

AD_____

Award Number: W81XWH-08-1-0114

TITLE: Mechanisms of Radiosensitization by the Neurotensin Receptor Antagonist
SR48692 in Prostate Cancer Models

PRINCIPAL INVESTIGATOR: Jaroslaw Dziegielewski, Ph.D.

CONTRACTING ORGANIZATION: University of Virginia
Charlottesville, VA 22904

REPORT DATE: April 2009

TYPE OF REPORT: Annual

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 (DD-MM-YYYY) 01-04-2009		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 1 APR 2008 - 31 MAR 2009	
4. TITLE AND SUBTITLE Mechanisms of Radiosensitization by the Neurotensin Receptor Antagonist SR48692 in Prostate Cancer Models				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-08-1-0114	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jaroslaw Dziegielewski, Ph.D. ; Email: JD5KF@VIRGINIA.EDU *				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Virginia Charlottesville, VA 22904				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 Radiation is one of the most used treatments for prostate cancer. However, it causes several negative sideeffects, including the damage to surrounding normal tissues. An agent which selectively sensitizes prostate tumor but not normal prostate could improve the therapeutic ratio of radiation. Neurotensin is a one of the factors secreted by neuroendocrine cells in prostate, which stimulates and promotes cancer cell growth and proliferation. In this project we are investigating if the inhibition of neurotensin receptor by SR48692 drug could sensitize cancer cells to radiation. SR48692 activity was measured in PC3, C42 and LNCaP prostate cancer cells, as well as in RWPE1 normal prostate epithelial cells, using clonogenic survival and growth inhibition assays. PC3Mluc orthotopic xenografts in nude mice were treated with SR48692, radiation, or in combination, and tumor growth was determined by bioluminescence imaging. Our results show that inhibition of neurotensin receptor by SR48692 effectively sensitizes human prostate cancer cells to radiation, while the effects on normal prostate epithelial cells are minimal. Importantly, the combination therapy (SR48692 and radiation) suppressed xenograft tumor growth in nude mice significantly more than either treatment alone. Our study suggests that NTR1 receptor, or neurotensin signaling pathway, are viable targets for combined chemo/radiotherapy of prostate cancer.					
15. SUBJECT TERMS radiotherapy, prostate cancer, drug development, neuroendocrine signaling					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 10	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>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	10
Reportable Outcomes.....	10
Conclusion.....	10

INTRODUCTION

Prostate cancer is the most common cancer in men in the United States, and the second leading cause of cancer deaths. Radiotherapy of prostate cancer is limited by both radioresistance of prostate cancer cells and the adverse radiation effects on surrounding normal tissue. Thus, an agent that radiosensitizes prostate tumor cells but spares normal prostate tissue would have clinical significance. Based on our previous studies concerning the role of neuroendocrine cells (NE) in prostate cancer, we hypothesized that SR48692, a specific inhibitor of neurotensin receptor 1 (NTR1), (1) will radiosensitize cancer cells and tumors, (2) that normal prostatic tissue will be spared *in-vivo*, and (3) that the mechanism involves EGFR Tyr845/Src/Stat5b. These hypotheses are tested in two Specific Aims: (1) to study the radiosensitizing properties of SR48692 using androgen-sensitive and androgen-insensitive prostate cancer models vs. normal tissue effects *in-vitro* and *in-vivo*; and (2) to examine the molecular mechanisms of radiosensitization by SR48692 in human prostate cancer cell lines by studying novel effectors downstream of the neurotensin receptor (NTR1). Successful completing of this project will allow us to develop an efficient radiosensitizing agent, with potential clinical application in prostate cancer radiotherapy.

BODY

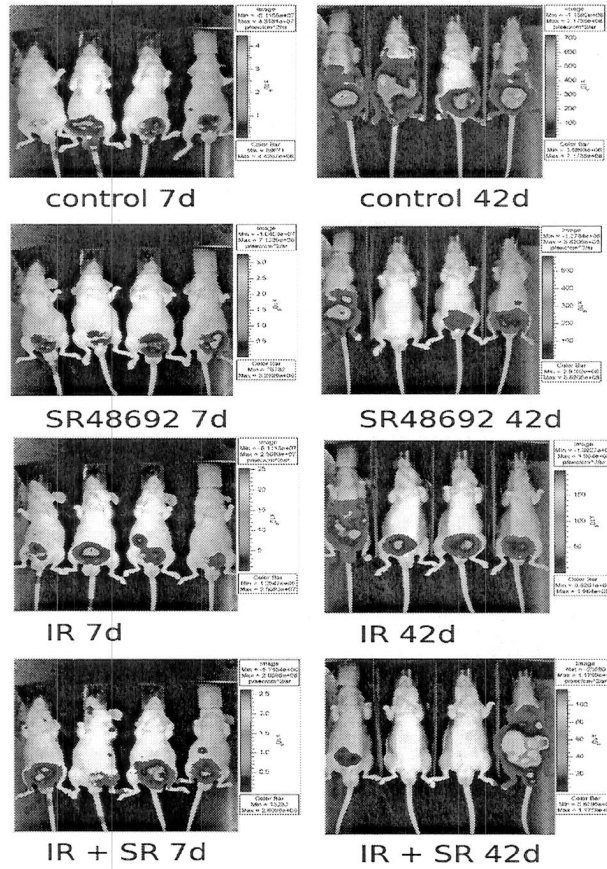
This is the first year report for grant W81XWH-08-1-0114 covering the initial phase of the project. During this phase we have concentrated on Specific Aim 1, investigating the radiosensitizing activity of SR48692 in both *in-vivo* and *in-vitro* model systems.

For *in-vivo* studies nude mice harboring human orthotopic (intra-prostate) xenografts were used. The xenografts were established from PC-3M-luc cells, a metastatic variant of human prostate adenocarcinoma PC-3 cells, engineered to stably express luciferase. This system allows for non-invasive continuous measurements of cancer volume/size, even for xenografts located inside the body. In addition, our preliminary experiments demonstrated that in this system bioluminescence intensity correlates very well with tumor size as measured by calipers.

NCI nu/nu mice aged 5 weeks were injected in the posterior ventral lobe of the prostate (orthotopically) with 5×10^6 PC-3M-luc cells. The mice were divided into 4 groups (control, radiation only, drug only, and IR + drug). On days 4-8 post-injection, the groups receiving SR48692 were gavaged with 25mg/kg drug. Mice receiving radiation were subjected to a 2.5 Gy dose of ionizing radiation on days 5 and 7 post-injection. The mice were anesthetized, injected with luciferin and imaged on a Xenogen IVIS system every 7 days post-injection.

As demonstrated in **Fig 1** mice receiving the combination therapy (SR48692 and radiation) showed significant reduction in tumor size over either treatments alone. Interestingly, the radiation alone regime used here delayed tumor growth for approximately two weeks, but did not produce significant decrease in tumors number or size. However, the combination treatment with SR48692 not only delayed the first occurrence of measurable tumors, it also seemed to eradicate tumors in some mice. Further studies to confirm these observations are under way.

A



B

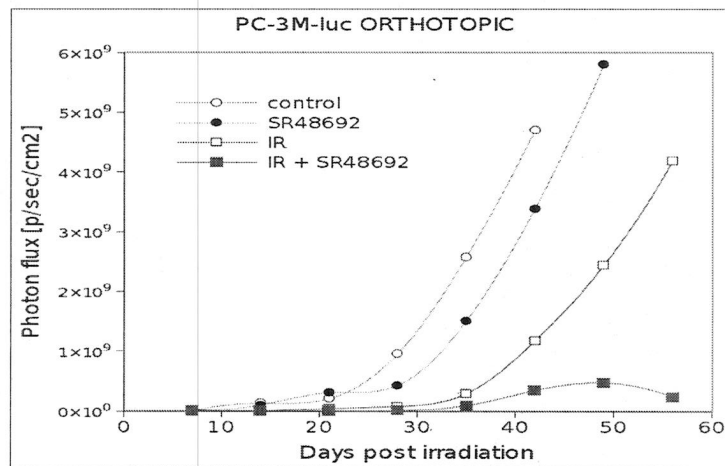


Figure 1. SR48692 sensitizes human orthotopic xenografts in mice *in-vivo* to therapeutic doses of ionizing radiation. Tumor size was measured by non-invasive bioluminescence imaging.

In *in-vitro* studies in this first phase we have compared the radiosensitizing properties of SR48692 in prostate cancer cell line (PC-3M, highly metastatic human prostate adenocarcinoma cells) and apparently normal human prostate epithelial cells (RWPE-1).

Both cell lines were treated with SR48692 at 1 μ M, the drug concentration known to efficiently block neurotensin-dependent cells' growth stimulation, as established in our preliminary experiments. Following 24 h treatment the cells were irradiated with x-rays doses in the range from 0 to 6 Gy and re-plated for colony formation. Colonies containing more than 50 cells were fixed, stained and scored, and the surviving fraction was calculated according to standard method.

As shown in **Fig 2A** SR48692 effectively sensitizes prostate cancer cells PC-3M to ionizing radiation at all studied doses. Based on the shape of clonogenic survival curve obtained from drug-treated cells, i.e. steep curve lacking broad shoulder in the low dose range, we expect that SR48692 activity could be mediated by one of the following mechanisms, all of which result in steep shape of clonogenic survival curve: (1) inhibition of DNA damage repair, (2) synchronization of cells in M phase of cell cycle, (3) efficient induction of apoptotic cell death, and/or (4) any combination of the above. These possible mechanisms of SR48692-induced radiosensitization are currently under investigation.

Importantly, pre-treatment with SR48692 does not have any significant effect on radiosensitivity of normal epithelial cell line RWPE-1, as shown in **Fig 2B**. This striking difference between normal (RWPE-1) and cancer (PC-3M) prostate cells is emphasized on **Fig 2C**, by comparing survival curves of both drug-treated and irradiated cell lines. As demonstrated on the graph, at the clinically relevant dose of 2 Gy, the difference between surviving fractions (SF2) in RWPE-1 and PC-3M cells treated with SR48692 and irradiated is approximately 2.1. Therapeutic gain of this magnitude has high clinical significance for radiotherapy of prostate cancer.

The difference between these two cell lines can be explained by our hypothesis that only cells expressing neurotensin receptor 1 (NTR1) will respond to SR48692 treatment. Our preliminary results clearly demonstrated that normal prostate cells (RWPE-1) do not express NTR1, as opposite to cancer cells (PC-3M). Since there is evidence that majority of human prostate tumors express NTR1, while normal surrounding tissues do not, SR48692 could become a very important clinically radiosensitizer for prostate radiotherapy.

In additional experiments we have tested the possibility that SR48692 alone is cytotoxic to prostate cancer cells (not the slight delay of xenografts development in mice treated with the drug alone, **Fig 1**). PC-3M cells were treated with the drug at doses ranging from 0 to 10 μ M for 24h, and then counted directly for short-term growth inhibition assessment. For long-term clonogenic survival the cells were plated at 100 cells per dish, treated continuously for 7-10 days, and the surviving colonies were stained and scored. As shown in **Fig 3**, the drug alone is relatively non-toxic to PC-3M cancer cells.

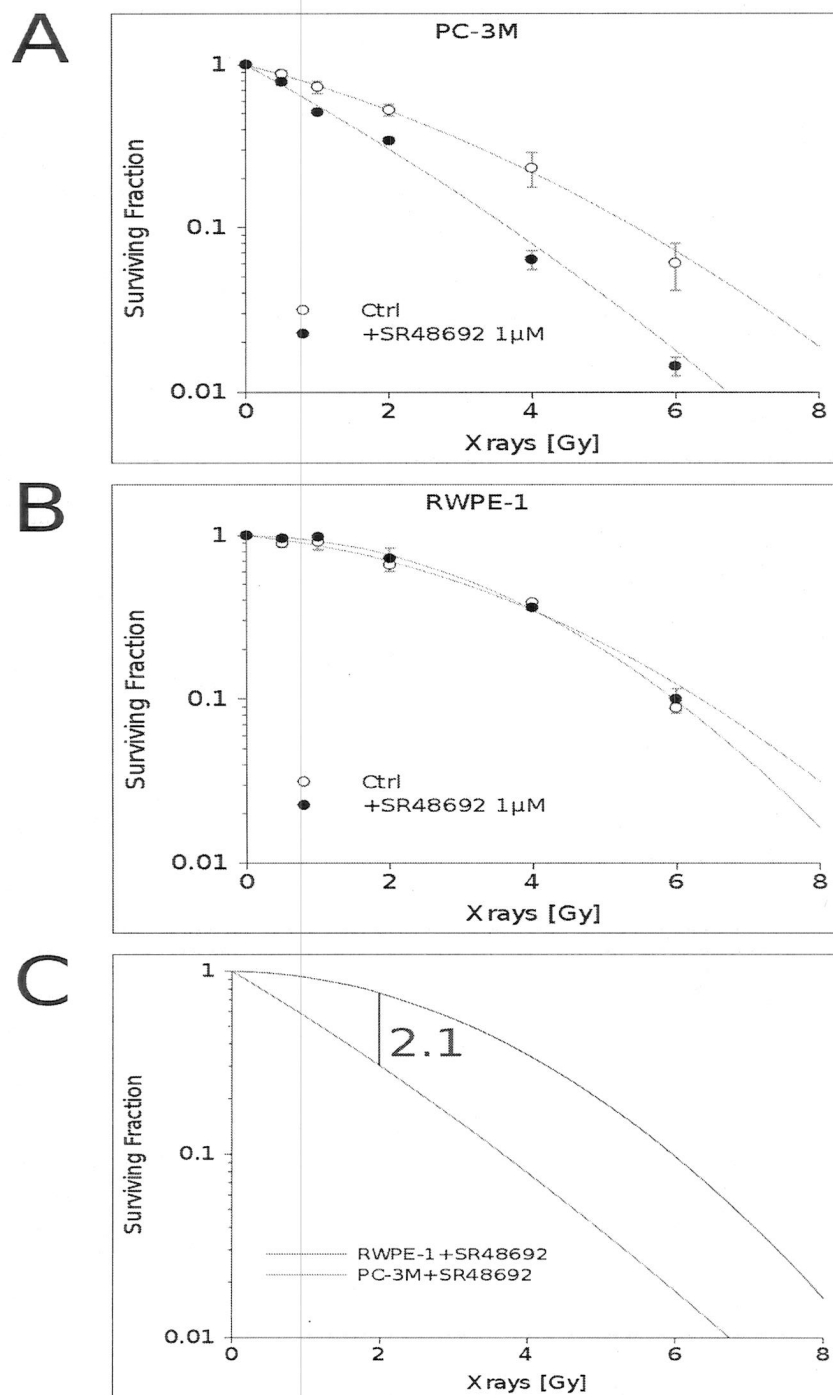


Figure 2. SR48692 sensitizes human prostate cancer cells (**A**, PC-3M) to ionizing radiation *in-vitro*, but does not have any significant radiosensitizing effect in normal prostate epithelial cells (**B**, RWPE-1). (**C**) Comparison of radiosensitivity of SR48692-treated normal (RWPE-1) and cancer (PC-3M) prostate cells.

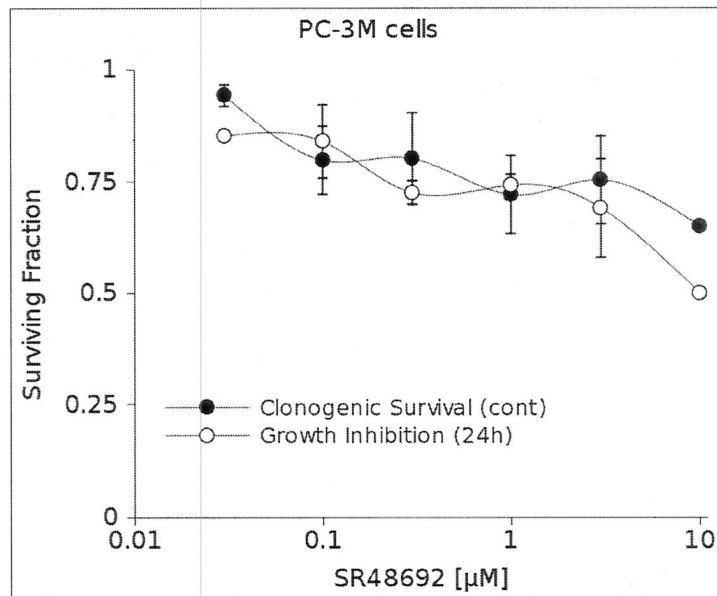


Figure 3. SR4869 alone is relatively non toxic to PC-3M cancer cells.

We have also started to explore the role of cells/cancers' androgen-dependence in their sensitivity to SR48692 and radiation treatment. Three cell lines were selected for the preliminary experiments: (1) androgen-sensitive LNCaP, (2) androgen-independent C4-2, and (3) normal prostate cells RWPE-1. Cells were grown in medium depleted of androgen (and other steroid hormones) by charcoal treatment, and supplemented with R1881 (androgen substitute), NT (neurotensin), or full fetal bovine serum (full medium). Following 24 h incubation the cells were treated with increasing concentrations of SR48692, and the cells' viability was measured 5 days later by XTT assay. As demonstrated in **Fig 4** normal prostate cells (RWPE-1, **Fig 4A**) as well as androgen-independent cancer cells (C4-2, **Fig 4C**) grow relatively well in androgen-free medium, and do not respond significantly to SR48692. In contrast, LNCaP cells (androgen-sensitive, **Fig 4B**) not only require androgen to proliferate, but also their response to SR48692 treatment is more pronounced. Interestingly, stimulation with NT can induce proliferation (up to ~50% of normal rate) in androgen-depleted LNCaP cells. The follow-up studies testing the radiosensitivity of LNCaP, C4-2, PC-3M and RWPE-1 cells in the presence or absence of androgen are in progress.

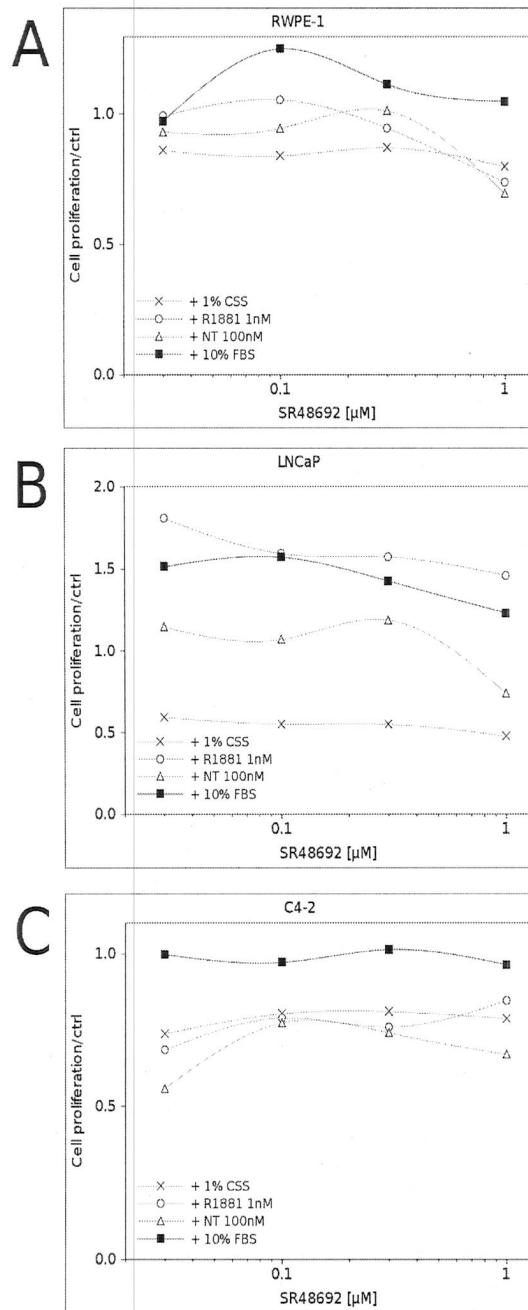


Figure 4. SR4869 cytotoxic activity on prostate cells differs depending on their androgen dependence.

KEY RESEARCH ACCOMPLISHMENTS

- SR48692 **sensitizes** human prostate **cancer xenografts** to ionizing radiation *in vivo*
- SR48692 **sensitizes** human prostate **cancer cells** to ionizing radiation *in vitro*
- SR48692 **does not sensitize** human prostate **normal epithelial cells** to ionizing radiation *in vitro* (the therapeutic gain of about 2)

REPORTABLE OUTCOMES

The results obtained during the first year of this project were presented at the 100th Annual Meeting of the American Association for Cancer Research (April 18-22, Denver, CO) [1]. In addition two presentations describing our results were accepted for presentation during this year annual meetings of Radiation Research Society (October 4-7, Savannah, GA) [2], and American Society for Therapeutic Radiology and Oncology (November 1-5, Chicago, IL) [3].

[1] G Amorino, B Pemberton, M Dunlap-Brown, S Parsons; Neurotensin receptor antagonist SR48692: potential radiosensitizer in mice.

[2] J Dziegielewski, BL Pemberton, ME Dunlap-Brown, JM Larner, SJ Parsons, GP Amorino; SR48692, a specific neurotensin receptor (NTR1) antagonist, sensitizes prostate cancer cells to ionizing radiation, in both in-vitro and in-vivo models.

[3] J Dziegielewski, BL Pemberton, ME Dunlap-Brown, SJ Parsons, GP Amorino, JM Larner; The specific neurotensin receptor (NTR1) antagonist, SR48692, sensitizes prostate cancer cells to ionizing radiation, in both in vitro and in vivo models.

CONCLUSION

The results obtained during the first year of this project convincingly demonstrate that SR48692, a specific inhibitor of NTR1 receptor, sensitizes human prostate tumors and cancer cells to ionizing radiation at doses used in clinical radiotherapy. At the same time SR48692 does not sensitizes normal prostate cells to radiation. The experiments aiming to elucidate the molecular mechanism(s) of SR48692 radiosensitizing activity and selectivity are in progress, and will provide a solid foundation for future selective radiotherapy of human prostate cancers.