Radiation Therapy Symposium: Introduction

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*On March 6, 2000 a joint symposium was organized by the *District of Columbia Section of the Society for Experimental Biology and Medicine and †Oxygen Club of Greater Washington, D.C.*

The topics presented in this mini-symposium encompassed the whole spectrum from basic research to clinical applications of different qualities of radiation. Dr. Usha Kasid's laboratory uses cellular, molecular, biochemical, and *in-vivo* approaches to study the role of Raf-1, a cytoplasmic protein kinase, in cell growth, proliferation, and cell survival. The potential role of Raf-1 in tumor response to ionizing radiation (IR) is investigated. Dr. Kasid showed that exposure of human carcinoma cells to IR activates Raf-1, stimulates the association of Ras with Raf-1, and downstream activation of MKK, MAPK, and AP-1. These findings suggested that Raf-1 is an effector of Ras in IR-stimulated MAPK pathway. The data from this lab suggest a basis for clinical investigation of radiation and antisense-raf oligodeoxyribonucleotide in cancer therapy. Dr. Mark H. Whitnall presented very interesting and promising data on the use of 5-androstenediol (AED) as a radioprotectant to enhance the immune function and promote survival following wholebody exposure to IR. It is known that acute exposure to IR causes failure of the hemopoietic progenitors to proliferate leading to death due to infection and hemorrhage. Free radical scavengers and antioxidants are the common pharmacologic tools used to minimize injury by reducing reactive free-radical species and, in certain cases by acting as immunomodulators, cytokines can stimulate hemopoietic recovery. It has been shown that pretreatment with dehydroepiandrosterone and its metabolite AED, increases survival during bacterial and viral infections in un-irradiated animals. Dr. Whitnall is studying this family of steroids and their application as countermeasures to whole-body IR at doses that can cause death attributed to reduced immunity to infection. In contrast to free radical scavenger therapy, AED seems to be not cytotoxic. Dr. Martin W. Brechbiel's research focuses on investigating the challenging technology and the promising use of radiolabeled monoclonal antibodies in their clinical cancer therapy applications. These macromolecules localize and bind to malignant cells to selectively target, as well as deliver, either an imageable isotope or a localized radiation dose. Dr. Brechbiel discusses the pros and cons of the various emitters and the potential advantage of using a-emitting radionuclides in treating leukemias and lymphomas.

Raf-1 Protein Kinase, Signal Transduction, and Targeted Intervention of Radiation Response

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Raf-1, a cytoplasmic protein serine-threonine kinase, plays an important role in cell growth, proliferation, and cell survival. State-of-the-art cellular, molecular, biochemical, and pre-clinical approaches have been used in our laboratory to elucidate the role of Raf-1 in tumor response to ionizing radiation (IR). Our data demonstrate that exposure of human carcinoma cells to IR results in tyrosine phosphorylation, membrane-recruitment, and activation of Raf-1 protein kinase. In addition, IR stimulates the association of Ras with Raf-1, and downstream activation

of MKK, MAPK, and AP-1. These observations indicate that Raf-1 is an effector of Ras in IR-stimulated MAPK pathway.

Previously, we have demonstrated a correlation between antisense *c-raf 1* cDNA mediated inhibition of Raf-1, delayed tumor growth, and enhanced radiosensitivity of relatively radioresistant laryngeal squamous carcinoma cells (SQ-20B). Furthermore, *c-raf-1* cDNA transfection results in a relatively radioresistant phenotype of human immortalized bronchial epithelial cells and human squamous carcinoma cells. Consistent with these data, antisense *raf* oligodeoxyribonucleotide (AS-*raf*-ODN)-specific inhibition of Raf-1 protein has been associated with radio sensitization of SQ-20B tumor cells. These and other studies utilizing mouse embryo fibroblasts containing targeted disruption of the c-raf-1 gene suggest that Raf-1 is an important cell survival factor.

Translation of laboratory observations into clinically relevant protocols is an important step towards the ultimate goal of developing safer and more effective cancer therapies. Along these lines, novel cationic liposomes have been designed to systemically deliver AS-*raf*-ODN to tumor tissues. Safety and pharmacokinetics profile of liposomes carrying AS-*raf*-ODN have been established in different animal species. It should be noted that unmodified AS-*raf*-ODN is nontoxic *in vivo* and cationic liposomal composition that we designed is also non-toxic. Plasma and normal tissue pharmacokinetics of liposome-encapsulated unmodified AS-*raf*-ODN (LE-AS-*raf*-ODN) is significantly better compared to "free" AS-*raf*-ODN. In addition, systemic delivery of the liposomal formulation of a phosphorothioated AS-*raf*-ODN (LE-5132) provides an improved tumor control compared to "free" phosphorothioated AS-*raf*-ODN (5132) ($P < 0.001$). Intravenous administration of LE-5132 leads to radiosensitization of SQ-20B tumor xenografts. Histopathologic evaluation of these tumor specimens revealed a significant proportion of cells containing fragmented chromatin compared to single agent treated or control groups. These data demonstrate that AS-*raf*-ODN is an effective radiosensitizer *in vivo*.

To identify new cellular targets of cancer gene therapy, we have used the differential gene expression technology and selected for genes aberrantly expressed in metastatic or radiation-resistant tumor cells but not in the matched primary tumor-derived cells or radiosensitive tumor cells. In addition, candidate genes downstream of Raf-1 have been identified using AS-*raf*-ODN and comparing the gene expression patterns in isogenic tumor cell populations expressing differential steady state levels of Raf-1. Because AS-*raf*-ODN treatment of a variety of human tumor cells causes apoptosis, characterization of novel effectors downstream of Raf-1 should provide further insight into the mechanisms regulating cell death or cell survival.

In conclusion, these studies establish an important role of Raf-1 in radiation resistance and radiation-initiated intracellular signal transduction process. In addition, our data provide a basis for a clinical study of the combination of AS-*raf*-ODN and radiation in cancer patients.

In Vivo Protection Against Gamma-Irradiation with 5-Androstenediol

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5-androstenediol (AED) is a representative of a novel class of radioprotectants, the 5-androstene steroids, that enhance immune function and promote survival after whole-body exposure to ionizing radiation. Improvements in survival were observed when AED was administered by sc injection between 24 h before and 2 h after gamma-irradiation of mice. A dose reduction factor of 1.3 was calculated from probit survival curves. Protection was observed in both male and female mice, with and without subsequent inoculation with lethal doses of *Klebsiella pneumoniae*. No protection was observed with a number of other steroids: dehydroepiandrosterone (DHEA), 5-androstene-3B,7B, 17B-triol (AET), androstenedione, or estradiol. AED induced increases in circulating neutrophils, platelets, and NK cells in normal or irradiated mice, with no effect on numbers of circulating B cells or T cells. Numbers of granulocyte-monocyte progenitors in bone marrow were also increased with AED. No signs of toxicity were observed in