This is the final technical report for Project DE-FG02-01ER63238, Molecular Mechanisms of Radiation-Induced Genomic Instability in Human Cells.

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This project was conducted from September 15, 2001 through January 31, 2002. At that point, Howard Liber moved to Colorado State University, Fort Collins, CO. The project is being continued there as DE-FG03-02ER63365.

The overall strategy for our research project was to create a series of isogenic human cell lines that differ in key elements of cell cycle checkpoints, apoptosis, or DNA repair in response to radiation-induced damage. The goal then was to quantify the fractions of cells within a population that exhibit reduced telomere lengths and relate this to the genetic background of the cell, as well as to the response to ionizing radiation.

Associations between telomere length and degree of genomic instability in the population is being examined for seven closely-related cell lines, that vary in p53 status, bcl-2 status, or ability to repair double strand breaks. Experiments utilize γ -rays, treating cells at doses of 0, 10 and 200 cGy. For each line, 50 independent surviving clones are being isolated. In each clone, telomere lengths are to be established, and instability is to be assessed in two distinct ways, as (i) frequency of chromosome aberrations and (ii) spontaneous mutation frequency and rate at the autosomal thymidine kinase locus. Thus we are studying spontaneous genomic instability as well as instability induced by a low or high dose of ionizing radiation.

In the 4.5 months between September 15, 2001 and January 31, 2002, we concentrated on generating data with TK6 (p53 wild-type) and NH32 (p53 null), two cell lines that vary in their status of p53. Approximately one-third of the required clones (≥ 50 for each dose and each line, or more than 300) were isolated, and analyses for mutagenesis and chromosome aberrations were undertaken.

These analyses were continued and are described in detail in the first progress report for DE-FG03-02ER63365.

DOE Patent Clearance Granted

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Molecular Mechanisms of Radiation-Induced Genomic Instability in Human Cells

Abstract Knowledge of the signal and target through which radiation induces genomic instability, which we propose to investigate herein, will allow us to model effects at low doses. Such knowledge will aid in understanding variations in the induction of this genomic instability, both among cells and among individuals. This information could also lead to more sensitive measures of instability that could yield accurate measures of instability induction at low doses.

We have developed an *in vitro* cell model, in which radiation-induced chromosome instability develops in a two-stage process. The first stage requires telomeres to be reduced in size to some critical length. This occurs in normal somatic cells as a consequence of replication-dependent telomere shortening. Normally, when telomeres reach this critical size, they signal for a p53-dependent cell cycle arrest or apoptosis. Failure to arrest leads to continued telomere shortening and ultimately telomere fusions. The second step in instability development is the loss or alteration in this p53-dependent cell cycle checkpoint control that then allows for cells with short telomeres to survive. The telomere fusions that subsequently develop lead to the aneuploidy, chromosome deletions, duplications, and translocations and gene mutations that characterize genome instability. In the model, then, instability develops after radiation-induced DNA damage [the signal] alters the normal p53-dependent cell cycle checkpoint controls that signal for elimination of cells with short telomeres [the target]. We propose to explore this model in more depth by testing the following hypotheses.

1. All unstable cells, whether they develop spontaneously or are radiation-induced, will exhibit short telomeres (relative to telomere lengths in stable cells).
2. Loss of the tumor suppressor p53 will be associated with greater levels of spontaneous instability but reduced sensitivity to radiation-induced instability because these clones will not eliminate unstable cells even in the absence of radiation exposure.
3. Suppression of apoptosis by over-expression of bcl-2 will mimic the effects of p53 inactivation because both genetic alterations act on instability through the same mechanism.
4. Radiation-induced genomic instability will be greater in cells deficient in DNA double strand break repair.

The main focus of the research will be to investigate whether radiation exposure acts at the level of disrupting the signaling pathways that connect telomere metabolism to cell cycle arrest and apoptosis. We also will investigate whether radiation-induced DNA double strand breaks produce alterations in these signal transduction pathways, which allows cells to continue to grow despite containing these critically short telomeres. The overall strategy for the research is to create a series of isogenic human cell lines that differ in key elements of cell cycle checkpoints, apoptosis, or DNA repair in response to radiation-induced damage. The goal then will be to quantify the fractions of cells within a population that exhibit reduced telomere lengths and relate this to the genetic background of the cell, as well as to the response to ionizing radiation.

Associations between telomere length and degree of genomic instability in the population will be examined for seven closely-related cell lines, that vary in p53 status, bcl-2 status, or ability to repair double strand breaks. Experiments will utilize X- or γ -rays, treating cells at doses of 0, 10 and 200 cGy. For each line, 50 independent surviving clones will be isolated. In each clone, telomere lengths will be established, and instability will be assessed in two distinct ways, as (i) frequency of chromosome aberrations and (ii) spontaneous mutation frequency and rate at the autosomal thymidine kinase locus. Thus we will study spontaneous genomic instability as well as instability induced by a low or high dose of ionizing radiation.