cIAP-1 were pulled down by the IAP inhibitors.

we employed proteomics technology to analyze the proteins pulled down by the biotin-labeled IAP inhibitors. The pull-down assay samples were run in a 2-D gel and the spots that were outstanding over that from the control were picked for LC-MS/MS analysis and identification. Our preliminary proteomics analysis indicated that several members of IAPs were pulled down by the biotin-labeled IAP inhibitors, consistent with the Western blot data shown in **Figure 6**. More importantly, our proteomics study identified several unknown proteins and a few known proteins that have not been reported before to be able to bind to IAPs or Smac. Here are a few examples from the hit list (numbers are MS/MS Ion Score C.I.%): XIAP (99.16%), beta-tubulin (99.977%), heat shock protein 27 (96.03%), apoptosis inhibitor IAP homolog (99.79%), beta-actin (99.99%), GTPase-activating protein (96.48%), etc., as well as several unknown protein products. We are currently repeating and refining our proteomics assays and trying to confirm the findings through biochemical, immunological and genetic methods, which are still ongoing.

C.4. Use immunoprecipitation (IP)-pulldown assay to investigate the effects of Smac-mimetics on the interactions of XIAP with Smac and caspase-9.

To further examine whether the small molecule IAP-inhibitor, SH-130, can really bind to IAPs in cells as designed, we carried out IAP-immunoprecipitation (IP) pull-down assay to see if SH-130 would interfere with the XIAP (or cIAP-1) and Smac interaction. As shown in **Fig.7**, SH-130 potently and dose-dependently interrupted the interaction between XIAP/cIAP-1 and Smac. Interestingly, under non-reducing and non-denaturing conditions, XIAP and Smac formed the complex at molecular weight from approximately 80 kD to 160 kD, consistent with the tetramer model of Drs. Shi and Alnemri¹. Such XIAP-Smac complex level was clearly reduced by adding the Smac-mimic compound SH-130 (**Fig.7A** upper panel), with the increasing level of free XIAP and Smac (**Fig.7A**, middle panels). This reflects a desirable activity of SH-130, but not SH-123, on blocking the XIAP-Smac interaction in a dose-dependent manner. Such effect of SH-130 was further confirmed by immunoprecipitation with Smac antibody (**Fig.7B**). When Smac was pulled down, XIAP together with cIAP-1 were co-precipitated (**Fig.7B**, upper panels), and reversely, Smac could also be pulled down together with XIAP (**Fig.7B**, lower panels). SH-130 interrupted XIAP/cIAP-1 and Smac interactions dose-dependently, since the levels of IAP proteins pulled down were reduced with increasing SH-130. These data provided strong support that the IAP-inhibitor can indeed bind to XIAP/cIAP-1, thus interrupt the interaction between endogenous XIAP/cIAP-1 and Smac in prostate cancer cells.

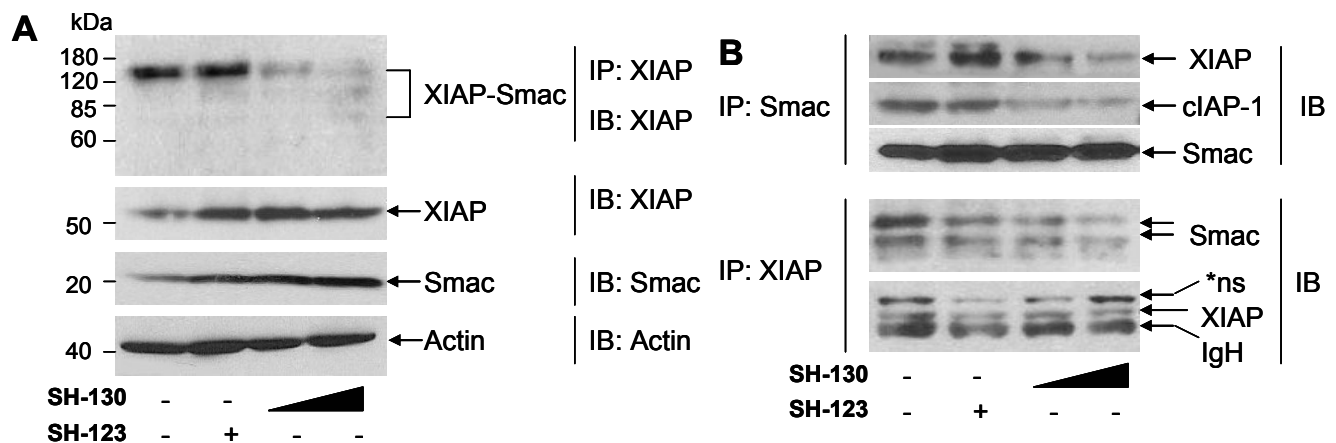


Figure 7. SH-130 interrupted the interaction between XIAP/cIAP-1 and Smac. **A.** DU-145 cell lysates were incubated with 1 μ M and 10 μ M SH-130, or 10 μ M SH-123 for 1 h. Lysates (20 μ l) were mixed with sample buffer (no reducing agent) and directly loaded on a 12% SDS-PAGE gel without boiling before antibody addition. Anti-XIAP and anti-Smac antibodies were used. Actin was a loading control. **B.** DU-145 cell lysates from **A** above were further incubated with anti-Smac or anti-XIAP antibody (Cell Signaling), and mixed with Protein A/G-Agarose beads (Invitrogen). Final eluents were processed to Western blot and probed with antibodies against XIAP, cIAP-1 and Smac. IgG heavy chain (IgH) was also probed as a control. PD, pull-down; IP, immunoprecipitation; IB, immunoblotting. *ns, non-specific. Data shown was one representative result of three independent experiments.

C.5. IAP-inhibitors inhibit NF-κB activation

A recent study suggests that IAP-inhibitor, Embelin, also inhibits NF-κB activation by TNFα. We confirmed this finding in prostate cancer cells. To investigate whether this is true only for natural compound such as Embelin that may have multiple targets, we analyzed the effect of SH-130, our more potent and specific IAP-inhibitor, on the NF-κB activation in prostate cancer cells. As shown in **Figure 8**, SH-130 inhibited TNFα-induced IκBα degradation, an indication of NF-κB activation by the canonical pathway. The negative control compound SH-123 that does not bind to IAPs has no effect on NF-κB. SH-130-mediated NF-κB inhibition is partial and dose-dependent, without significant change of Rel A translocation, suggesting that SH-130-mediated inhibition of NF-κB activation is indirect.

Above data were confirmed by a quantitative, luciferase-based NF-κB reporter assay. As shown in **Figure 9**, both SH-130 and Embelin inhibited TNFα-induced NF-κB activation in NF-κB luciferase reporter assay. This inhibition could not be blocked by Pan-Caspase inhibitor zVAD, indicating that the IAP-inhibitor-mediated NF-κB inhibition does not involve Caspases. This NF-κB inhibition is partial, even at high doses of IAP-inhibitors, comparing with celastrol, a natural proteasome inhibitor that completely blocked NF-κB activation, consistent with the recent literature. In a separate qRT-PCR assay of NF-κB target gene expression, Embelin also partially block NF-κB activation (**Figure 10**).

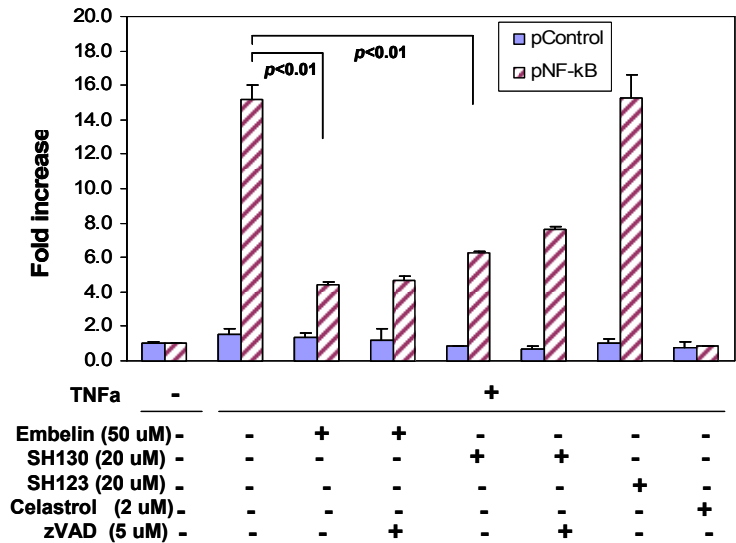


Figure 9. SH-130 inhibits TNFα-induced NF-κB activation in NF-κB luciferase reporter assay. DU-145 cells were co-transfected the NF-κB luciferase reporter (pNF-κB) or a vector control (pControl), together with β-galactosidase plasmid. 24 h later, cells were pre-treated with different drugs in duplicates for 1 hr, followed with TNFα stimulation for 4 h, and then lysed to measure the luminescence and normalized to β-galactosidase activity. Fold increase was calculated for each treated sample by dividing the normalized luciferase activity with that of untreated control. Celastrol: positive control compound.

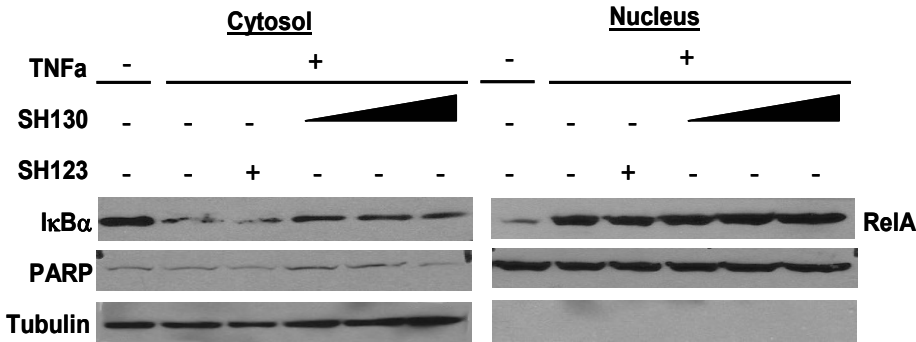


Figure 8. Effect of IAP-inhibitor SH-130 on TNFα-induced NF-κB activation in DU-145 cells. Cells were pre-treated with SH-130 for 1 hr, then TNFα was added for 10 min. TNFα was removed and cells were incubated with SH-130-containing medium for another 30 min. Cells were then collected for cytosol-nucleus fractionation. Subcellular fractions were subjected to Western blot with antibodies against the indicated proteins. SH-123: negative control compound.

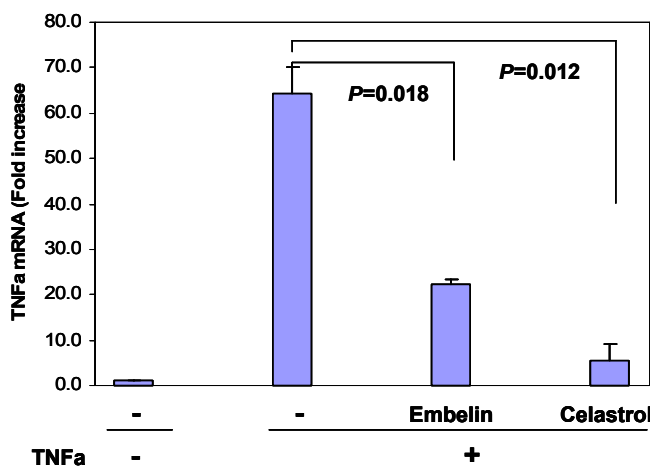
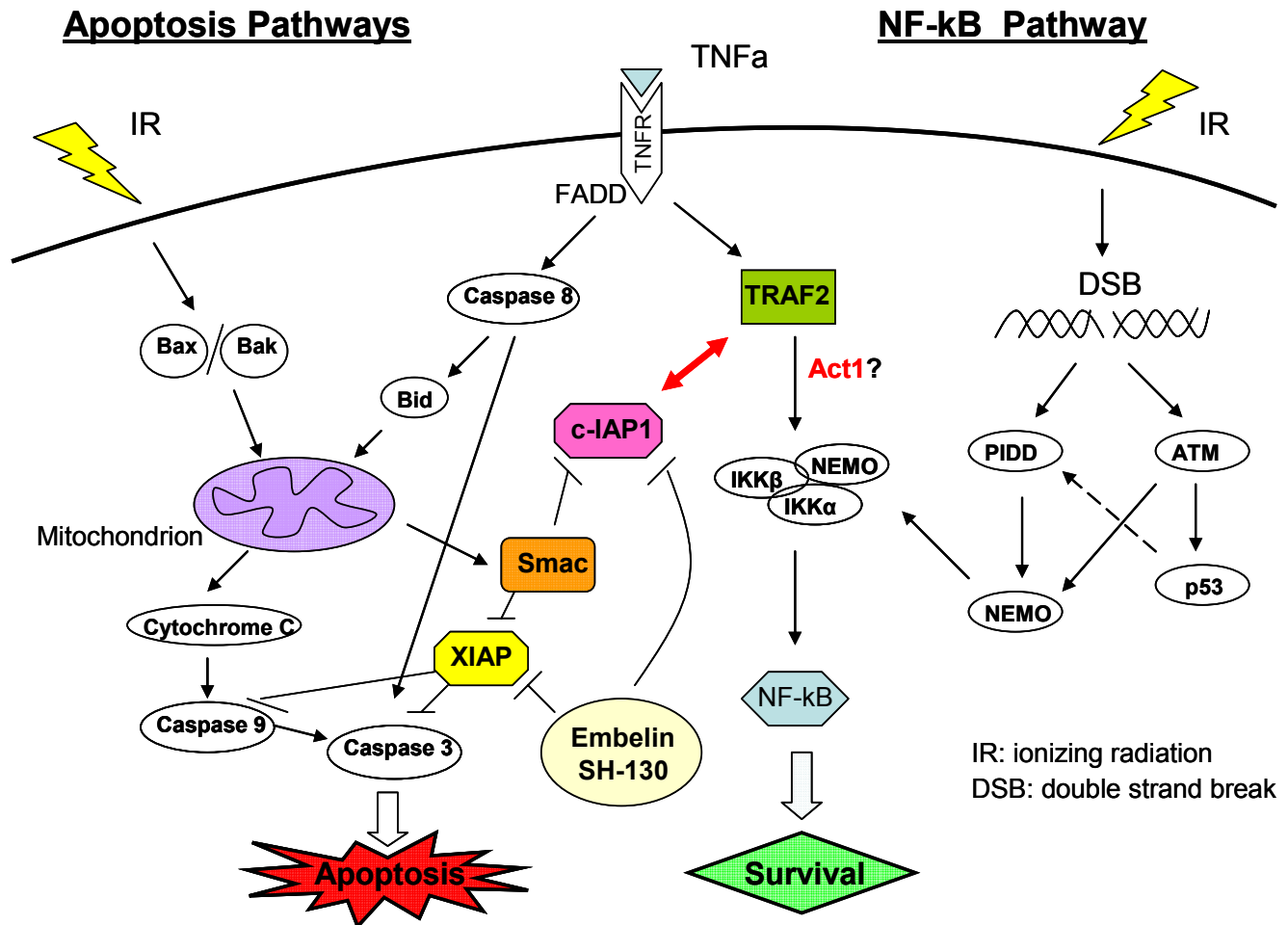


Figure 10. Embelin inhibits of NF-κB activation as analyzed by qRT-PCR of NF-κB target gene expression. DU-145 cells were treated embelin (50 uM) or celastrol (4 uM) for 0.5 hr, then TNFα was added for 10 min pulse stimulation. Then TNFα were removed and cells were incubated with drug-containing medium for another 1 hr. RNA was extracted and Quantitative RT-PCR was performed to detect TNF as a NF-κB target gene and GAPDH as a control, using Taqman kits. Fold increase of gene expression was calculated for each treated sample by dividing the normalized TNF expression activity with that from the untreated control.

Taken together, our preliminary data with NF- κ B activation studies demonstrate that IAP-inhibitors Embelin and SH-130 can block NF- κ B activation without involving Caspases. This partial and apparently indirect inhibition of NF- κ B activation indicates a potential role of IAP – Smac in NF- κ B pathway other than classical apoptosis pathway. Based on these exciting new finding and recent publications in the literature, we propose a potential link or crosstalk between IAP-apoptosis pathway and NF- κ B pathway: the TRAF-cIAP–(Act1)–NEMO loop. See **Chart III** for more details. Delineation of this link/crosstalk is beyond the current funded project and is one of the aims in the R01 proposal submitted.

Chart III: Working hypothesis: Crosstalk between Apoptosis and NF- κ B pathway



III. Reportable outcomes:

A. One manuscript was submitted to Cancer Research and under revision.

Yao Dai, et al, and Liang Xu*. Molecularly targeted radiosensitization of human prostate cancer by potent small molecule inhibitor of IAP. (*Corresponding author)

B. The second manuscript on Embelin radiosensitization will be submitted soon.

C. One R01 grant applied in 2006 and resubmitted in 2007:

Based on the data partly obtained from this PRCP grant, we applied an R01 grant in 2006. Scored **188, 24.2 percentile**. Will be resubmitted soon.

1 R01 CA118367-01A2 (Liang Xu, P.I.)

NIH/NCI R01

\$250,000

“Radiosensitization by modulating IAP family of proteins”

The major goal of the proposal is to evaluate the small-molecule inhibitors of IAP as a novel approach for radiosensitization of human cancers.

Role: Principal Investigator

D. Funded from this PRCP grant, two abstracts were presented in national or international meetings, and one abstract will be submitted to 2007 AACR annual meeting.

- Xu L, et al. Molecularly targeted cancer chemo/radiosensitization in vitro and in vivo by modulating apoptotic pathways. *The American Association for Cancer Research 98th Annual Meeting 2007*, Los Angeles, California, April 14-18, 2007. (Poster)
- **Xu, L.** Molecular radiosensitization of prostate cancer by targeting apoptosis pathways. *DOD PCRP Innovative Minds in Prostate Cancer Today (IMPACT) Meeting*, Atlanta, GA, September 05-08, 2007. **(Oral presentation)**
- **Xu L**, et al. Molecular radiosensitization of prostate cancer by modulating cell death pathways. *AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics*, San Francisco, CA, October 22-26, 2007. (Poster)

IV. Conclusions:

The major goal in the second year of the project is to investigate the radiosensitization activity in vivo and the mechanism of action of novel IAP-inhibitors in prostate cancer models. We have investigated the *in vivo* radiosensitization activity of our lead IAP-inhibitors, SH-130 and Embelin, in human prostate cancer xenograft models. IAP-inhibitors bind to XIAP BIR3 and potently antagonize XIAP BIR3, and promote activation of Caspase-3 and Caspase-9 in a dose-dependent manner. They potently enhanced apoptosis induction in cancer cells and sensitized cancer cells to radiation. Biotin-labeled compound pull-down assay showed that XIAP, cIAP-1 and cIAP-2 proteins can be pulled down by the IAP-inhibitors. In nude mouse xenograft models, IAP-inhibitors Embelin and SH-130 potently sensitized the DU-145 tumors to X-ray radiation. Bioluminescence imaging confirmed SH-130 plus radiation resulted in complete tumor regression in 6 out of 10 tumors, comparing 2/10 tumors with radiation alone, and 0/10 tumors with SH-130 alone. Mechanism studies show that NF- κ B pathway activation was also inhibited in the combination therapy. Based on our exciting data obtained from this PCRP project, together with data from other collaborators, we are working with FDA for IND filing aiming for Phase I clinical trial with SH-130 as radiosensitizer for prostate cancer.

VI. APPENDICES: