DOE/ER/62724-1

ER
DE-FG02-99[illegible]62724)

Boothman, David, A.

Nuclear apoJ: An X-ray-inducible cell death signal
Final Technical Report *(Revised STI Product)*

Description/Abstract

The complex interactions between apoptosis, genomic instability and carcinogenesis induced by low dose ionizing radiation (IR) are poorly understood. Tissues differentially withstand IR based on complex processes that include DNA repair, altered gene expression, and apoptosis. This proposal is based on the investigators' discovery of a protein, apoJ (initially designated xip8/XIP8), that is dramatically IR-induced. The protein is also known as a marker for apoptosis, but its function is unknown. The "nuclear" form of the apoJ protein, undergoes dramatic accumulation in the nucleus following IR, and then strongly associates with the C-terminus of Ku70, a key factor in DNA-PK-dependent nonhomologous DNA double strand break (DSB) repair.

Based on their preliminary data, the investigators' hypothesize that the accumulation of nuclear apoJ is regulated by the IR-inducible p53 tumor suppressor protein. Expression of the E6 papillomavirus protein abrogated apoJ induction responses. p53-mediated, elevated levels of nuclear apoJ then sequesters the Ku70/Ku80 heterodimeric subunit and prevents the repair of chromosomes that have been severely damaged by trans-lesion DNA synthesis, which may accumulate over several generations after exposure to low dose IR. The implication of the hypothesis is that cell death (apoptosis) associated with delayed genomic instability after IR is strongly enhanced by nuclear apoJ.

The goals of this proposal are to (1) define the molecular mechanisms responsible for apoJ induction and accumulation in damaged nuclei after IR using biochemical, cell transfection, and transgenic mouse analyses. The investigators have generated a series of molecular and transgenic animal reagents that can define transcriptional and post-transcriptional mechanisms responsible for apoJ induction by low dose IR; and (2) assess apoJ's potential to protect against radiation-mediated carcinogenesis in cells and tissues using apoJ knock-out mice. Preliminary evidence indicates that apoJ-deficient animals exhibit decreased apoptosis and decreased mortality after IR injury. The hypothesis is that knock out animals will be more permissive of genomic damage and will be prone to carcinogenesis following low dose IR. Experiments to test these hypotheses will be broadly divided into whole animal and cell culture model systems. In addition to defining the importance of apoJ in response to low dose IR, these studies will improve our understanding of gene regulatory mechanisms induced by low dose IR and provide a substantial foundation for understanding the role of apoptosis in genomic instability and carcinogenesis within the intact animal.

These studies suggest that the nuclear form of apoJ is a major determinant in the elimination of carcinogenic cells and that it contributes strongly to nonlinearity threshold responses for *survival and carcinogenesis. ApoJ also has a secreted form which is dramatically induced following IR. The investigators propose that circulating levels of this protein will be an outstanding marker of low dose IR exposure in humans, and its levels could be monitored for the evaluation of radiation exposures in persons working in high risk areas.*

Overview:

DISCLAIMER

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Aim 1: **Elucidating CLU promoter elements responsible for low dose IR induction.** Our prior findings from last year were found to be not complete. The sequence data that indicated that Sp1 and NF-κB sites may be important for clusterin (CLU) induction after low doses of IR were not the entire story. The results in MCF-7 cells were complicated by the influence of p53, and the more serious matter of a high background of CLU expression in transfected cells irregardless of inclusion of DNA in the transfections. This 'high noise' forced us to develop alternative methods of investigating CLU induction responses at the promoter level. These new approaches involved the development of (a) stably integrated and transfected CLU-promoter-luciferase (CLU-LUC) MCF-7 cells for the investigation of IR-inducible gene expression; (b) CLU-LUC transient transfection systems using mouse and human cells; and (c) the generation of a transgenic mouse containing CLU-LUC, as well as MCF-7-CLU-LUC xenografts in athymic nude mice. These MCF-7 xenografts and CLU-LUC transgenic mice will be developed into the first biodosimeter that can be analyzed as a indicator of exposure to low doses and dose rates of IR in real-time using liquid N_2-cooled CCD camera-based bioimaging.

Aim 2: **Elucidating IR-inducible signal transduction processes, as well as p53-mediated repression.** We have continued our work on the role of the p53 tumor suppressor protein to suppress CLU gene expression after low doses of IR exposures. In Criswell et al (Cancer Biology and Therapy, 2003) we demonstrated that p53 was a negative regulator of CLU gene expression, suppressing IR-inducible secretory CLU (sCLU) expression. sCLU is a pro-survival factor, and we have demonstrated that siRNA expression to sCLU can augment IR-mediated lethality (Leskov et al., In Preparation, 2003). In contrast, new results indicate that p53 does not regulate the levels of nuclear clusterin (nCLU), a pro-death protein (Leskov et al., J.B.C, 2003). nCLU is produced by alternative splicing and its expression and IR-induced activation can result in increased lethality (Leskov et al., J.B.C., 2003). Recent data using an siRNA to the nCLU junction site suggest the specific elimination of nCLU from cells, resulting in dramatically increased resistance to low dose IR exposures. These data suggest that p53 may regulate nCLU production and activation after IR exposures. Finally, we have begun to investigate the signal transduction pathways stimulated in cells after low doses of IR using specific inhibitors, dominant-negative constructs and specific siRNAs corresponding to various signal transduction processes.

Aim 3. **Investigate the role of sCLU in eliminating TGF-ß1 signal transduction processes.** Using human breast and colon cancer cell lines altered in TGFß1 RII expression, we demonstrated that TGF-ß1 exposure can dramatically increase sCLU expression (Klokov et al., In Preparation, 2003). Cells deficient in RII are devoid of TGF-ß1-induced sCLU expression. These data strongly support our hypothesis that sCLu can be induced by bystander IR-inducible TGF-ß1 levels, and in turn, can impart a bystander sCLU expression that could have effects on irradiated or non-irradiated cells at a far distance. We are examining the final loop of this proposed feedback system: Can sCLU suppress TGF-ß1-induced gene expression and growth suppression. Thus, sCLU could be a carcinogenic factor induced by low doses of IR.

Specific Progress:

Aim 1: Initial results indicated that NF-κB and Sp1 were involved in the low dose IR-induced up-regulation of sCLU in human cancer cells. Further analyzes revealed a more complicated pattern of gene regulation, as well as a complicating issue of an elevated noise of expression

caused by the transfection procedure. We believe that these data actually indicate that membrane and lipid peroxidation are triggering factors in the induction of sCLU levels after low doses of IR (Criswell et al., In Preparation, 2003). This led to the hypothesis that low doses of IR initiated a Ca2+ release from the ER, resulting in the IR-inducible expression of sCLU. Indeed, co-treatment of cells with a calcium chelator, BAPTA-AM suppressed sCLU expression. Later, we will return to the signal transduction processes induced by low dose IR exposure in human cells.

CLU Sequence Analyses: We developed a new transient transfection system using mouse and human cells. Nested deletion and point mutants of the CLU promoter have been generated, sequenced, and are being used for analyses of DNA sequences in the CLU promoter involved in the induction of CLU after low doses of IR. These analyses will be performed in conjunction with the signal transduction studies described below. These studies should allow us to elucidate the DNA sequences within the CLU promoter, allow us to identify unique transcription factors activated by low doses and dose rates of IR; see a recent review of the transcription factors known to be induced by low doses of IR in human cancer cells (Criswell et al., Oncogene, In Press, 2003).

Generation of a CLU Biodosimeter analyzed in real-time: CLU Promoter-Luciferase (CLU-LUC) transgenic mice and stably transfected MCF-7 xenografts were developed and are being used for the development of CLU biodosimeters. We have developed technology to monitor CLU gene expression after low doses of IR (Klokov et al., In Preparation, 2003). CLU Promter-LUC transgenic mice have been developed and are being analyzed for use as sentinel mice for monitoring low doses of IR exposure experienced during radiation clean-up and space travel.

Aim 2: We have completed and published our initial analyses of the negative regulation of sCLU by the p53 tumor suppressor protein (Criswell et al., Cancer Biology and Therapy, 2003). Recent data indicate that the clusterin (CLU) protein has both cytoprotective and cytotoxic activities. Our previous data strongly suggested that the secretory form of CLU was cytoprotective and induced after very low, nontoxic doses of ionizing radiation (IR: $\geq$0.02 Gy). In contrast, a nuclear form of CLU was induced by higher doses of IR (>1 Gy) and elicited apoptotic responses. Thus, CLU appears to represent a molecular switch between cell death and survival. However, the mechanisms regulating stress-inducible expression have not been elucidated. A screen of various cell lines indicated an inverse relationship between CLU expression and wild-type p53 status. IR stimulated CLU promoter activity, with concomitant increases in mRNA and protein, in log-phase MCF-7 cells 24-72 h post-IR. Expression of HPV E6 protein in human breast MCF-7 or RKO human colon cancer cells enhanced basal CLU levels, and augmented CLU synthesis after IR. Isogenically matched HCT116 human colon cancer cell lines that differ only in p53 or p21 status, confirmed a role for p53 in the transcriptional repression of secretory clusterin. Repression of sCLU protein levels by p53 may be important for the cascade of p53-mediated events leading to cell death after IR exposure.

Signal transduction responses: Our recent data suggest that low doses of IR stimulates the PKC-Ca^{2+}-src-jnk-erk-mapk signal transduction pathway in various mammalian cells. p53 suppresses this pathway, although further analyses is required for elucidation of the exact mechanism of suppression. These analyses are being confirmed using temporal analyses of specific intracellular phosphorylation sites using Western blot analyses, as well as the use of specific and general signal transduction inhibitors and expression of dominant-negative signal transduction proteins.

DE-FG02-99ER62724) Boothman, David, A.

Aim 3. Our data demonstrate that sCLU can be specifically induced by TGFß1 in RII-expressing human cancer cells. Human Vaco400 colon, MCF-7 breast and HCT116 colon cancer cells deficient in RII expression were compared to genetically matched cells expressing the TGF-ß RII receptor. Treatment of these cells with TGFß1 resulted in massive levels of sCLU expressed in cells as well as in the medium of exposed cells. An ELISA method is being developed for quantitation of sCLU expression after IR. We are using these genetically matched cells to explore the role of sCLU expression in suppressing TGF-ß1 signal transduction processes in vitro and in vivo. Additionally, we have analyzed the levels of sCLU levels induced in normal, tumor and blood of p53 wild-type, heterozygote and homozygote-deficient mice before and after low dose IR exposures.

Peer-reviewed Publications Resulting From This Research:

1. Yang, C-R., Yeh, S-Y, Odegaard, E.,, Leskov, K., Hsin, H-S,, Chang, C., Chen, D., Kinsella, T.J., and **Boothman, DA.** Isolation of Ku70 binding proteins (KUBs) 1999; Nucleic Acids Research, 27: 2165-2174.
2. Mendonca, M.S., Howard, K.L., Farrington, D.L., Desmond, L.A., Temples, T.M., Mayhugh, B.M., Pink, J.J., and **Boothman, D.A.** Delayed apoptotic responses associated with radiation-induced neoplastic transformation of human hybrid cells. 1999; Cancer Res., 59 (16): 3972-3979.
3. Yang, C-R., Wilson-Van Patten, C., Planchon, S.M., Wuerzberger-Davis, S.M., Davis, T.W., Cuthill, S., Miyamoto, S., and **Boothman, D.A.** Coordinate modulation of Sp1, NF-kappa B, and p53 in confluent human malignant melanoma cells after ionizing radiation. 2000; FASEB J., 14 (2): 379-390.
4. Huang, T.T., Wuerzberger-Davis, S.M., Seufer, B.J., Shumway, S.D., Kurama, T., Boothman, D.A., and Miyamoto, S. NF-κB activation by camptothecin: A linkage between nuclear DNA damage and cytoplasmic signaling events. 2000; J. Biol. Chem., 275 (13): 9501-9509.
5. Yang, C-R., Odegaard, E., Leskov, K., Hosley-Eberlein, K., Criswell, T., Kinsella, T.J., and **Boothman, D.A.** Nuclear clusterin/XIP8, an x-ray-induced Ku70-binding protein that signals cell death. 2000; Proc. Natl. Acad. Sci., USA, 97(11): 5907-5912.
6. Kalka, K., Ahmad, N., Criswell, T., **Boothman, D.A.,** and Mukhtar, H. Upregulation of clusterin during photodynamic therapy-mediated apoptosis of tumor cells and ablation of mouse skin tumors. 2000; Cancer Research, 60(21): 5984-5987.

Recent and Relevant Publications From Beyond the This Grant Period

7. Leskov, K.S., Klokov, DY., Li, J., Kinsella, TJ., and **Boothman, D.A.** Synthesis and functional analyses of nuclear clusterin, a cell death protein. J. Biol. Chem., 278 (13): 11590-11600, 2003.
8. Sun, W, Sawada M, Hayes P, Leskov, K, **Boothman, DA,** and Matsuyama, S. Ku70 suppresses the apoptotic translocation of Bax to mitochondria. Nature Cell Biology 5(4): 320-329, 2003.
9. Criswell, T., Klokov, D. Lavik, J.P. Beman, M. and **Boothman, D.A.** Transcriptional Repression of Clusterin by the p53 Tumor Suppressor Protein. *Cancer Biology and Therapy,* 2(4): 25-31, 2003.
10. Klokov, D., Criswell, T., Sampath, L., Leskov, KS., Frinkley, K., Araki, S., Beman, M., Wilson, D.L., and Boothman, D.A. Clusterin: a protein with multiple functions as a potential ionizing radiation exposure marker. International Congress Series. 2784: In Press, 2003.

ER

DE-FG02-99ER62724) Boothman, David, A.

Papers Published in Non-Peer-Reviewed Journals.

1. **Boothman, D.A.,** Odegaard, E., Sahijdak, W.M., Meyers, M., and Yang, C-R. Role of cyclin A in adaptive survival responses. Contributed as part of the 10th International Congress of Radiation Research, 1997.
2. **Boothman, D.A.**, Odegaard, E., Yang, C-R., Hosley, K., and Mendonca, M. Molecular analyses adaptive survival responses (ASRs): role of ASRs in radiotherapy. 1998; Belle Newsletter.
3. Davis, T.W., Wilson-Van Patten, C., Yang, C-R., Sharda, N., Meyers, M., Kinsella, T.J., and **Boothman, D.A.** Trasncriptional Responses to damage created by ionizing radiation. In: "DNA Damage and Repair, Vol. 2:DNA Repair in Higher Eukaryotes, (Nickoloff, J.A. and Hoekstra, M., eds.). Humana Press, Inc., Totowa, NJ. pp. 223-261, 1998.
4. Yang, C-Y., Leskov, K., Hosley-Eberlein, K.J. Criswell, T.L., Mooney, M.A., Pink, J.J. and **Boothman, D.A.** Ku70-binding proteins. Radiation Research 2: 426-429, 2000.
5. Leskov, K., Criswell, T.A., Antonio, S. Li, J., Yang, C-R., Kinsella, T.J., and **Boothman, D.A.** When X-ray-inducible proteins meet DNA double strand break repair.. Seminars in Radiation Oncology *11 (4):* 352-372, 2001.

Non-Peer-Reviewed Publications Beyond the Scope of the Prior Grant Period

6. Leskov, K, Antonio, S., Criswell, T., Yang, C-R., Kinella, TJ, and **Boothman, D.A.** Radiation-inducible clusterin (CLU): A molecular switch between life and death. Radiation Research *156 (4):* 441-442, 2001.
7. Criswell, T., Leskov, K., Miyamoto, S., Luo, G., and **Boothman, DA.** Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiaiton. Oncogene, *22:* 5813-5827, 2003.
8. Klokov D, Criswell T, Sampath L, Leskov K, Frinkley K, Araki S, Beman M, Wilson D, Boothman DA. Clusterin: a protein with multiple functions as a potential ionizing radiation exposure marker. In: 1st Nagasaki Symposium of International Consortium for Medical Care of Hibakusha and radiation Life sciences. (Shibata Y, Yamashita S, Watanabe M, Tomonaga M Eds.) Elsevier, Amsterdam, 2003 In Press.