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13. ABSTRACT (Maximum 200 Words)

During first three years of funding we have developed recombinant plasmids expressing the DNA-binding domain of poly(ADP-ribose) polymerase (PARP-DBD) under the control of the 5'-flanking sequences of the human prostate-specific antigen (PSA) gene, and demonstrated that PARP-DBD sensitized prostate cancer cells to DNA-damaging agents *in vitro*. Present study is designed to assess the efficiency of this strategy *in vivo*. Towards this end, we developed a cationic liposome-mediated gene delivery of the PARP-DBD expressing plasmid in tumor xenografts of PSA producing and androgen sensitive prostate cancer cells (22Rv1 cell line). Tumor bearing mice were treated intratumorally with liposome-complexed plasmid for PARP-DBD (LE-PARP-DBD), ionizing radiation (IR) or a combination of LE-PARP-DBD and IR. We found that administration of LE-PARP-DBD resulted in expression of dominant-negative mutant of PARP in 22Rv1 tumor xenografts and enhanced radiation-induced inhibition of tumor growth in vivo. These data indicate that expression of the PARP-DBD in PSA producing prostate cancer cells show potential to radiosensize prostate tumor cells *in vivo*.

14. SUBJECT TERMS
Prostate Cancer; Poly (ADP-ribose) polymerase; Radiation; DNA damage; Liposomes

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Table of Contents

ADDENDUM TO FINAL REPORT

INTRODUCTION

The central objective of the proposal is to express the DNA-binding domain of PARP under control of prostate tissue-specific promoter in prostate cancer cells and sensitize them to radiotherapy or chemotherapy. Using PSA-producing cells (LNCaP) as the primary experimental model system we 1) developed prostate carcinoma cell sublines which allow androgen-inducible, high-level expression of the PARP-DBD and 2) tested the DNA-binding domain of PARP as a molecular sensitizer for improving responses of prostate tumor cells to gamma radiation and DNA-damaging drugs. Our results (Final Report) provide a proof-of-principle for a novel therapeutic strategy to control prostate cancer. Present study is designed to assess the efficiency of this strategy in vivo.

RESEARCH ACCOMPLISHED

PARP-DBD enhances radiation-induce growth inhibition of prostate tumor in the 22Rv1 xenograft model.

During first three years of funding we have developed recombinant plasmids expressing the PARP-DBD under the control of the 5'-flanking sequences of the human prostate-specific antigen (PSA) gene. Further, we demonstrated *in vitro* that expression of PARP-DBD in prostate carcinoma cells might offers a strategy to achieve sensitization to genotoxic treatments (Trofimova et al., 2002). In the present study we examined efficiency of this strategy *in vivo* using 22Rv1 human prostate cancer xenograft. 22Rv1 human prostate carcinoma epithelian cell line (ATCC, CRL-2505) was established from tumor after castration-induced regression and relapse of the parental CWR22 xenograft (Sramkoski et al., 1999). We have selected 22Rv1 cells for their ability to express prostate specific antigen (PSA), responsiveness to androgens (Sramkoski et al., 1999), and potential to produce xenograft tumors in nude mice.

First set of experiments was set out to evaluate sensitivity of 22Rv1 human prostate cancer cells to ionizing radiation *in vivo*. Towards this end we have established a 22Rv1 xenograft model in male BALB/c nude (*nu/nu*) mice (NCI). All Georgetown University policies concerning the care and use of laboratory animals were strictly adhered during entire study. Approximately $2x10^6$ 22Rv1 cells in 0.2 ml of 50% Matrigel (Becton Dickinson)-50%PBS were injected subcutaneously in the sacral region of 4-6 weeks old male nude mice. Tumors started to develop by 3-4 weeks. When palpable tumors grew to mean tumor volume of 150 mm^3, mice were divided into various treatment groups. Irradiations at doses 5-20 Gy were performed using the Mark I Model 30 irradiator equipped with cesiun-137 source. For radiation of tumors, animals were be secured in a lead restraint that permits only the tumor area to be exposed to γ-irradiation. Tumor volumes will be determined from caliper measurements of the three major axes (a,b,c) and calculated using abc/2, an approximation for the volume of an ellipse (πabc/6) as described (Gokhale et al., 1997). Based on results from this experiment (Fig.1), we have chosen 5Gy as a dose of ionizing radiation suitable to assess the effect of the PARP-DBD on radiosensitization of 22Rv1 cells *in vivo*.

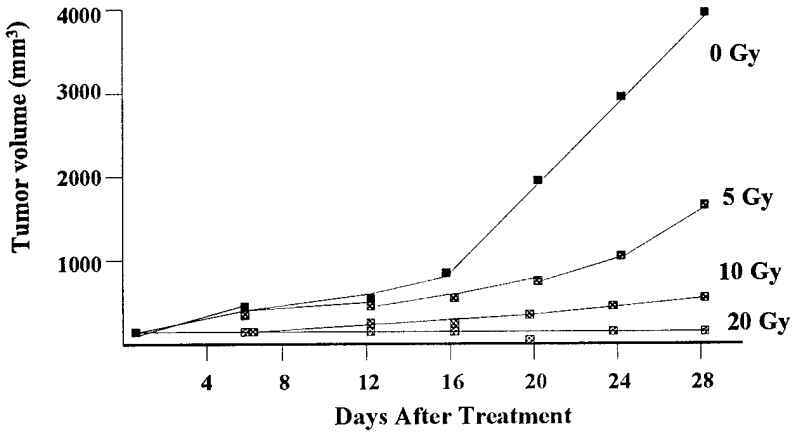

Figure 1. Radiation-induced inhibition of 22Rv1tumor xenograft growth. Mice were irradiated when tumors were established to average volume of 150-200 cubic mm. Tumors were measured in three dimensions daily and data are presented as the mean tumor volume of the surviving animals in each treatment group. Four mice were included in each group.

We next examined whether PARP-DBD would increase the susceptibility of human prostate carcinoma to DNA-damaging treatments *in vivo*. To deliver the vector for PARP-DBD expression under control of the PSA promoter/enhancer in prostate tumor we employed a cationic liposome delivery system. Cationic liposomal formulation was prepared as described previously (Gokhale et al., 1997). For PARP-DBD expression studies, nude mice bearing subcutaneous tumors were treated intratumorally with 100 μg liposome-complexed PARP-DBD (LE-DBD). Tumors were excised on day 1, 2 and 3, sectioned onto slides and used for immunohistochemical detection of Flag-fused PARP-DBD protein with subsequent counterstaining of slides with H&E. Figure 2 shows that PARP-DBD protein was expressed in R22v1 tumor xenografts, and that its expression levels were appreciably high in prostatic carcinoma cells upto 72 hours after injection. These data are consistent with previously reported findings that PSA promoter driven expression of protein shows tissue specificity both *in vivo* and *in vitro* (Cleutjens et al., 1997).

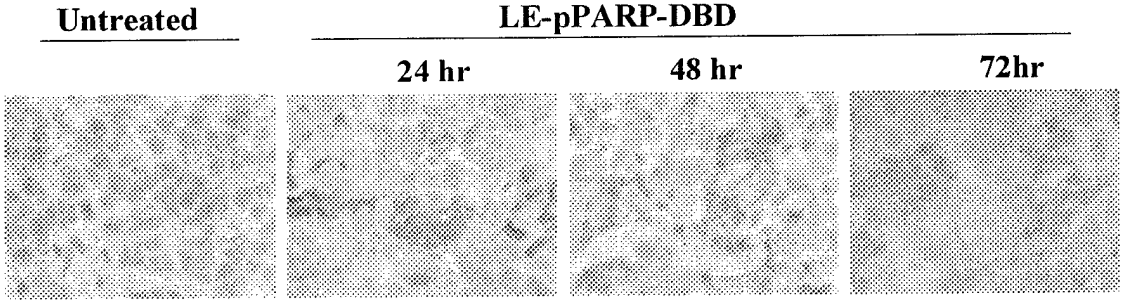

Figure 2. PARP-DBD Flag-fused protein expression in R22v1 prostate tumor xenografts. Cryostat tissue sections were Ethanol-fixed and stained with monoclonal anti-Flag primary antibody (Sigma). Immunohistohemistry was performed using the Vector ABC peroxidase kit. Enzymatic activity of horseradish peroxidase was visualized with the AEC staining kit (Sigma) with subsequent counterstaining of slides with H&E. Representative fields at 400x magnigication were documented using the Olympus AH2 Vanox microscope system.

The efficacy of the PARP-DBD entrapped in liposomes (LE+DBD) and ionizing radiation (IR), alone or in combination, was studied using R22v1 xenografts, grown in Balb/c nu/nu mice. Nude mice bearing subcutaneous tumors were treated intratumorally with 100 μg liposome-complexed PARP-DBD (LE-DBD) at on days 0,1,2 and 3, or with control blank liposomes LE). Radiation (IR) was administered at a dose of 5 Gy on day 4. One group received the combination of LE+DBD and IR at dose 5 Gy. Tumors were measured in three dimensions and expressed as % of initial volume as described (Gokhale et al., 1997). Figure 3 shows that reduction of approximately 30-40% of tumor growth was observed in the male nude mice with R22v1 prostate tumor xenograft treated irradiation alone. Administration of liposome-complexed PARP-DBD did not result in noticeable inhibition of tumor growth. However, radiation-induced tumor growth suppression was significantly enhanced (approximately 3 fold) following combined treatment with IR and LE+DBD. These data indicate that expression of the PARP-DBD in PSA producing prostate cancer cells show potential to radiosensize prostate tumor xenografts.

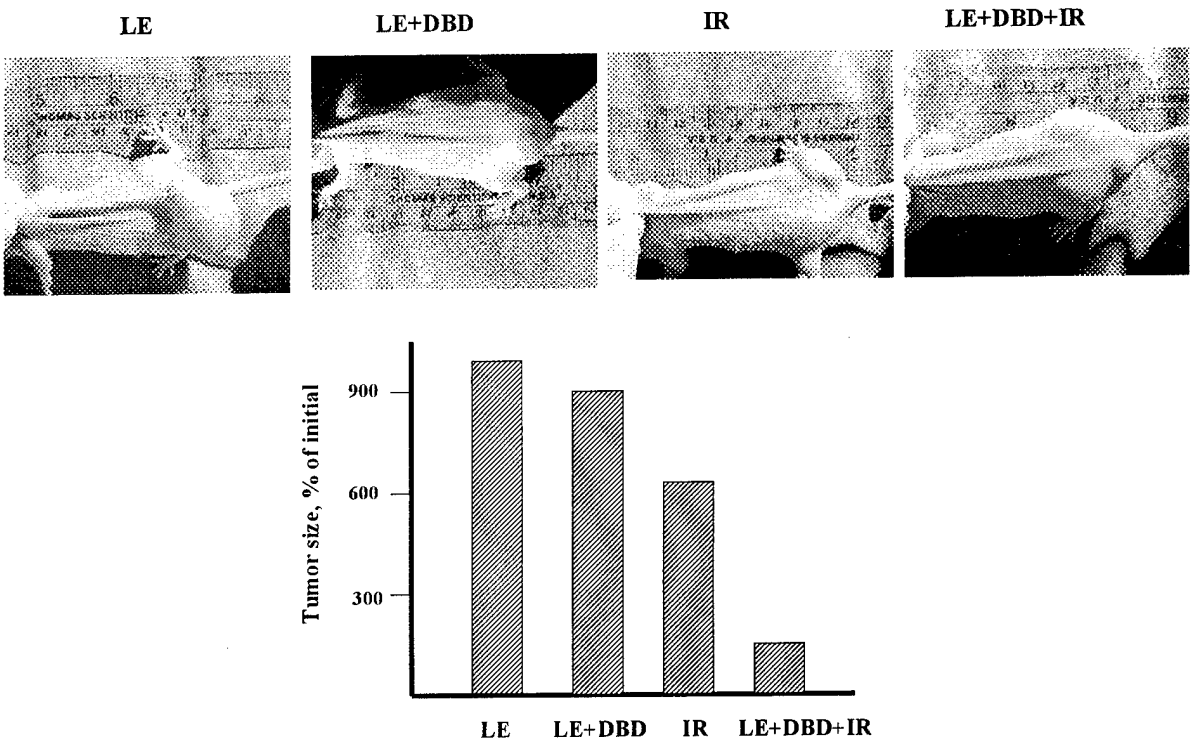

Figure 3. Cationic liposome-mediated gene delivery of PARP-DBD plasmid in tumor xenografts of PSA producing prostate cancer cells (22Rv1). Tumor bearing mice were treated intratumorally with 100 μg liposome-complexed PARP-DBD (LE-DBD) at on days 0,1,2 and 3, and /or 5Gy of ionizing radiation (IR) day 4. Control group received blank liposomes (LE). Fractional tumor volume is calculated on day 32 as percentage of original (day 0) volume

KEY RESEARCH ACCOMPLISHMENTS

- Established tumor xenografts of PSA producing and androgen sensitive prostate cancer cells (22Rv1 cell line) in nude mice
- Demonstrated that cationic liposome-mediated gene delivery of PARP-DBD expressing plasmid in tumor xenografts of 22Rv1 cells enhanced radiation-induced inhibition of tumor growth

REPORTABLE OUTCOMES

Papers presented:

1. Zang L., Gokhale P., Mewani R., Li B., Dritschilo A., and **Soldatenkov V**. Sensitization of prostate cancer to ionizing radiation by targeting poly(ADP-ribose) polymerase: preclinical studies. 12th Internatl. Congress Radiat. Res., Brisbane, Australia. Proceedings, 2003

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2. Sramkoski RM, Pretlow TG, Giaconia JM, Pretlow TP, Schwartz S, Sy MS, Marengo SR, Rhim JS, Zhang D, Jacobberger JW. A new human prostate carcinoma cell line, 22Rv1. In Vitro Cell Dev Biol Anim. 35: 403-409, 1999
3. Gokhale PC, Soldatenkov V, Wang F-H, Rahman A, Dritschilo A and Kasid U. Antisense *raf* oligodeoxyribonucletide is protected by liposomal encapsulation and inhibits Raf-1 protein expression *in vitro* and *in vivo*: implication for gene therapy of radioresistant cancer. Gene Therapy. 4: 1289-1299, 1997
4. Cleutjens, K.B., van der Korput, H.A., Ehren van Eekelen, C.C., Sikes, R.A., Fasciana, C., Chung, L.W., and Trapman, J. A 6-kb promoter fragment mimics in transgenic mice the prostate-specific and androgen-regulated expression of the endogenous prostate-specific antigen in humans. Mol. Endocrinol., 11: 1256-1265, 1997

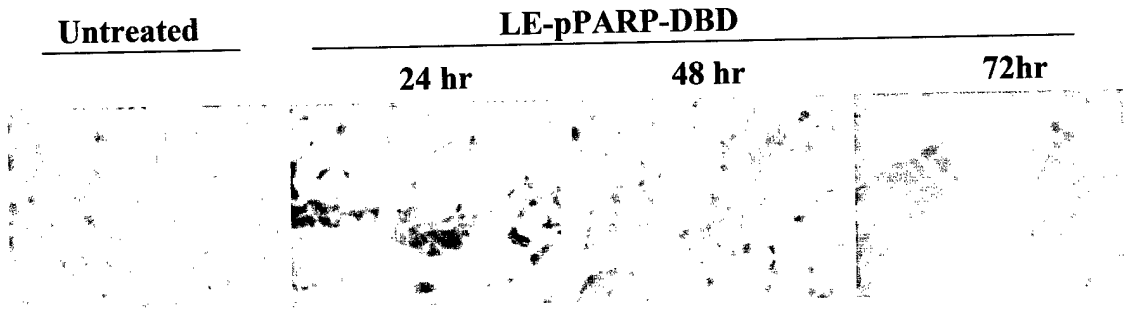
CONCLUSION

Previously we have developed recombinant plasmids expressing the PARP-DBD under the control of the 5'-flanking sequences of the human prostate-specific antigen (PSA) gene, and demonstrated that PARP-DBD sensitized prostate cancer cells to DNA-damaging agents *in vitro* (final report). Present study is designed to assess the efficiency of this strategy in vivo. Towards this end, we developed a cationic liposome-mediated gene delivery of PARP-DBD plasmid in tumor xenografts of PSA producing and androgen sensitive prostate cancer cells (22Rv1 cell line). Tumor bearing mice were treated with intratumoral liposome-complexed PARP-DBD (LE-PARP-DBD), ionizing radiation (IR) or a combination of LE-PARP-DBD and IR. We found that administration of LE-PARP-DBD resulted in expression of dominant-negative mutant of PARP in tumor cells and enhanced radiation-induced inhibition of tumor growth.

In summary, the plasmid vector developed in this study permits the expression of the human PARP-DBD in an androgen-inducible and PSA-dependent fashion and sensitizes prostatic adenocarcinoma cells to DNA-damaging treatments both *in vitro* and *in vivo*. These results provide a proof-of-principle for a novel therapeutic strategy to control prostate cancer.

APPENDIX

1. Figure 2 (color image)
2. Figure 3 (color Image)



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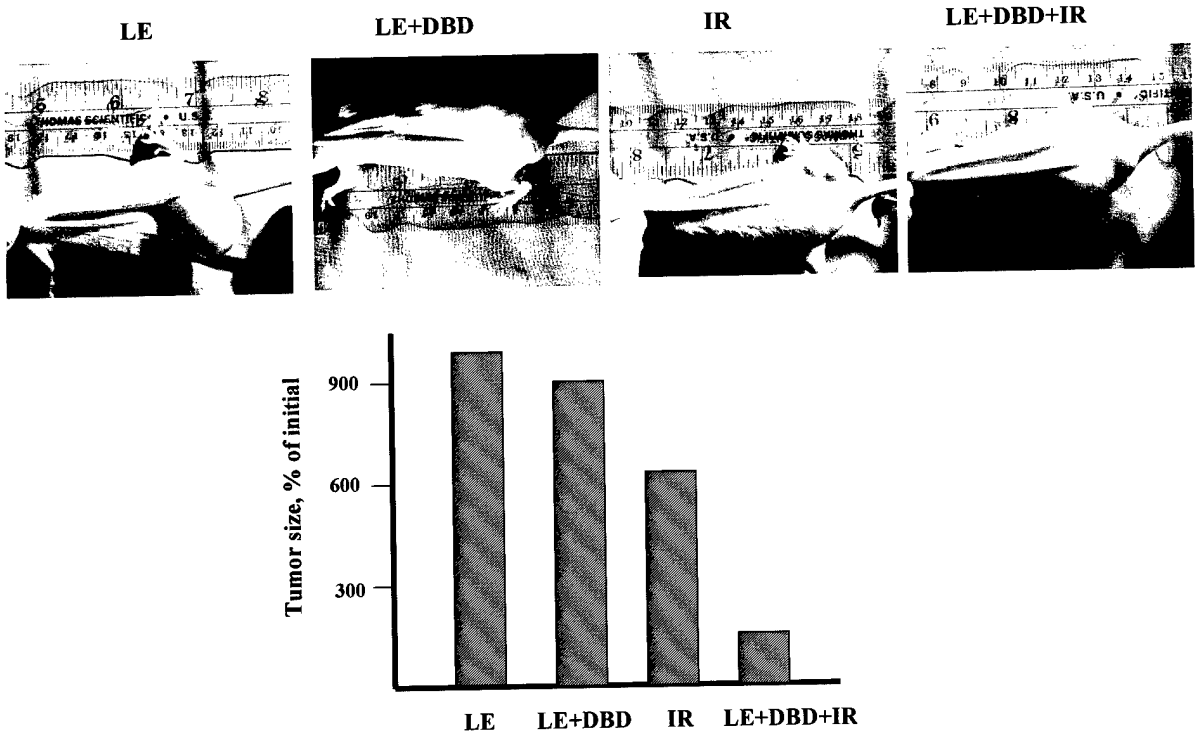

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