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14. ABSTRACT: Our focus of this project is to characterize a newly developed small peptide on its ability to function as a powerful radiosensitizer. Radio sensitivity is mainly controlled by a kinase named ATM and its phosphorylation of downstream targets, including Structural Maintenance of Chromosomal protein on (SMC1). Previously we have demonstrated that small fusion peptides containing SMC1 phosphorylation sequences can inhibit ATM activity. We have characterized the inhibitory effect of the THM-SMC1 peptide on cellular response to radiation and found the peptide can abolish radiation induced S-phase checkpoint and decrease prostate tumor cell clonogenic survival. During the last performance period, we further performed experiments focusing of the magnitude of peptide sensitization and the effect on the other cell cycle checkpoints. We have demonstrated the wild type SMC1 peptide linked with a tumor homing motif can significantly increase prostate tumor radiosensitivity. Future experiments will be focusing on mechanisms and the in vivo activity of the peptides.					
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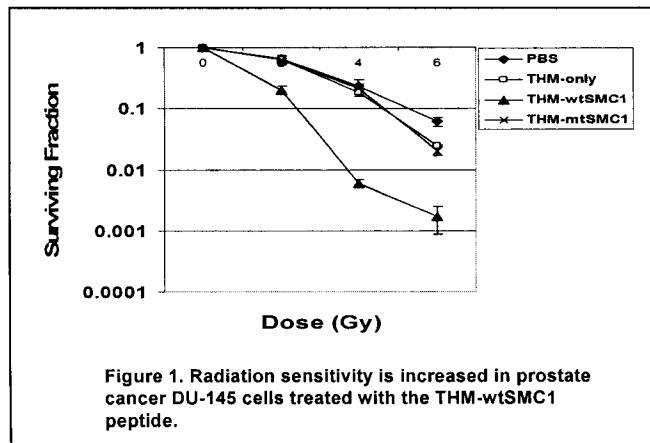
Introduction:

Previously, we, and others, demonstrated that the cellular sensitivity to ionizing radiation (IR) is controlled by the Ataxia-Telangiectasia-Mutated (ATM) protein kinase and its downstream target the Structural Maintenance of Chromosome protein one (SMC1)(Kim et al., 2002; Kitagawa et al., 2004; Yazdi et al., 2002). ATM phosphorylation of SMC1 at two serine sites is required for limiting the amount of radiation sensitivity. Our preliminary data have demonstrated that a small peptide containing ATM-mediated SMC1 phosphorylation sequence has an inhibitory effect on SMC1 phosphorylation and S-phase checkpoint activation after DNA damage. Therefore we hypothesized that peptides containing this SMC1 short sequence, when linked with a tumor homing motif (THM), can be a specific inhibitor to the ATM-mediated signaling pathway and a powerful radiosensitizer for prostate tumor radiotherapy. This hypothesis has been tested by studying the effect of synthetic peptides that aim to block the *in vivo* phosphorylation events on prostate cancer cellular response to IR. We have generated three peptides, which include THM only (serving as a negative control), THM-wtSMC1 (as to-be-tested peptide), and THM-mtSMC1 (designed as a negative control, with a possibility of having some inhibitory effects). In year one, we have finished testing the inhibitory effect and radiosensitizing effects of the peptides (SOW Task1). We have found that only THM-wtSMC1 could inhibit ATM-mediated SMC1 phosphorylation and increase cellular radiosensitivity. Our goals in year two were to further characterize the peptides and gain mechanistic insight of the inhibitory effect.

Body:**Further characterization of the magnitude of peptide radiosensitization.**

Our experiments performed in Year One have suggested that the THM-wtSMC1 peptide can significantly improve radiation-induced cell killing in PC-3 cells. To confirm these observations in other cell lines, we utilized the DU145 cells, a commonly used prostate cancer cell line for the colony formation assay. The assays were performed in 6-well plates with radiation (0-6Gy). Cells were treated with the peptides for one hour and then be radiated. After 2-week incubation, the numbers of colonies (those with more than 50 cells were counted as one surviving colony) were obtained and surviving fractions were calculated. Figure 1 depicts the survival curves for DU145 cells treated with 10 μ M of the peptides. After treatment with 2Gy, the survival of cells treated with wtSMC1 was 20% compared to 65%, 63%, and 63% for PBS, THM only or THM-mtSMC1 treated cells. At 4Gy, survival of cells treated with wtSMC1 decreases to 0.6% compared to 18% and 21% for THM only or THM-mtSMC1 treated cells. A dose increase to 6Gy lead to a significant decline in survival of cells treated with wtSMC1, 0.2%, compared to 2% and 2% for cells treated with THM only or THM-mtSMC1 treated cells, respectively.

To establish statistical significance of peptide induced radiosensitivity, Student's t-test (Paired 2 sample for means) was incorporated. The data were first fit to each experimental group over a dose range of 0-6Gy. Significant differences in clonogenic survival were observed between cells treated with wtSMC1, and those treated with PBS, THM only or THM-mtSMC1 treated cells. Collectively, these results have provided strong evidence that the wtSMC1 peptide containing the phosphorylation sequence of SMC1 indeed sensitizes prostate tumor cells to ionizing radiation.



Key Research Accomplishment

The THM-wt SMC1 peptide containing the wild-type SMC1 phosphorylation sequence has an inhibitory effect on ATM activation and subsequent SMC1 phosphorylation after IR;

1. This peptide does not possess cellular toxicity to normal or tumoral prostate cells; and
2. It can increase radiation sensitivity of prostate tumor cells.

Reportable outcomes:

A. Publications and meeting presentations:

1. Xi Tang, Xiao-nan Sun, Renu Garg and Bo Xu. Regulation of ATM phosphorylation in the absence of DNA damage. Under review with *Journal of Biological Chemistry*.
2. Yali Cui, Shannon Callens and Bo Xu. DNA damage signaling and potential radiotherapeutic targets in prostate cancer treatment. Proc Amer Assoc Cancer Res, Volume 47, 2006

B. Invited seminars:

1. "Sensing DNA double strand breaks", Southern Research Institute, November 22, 2005
2. "DNA damage response mechanisms and new approaches to developing radiosensitizers" Methodist Hospital Residence Seminar Series, Baylor College of Medicine, March 2006
3. "Mechanisms of DNA damage induced cell cycle checkpoints", Nelson Institute of Environmental Sciences, NYU School of Medicine, March 21, 2006

Conclusions:

Our data have provided strong evidence that targeting ATM-SMC1 phosphorylation can be a novel approach for developing radiosensitizers. Furthermore, we have identified a novel peptide, the THM-wtSMC1, which can increase prostate tumor radiosensitivity significantly in vitro. This peptide will be further tested for its in vivo activity (Aim 3). We will also study how the small peptide works as a means to understanding the mechanisms of peptide inhibition.

It is noted that, our research program was significantly affected by Hurricane Katrina, which flooded our research building in New Orleans on August 29, 2005. Due to the power outage after the storm, we lost almost all cell lines, reagents, and peptides stored in the building. The lab had been transiently relocated to the Department of Genetics at Baylor College of Medicine in Houston, TX before the LSUHSC research buildings reopened in February 2006. During this period in Houston and the first two months after we returned to New Orleans, we had mainly focused on restoring cell lines, plasmids, and reagents. In addition to these recovering efforts, we were able to perform some of the experiments in the proposal. Our lab has recently relocated to the Southern Research Institute in Birmingham, AL. We will continue our research program focusing on developing therapeutic drugs for prostate cancer radiotherapy. The strength of Southern Research Institute in drug development and animal studies will further facilitate completion of this promising project.

References

- Kim,S.T., Xu,B., and Kastan,M.B. (2002). Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. *Genes Dev.* 16, 560-570.
- Kitagawa,R., Bakkenist,C.J., McKinnon,P.J., and Kastan,M.B. (2004). Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes Dev.* 18, 1423-1438.
- Xu,B., O'Donnell,A.H., Kim,S.T., and Kastan,M.B. (2002). Phosphorylation of serine 1387 in Brca1 is specifically required for the Atm-mediated S-phase checkpoint after ionizing irradiation. *Cancer Res.* 62, 4588-4591.
- Yazdi,P.T., Wang,Y., Zhao,S., Patel,N., Lee,E.Y., and Qin,J. (2002). SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev.* 16, 571-582.

Appendices

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