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Award Number: DAMD17-98-1-8546

TITLE: Radiosensitization of Prostate Tumor Cells by Prenyltransferase Inhibitors

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REPORT DATE: October 1999

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

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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1999	Annual (1-Oct-98 - 30-Sep-99)

4. TITLE AND SUBTITLE
Radiosensitization of Prostate Tumor Cells by Prenyltransferase Inhibitors

5. FUNDING NUMBERS
DAMD17-98-1-8546

6. AUTHOR(S)
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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. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

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13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this study is to determine whether ras oncogene activation contributes to radiation resistance in prostate tumor cells, and if so, whether this resistance can be reversed through the use of prenyltransferase inhibitors. We have examined both rodent and human prostate tumor cell lines *in vitro* and determined that radiation resistance is increased in some, but not all lines after expression of activated ras is induced by transfection or transduction with activated *ras* oncogenes. In cells where expression of activated ras was linked to increased radiation resistance, treatment with farnesyltransferase inhibitors resulted in radiosensitization *in vitro*. Preliminary *in vivo* results show that farnesyltransferase inhibitors have the effect of reducing tumor hypoxia in prostate tumors expressing activated ras. Our results imply that prenyltransferase inhibitors may be useful in the treatment of prostate tumors when used in conjunction with radiation therapy. These inhibitors appear to affect both intrinsic cell radiosensitivity (as measured *in vitro*) as well as altering the tumor micro-environment (as shown *in vivo*).

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate Cancer	16
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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Eric Jacques Bernard 9/10/99

PI - Signature Date

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Introduction:

Oncogenic ras expression has been shown to increase the radiation resistance of many human and rodent tumor cell lines(1-4). The focus of this grant is to examine whether the presence of activated ras in prostate tumors increases their resistance to killing by radiation, and if this is the case, to examine a means of reversing this radiation resistance. This grant was funded without modification of the specific aims which are:

Aim 1. To determine the effects of prenyltransferase inhibition on the radiosensitivity of H-ras transformed murine prostate tumor cells.
Aim 2. To define the effects of prenyltransferase inhibition on the radiosensitivity of human prostate tumor cell lines expressing either H- or K-*ras* oncogenes.
Aim 3. To determine the effectiveness of prenyltransferase inhibitors as prostate tumor radiosensitizing agents *in vivo*.

We previously demonstrated that transformation by oncogenic ras caused increased radiation resistance in rat embryo fibroblasts (5). We subsequently showed that inhibition of ras activity led to a decrease in the radiation survival in these cells accompanied by an increased radiation-induced apoptosis (6). In these studies, ras activity was inhibited by preventing the post-translational prenylation of ras required for its membrane association. Subsequent studies on human tumor cells with H- or K-ras mutations showed that these cells could also be radiosensitized by prenyltransferase inhibitors (7). Activating mutations of *ras* genes have been found in both human prostate tumors (8-13)] and have been studied in animal models of prostate tumorigenesis (14-16). We have therefore examined the role of ras activation in prostate tumor cell radioresistance.

Body:

As outlined in the plan of work, the majority of the work during the current year has focussed on specific aims 1 and 2. In order to characterize the influence of activated ras on the radiation resistance of prostate tumor cells we have inhibited H-ras activity in mouse prostate tumor lines expressing this oncogene using farnesyltransferase inhibitors. These inhibitors block the post-translational processing of the ras p21 protein required for its association with the cell membrane and its activity. Our studies have confirmed the preliminary results presented in the original application showing that treatment of H-ras + v-myc transformed mouse prostate tumor cells with farnesyltransferase inhibitors can result in increased apoptosis and significantly decreased long-term survival after irradiation as measured by clonogenic assay. These results have now been obtained with two different inhibitors. A total of 4 murine prostate tumor cell lines have been examined for induction of apoptosis by FTI plus radiation. Of these 4, three show significant enhancement of apoptosis at 24 h after irradiation although the levels of apoptosis varied between cell lines. Representative data are shown for two of the cell lines in Figure 1. One cell line showed no significant difference from irradiation alone after farnesyltransferase inhibitor treatment (not shown).

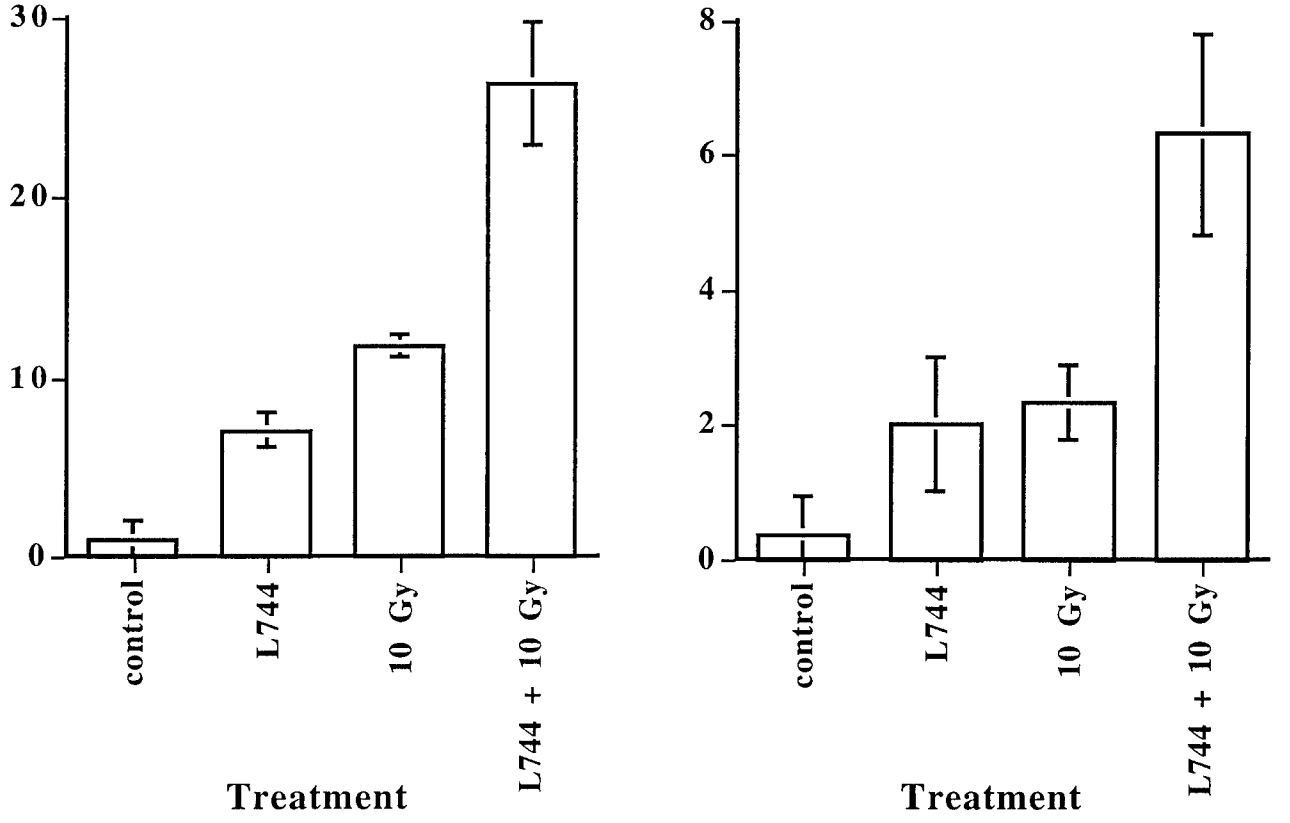


Figure 1. Murine prostate lines 148#1primary tumor (left panel) and 151#2 lung metastasis (right panel) were treated for 24 h prior to irradiation with 2μM L744,832. Apoptosis was determined by examining nuclear morphology after staining with propidium iodide.	Although both lines show significant increase in apoptosis after irradiation that is further increased by farnesyltransferase inhibitor treatment, the absolute levels of apoptosis vary between cell lines.

The clonogenic survival of mouse prostate tumor cell lines has also been examined after treatment with the L744,832 inhibitor (Figure 2). The radiation survival of prostate tumor cells as assessed by limiting dilution analysis was decreased after combined L744,832 inhibitor and 2 Gy irradiation treatment to a greater extent than was seen with either treatment alone. The radiation survival of tumor cell clonogens was reduced by 15-20% when corrected for the toxicity of the drug alone.

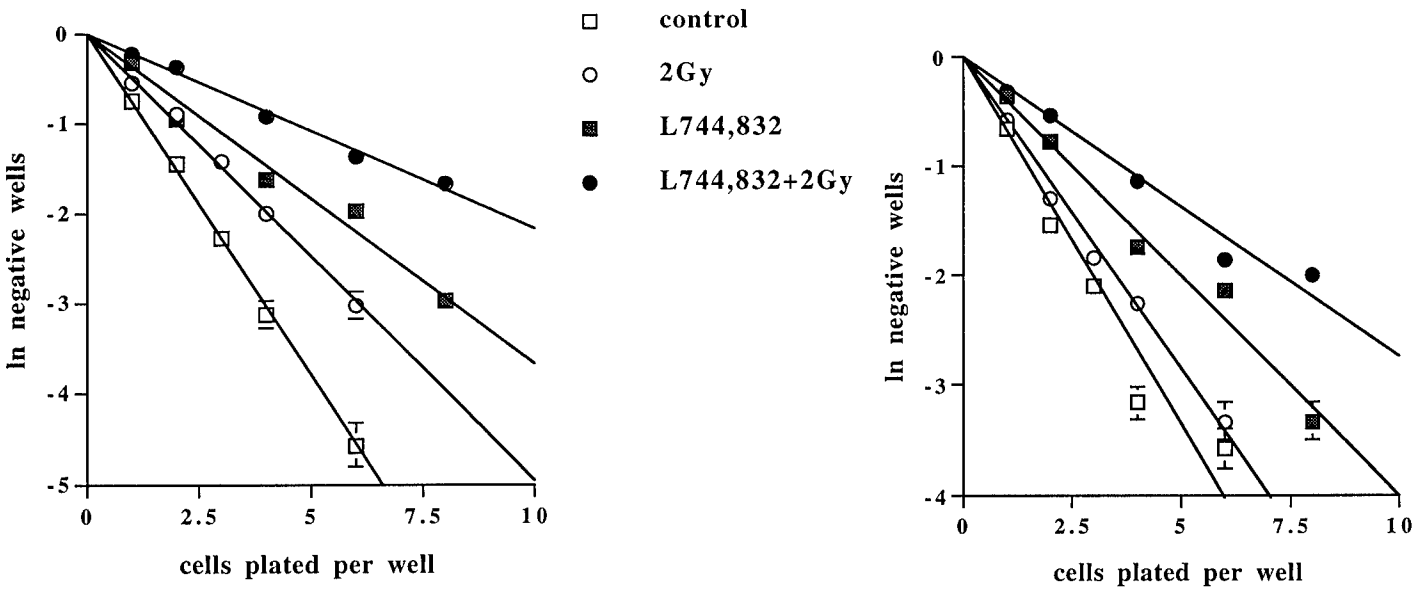
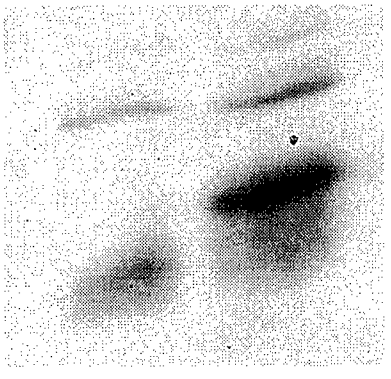


Figure 2. Clonogenic survival of two prostate tumor lines was determined by limiting dilution analysis after treatment with the farnesyltransferase inhibitor L744,832. The steeper the slope of the line, the greater the plating efficiency of the cells.	The surviving fraction at 2 Gy of prostate C (Left panel) was decreased from 0.61 to 0.52. The surviving fraction at 2 Gy of prostate D (right panel) was decreased from 0.85 to 0.68.

Although magnitude of the effect of farnesyltransferase inhibitor treatment on apoptosis and radiation survival varies between mouse cell lines, the data obtained confirm that farnesyltransferase inhibitors can increase radiation-induced apoptosis and decrease clonogenic survival in this model of prostate cancer.

We have extended our studies to human cells expressing oncogenic H- or K-ras as a result of transfection. We successfully obtained clones of the 267B1 cell line (17) after transfection with H-ras that expressed high levels of H-ras^{V12} (Figure 3). These clones showed alterations in morphology including greater refractility and rounding up of the cells. One of these clones has been tested for survival after irradiation with or without farnesyltransferase inhibitor treatment.

Figure 3. Western blot of H-ras protein expression. The 267B1pAL8 clone expressing high levels of H-ras^{V12} before (left lane) and after (right lane) farnesyltransferase inhibitor treatment at 5μM for 24 h. Unfarnesylated H-ras migrates more slowly.



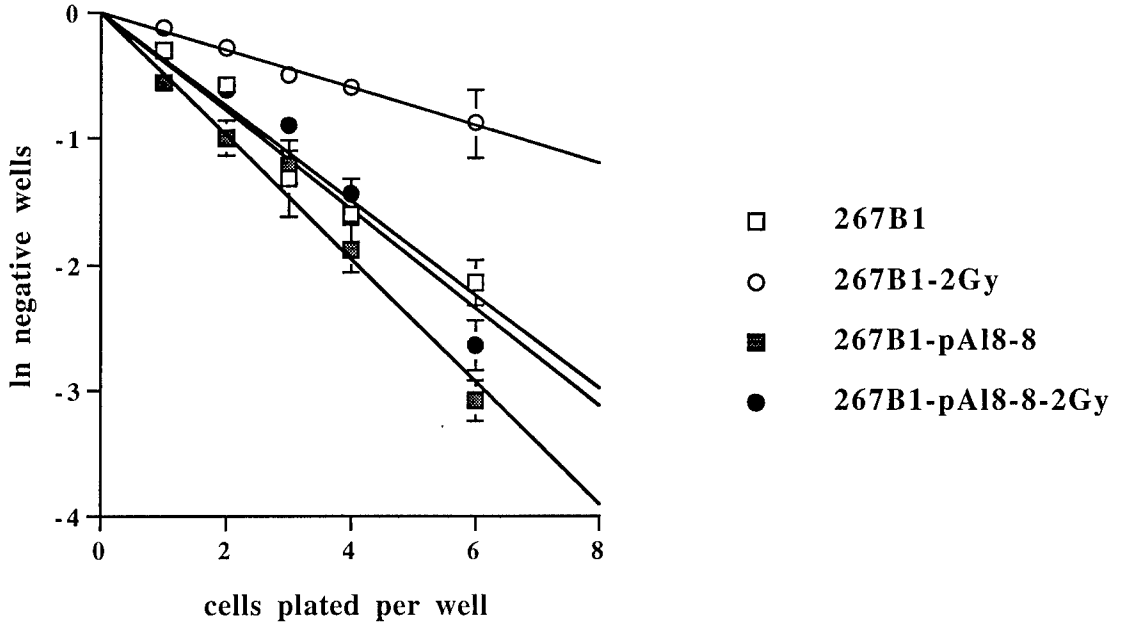


Figure 3. The radiation survival of parental 267B1 cells was compared to that of 267B1 cells transfected with the pAL8 plasmid encoding activated H-ras. Radiation survival, as determined by limiting dilution analysis as in Figure 2 was increased from a surviving fraction of 0.4 to 0.8 after transfection with a plasmid encoding H-ras^{V12}.

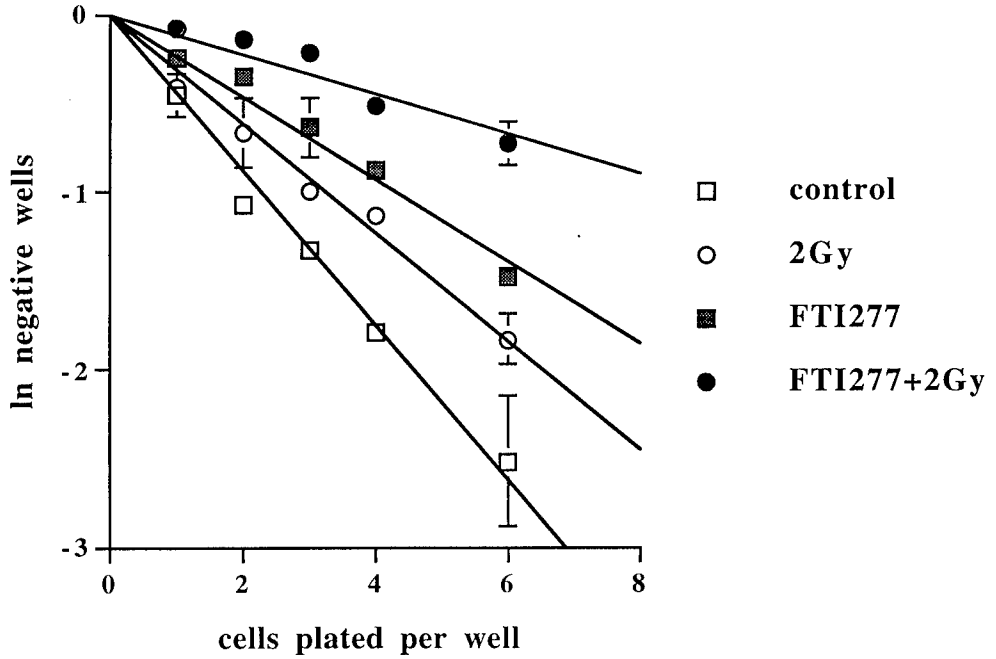


Figure 4. 267B1-pAL-8 cells expressing high levels of H-ras^{V12} were tested for clonogenic survival after treatment with FTI-277. Radiation survival as assessed by limiting dilution analysis in this experiment was reduced from a surviving fraction of 0.7 to 0.48 after FTI-277 treatment.

The surviving fraction of 267B1 cells after 2 Gy irradiation went from 0.4 to 0.8 after ras transfection. Thus demonstrating a significant increase in radiation resistance over the parental line (Figure 3). Treatment of this clone with farnesyltransferase inhibitor resulted in the reduction of this radiation resistance (Figure 4). Thus introduction of activated H-ras into prostate cells can increase radiation resistance, and this resistance can be reversed by inhibiting ras farnesylation. In contrast, the Ki267B1 cell line obtained from Dr. Johng Rhim, which expresses activated K-ras as a result of viral transduction showed no increase in radiation resistance over the parental cells. Radiation resistance in this line was not was not affected by prenyltransferase inhibitor treatment (not shown).

As a preliminary step to investigating the effects of farnesyltransferase inhibitors on prostate tumor xenograft radiosensitivity, we have examined the effects of treatment with these compounds on the tumor microenvironment. Both the vasculature of prostate tumor xenografts in nude mice, and the oxygenation status of these tumors has been examined. As shown in Figure 5A, prostate tumor xenografts are highly hypoxic. Treatment for 7 days with the L744,832 farnesyltransferase inhibitor resulted in a tumor with a markedly higher oxygenation state. This is important in that tumor cell oxygenation can have a large influence on the response of these cells to radiation. A severly hypoxic environment can result in a two-fold or greater reduction in tumor cell killing by radiation. Thus farnesyltransferase inhibitor treatment of these tumors resulted in a microenvironment that is predicted to result in improved tumor cell killing by radiation.

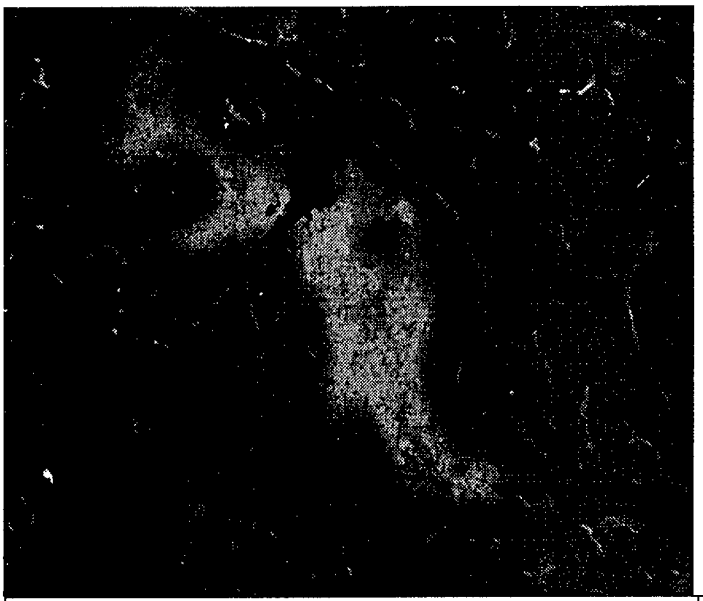


Figure 5A. Mouse prostate tumors growing subcutaneously were labelled with the hypoxic cell marker EF-5. Binding of this marker was detected with a cy-5 conjugated monoclonal antibody specific for EF-5. Vessels were subsequently stained with anti CD-31. Areas of hypoxia are seen as brightly staining cellular regions in the central portion of this section. Vessel distribution is detected as fillamentous staining surrounding the central region

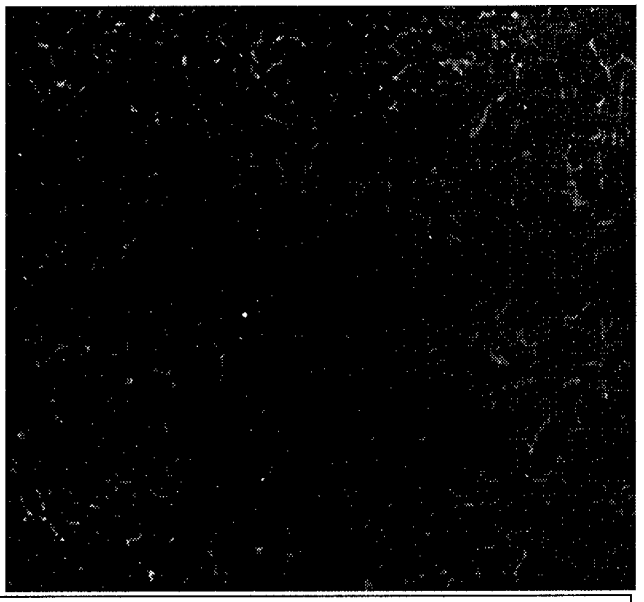


Figure 5B. Mouse prostate tumors were treated as in A except that the animals received continuous infusion of the FTI inhibitor L744,832 for seven days prior to sacrifice. Note that areas of hypoxia are not detectable in this tumor. Vasculature, in contrast, appears to be more abundant.

Key Research Accomplishments:

1. We have shown that the expression of activated ras can increase radiation resistance in both a rodent and a human cell system, although the increase in radiation resistance after ras introduction was not a universal finding.
2. When increased radiation resistance was found after activated ras introduction, this resistance was abrogated through inhibition of ras using farnesyltransferase inhibition.
3. Two inhibitors have now been tested on prostate cells, the FTI-276 inhibitor developed by Dr. Andrew Hamilton (now at Yale), and the L744,832 inhibitor developed by Merck and Co. Inc. Both inhibitors showed the same radiosensitizing effect.
4. Preliminary *in vivo* studies show reoxygenation of prostate tumors after treatment with farnesyltransferase inhibitors. This is a novel finding and predicts a positive effect on the response to radiotherapy.

Reportable Outcomes:

1. Development of new cell lines derived from immortalized human prostate epithelial that express high levels of H-ras^{V12}.
2. Training of medical student supported by an NIH short-term research grant for medical student training obtained on the basis of her work on this project. (Kun Huang, CV included in appendix)

Conclusions:

We have in the past year demonstrated that the expression of activated H-ras can lead to increased radiation resistance in both rodent and human prostate tumor cells. The radiation resistance induced by H-ras activation was reversed by treatment with farnesyltransferase inhibitors. These data support the hypothesis of the grant. The generalization of these findings to other prostate cell lines is one goal of future studies who's importance is underlined by the finding that one human prostate cell line transformed with K-ras by retroviral transduction did not show increased radiation resistance. In the cells studied the expression of ras was obtained after either transfection or retroviral transduction. We have now initiated the study of prostate tumor cell lines expressing naturally occurring mutations in *ras* oncogenes. In addition to these studies, we are initiating studies on the effects of farnesyltransferase inhibitors on the radiosensitivity of prostate tumors grown as xenografts in nude mice. As shown above, these inhibitors have a marked effect on tumor oxygenation. Our finding that farnesyltransferase inhibitors caused a reoxygenation of prostate tumor tissue is both novel and significant in that this predicts a favorable effect of *in vivo* farnesyltransferase treatment in combination with radiation.

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Education

1996 - 2000	University of Pennsylvania School of Medicine, Philadelphia, PA MD expected in May, 2000
1991 - 1993	University of Illinois at Urbana-Champaign, Urbana, IL MS in Biochemistry
1985 - 1989	Fudan University, Shanghai, China BS in Biochemistry

Honors and Awards

1999	Fellowship, NIH Short Term Research Grant
1997	Fellowship, Student Training Program, NCI, NIH
1996	Franklin Scholar, University of Pennsylvania School of Medicine
1989	First Award, Academic Excellence Award, Fudan University
1985	Acceptance into the Class for Young Talented Students, Fudan University

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Research Experience

1999	Evaluation of Computed Tomography in patients with peritoneal carcinomatosis and sarcomatosis Supervisor: Steven Hahn, MD, Department of Radiation Oncology, University of Pennsylvania School of Medicine (UPSM)
1999	Investigation of ras and its downstream signaling molecules in radiosensitization and radioresistance in prostate cancer cell lines Supervisor: Eric Bernhard, PhD and W. Gillies McKenna, MD.PhD, Department of Radiation Oncology, UPSM
1997	Participating in the phase II clinical trial of 96-hour infusional paclitaxel in patients with metastatic colorectal cancer. Supervisor: David J. Vaughn, MD, Department of Medicine, UPSM
1997	Participating in various aspects of ongoing clinical trials on new chemotherapeutic drugs and tumor vaccines. Supervisor: J. Michael Hamilton, MD, NCI-Navy Medical Oncology Branch, National Cancer Institute (NCI), NIH, Bethesda, MD

1993 - 1996 Studying the roles of T cell receptor subunits in T cell development using transgenic mice and gene targeting techniques.
Cloning and characterization of novel protein tyrosine kinases and phosphatases specific for signal transduction in T cells.
Supervisor: Paul E. Love, MD.PhD, NICHD, NIH, Bethesda, MD

1992 - 1993 Structural and functional analysis of integrins in cell adhesion and signaling.
Advisor: Alan F. Horwitz, PhD, University of Illinois at Urbana-Champaign.

1989 - 1991 Characterization of transcriptional regulatory elements of prostate steroid binding protein genes using footprinting technique.

1989 Reconstitution of purified mitochondrial respiratory chain proteins into liposomes to investigate the electron transfer chain.

Publications

1. Liu L, **Huang K** and Lin QS. (1991) Co-reconstitution of choline dehydrogenase and sub-mitochondrial preparation with liposomes. *Acta Biochimica et Biophysica Sinica* 23(1):62-69.

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8. **Huang K**, Vaughn DJ, Shaw LM, Recio A, Bonner H and Haller DG. (1998) A phase II trial and pharmacokinetic analysis of 96-hour infusional paclitaxel in patients with metastatic colorectal cancer. *Am J Clin Oncol* 21(6):548-552.

Employment

1993 - 1996 Biologist, Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development (NICHD), National Institute of Health (NIH), Bethesda, MD

1992 - 1993 Research Assistant, Department of Biochemistry, University of Illinois at Urbana-Champaign

1991 - 1992 Teaching Assistant, School of Chemical Sciences, University of Illinois at Urbana-Champaign

1989 - 1991 Research Assistant, Shanghai Institute of Biochemistry, Academia Sinica, Shanghai

Extracurricular Activities

1997 - 1998 Manager, University City Hospitality Coalition (UCHC) Homeless Health Clinic, Philadelphia, PA
Helping to run the weekly free clinic providing health care for clients at a local soup kitchen.

1997 - 1998 Co-organizer, Perspectives, University of Pennsylvania School of Medicine
Organizing this seminar series on various medically related topics which are complimentary to the existing curriculum.

1996 - 1997 Contact Person, Asian American Health Care Network (AAHCN), Philadelphia, PA
Addressing the health issues of the underserved Asian Pacific American community in the Greater Philadelphia area.

1994 - 1996 Volunteer, Clinical Oncology Center, Suburban Hospital, Bethesda, MD
Helping the medical staff to take care of patients.

1994 - 1996 Volunteer, Translator Registry, Clinical Center, NIH, Bethesda, MD
Interpreting for Mandarin speaking patients at the Clinical Center.

1993 - 1994 Volunteer, Pen Pal Program, Montgomery County Public Schools, Montgomery County, MD
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Memberships

1996 - 1999 Student Member, American Medical Association

1993 Member, American Association for the Advancement of Science,

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