Progress Report
Human Genetic Marker for Resistance to Radiations and Chemicals
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a. Specific Aims

The specific aims listed in the original application will essentially be pursued as indicated. The major goal of the grant is to characterize a human homologue of the fission yeast *Schizosaccharomyces pombe rad9* checkpoint control, radioresistance and chemoresistance gene, which is called *HRAD9*. The purpose is to gain information about the gene, including its structure and function, such that it can potentially be developed as a human genetic marker indicative of hypersensitivity to the deleterious effects associated with exposure to radiations or certain chemicals. The specific aims are divided into two major sections. The first section includes experiments designed to characterize the *HRAD9* gene at the molecular level. Specifically, the genomic version of the gene will be isolated and its DNA sequence determined, in vitro mutagenesis will be used to assess structure/function relationships, and expression in cells and tissues will be examined. The second major set of aims focuses on determining the role of *HRAD9* in radio/chemoresponsiveness and cancer. For this aim, human *HRAD9* mutants will be constructed and characterized. In addition, the status of *HRAD9* in cancer cells and tissues will be assessed.

b. Studies and Results

Progress has been made towards the completion of each of the specific aims. In regards to aim 1, we, in collaboration with Dr. Scott Davey at Queen's University in Kingston Ontario, Canada, isolated human genomic DNA clones bearing homology to *HRAD9*. Using the previously cloned and sequenced *HRAD9* cDNA as a reference point, we have begun to determine the DNA sequence of the genomic version of the gene. Thus far, we have obtained approximately 12 kbp of relevant sequence. This genomic region contains approximately 3/4 of the cDNA sequence and about half the coding region. Six introns and an equal number of exons have been identified to date.

Northern blot analysis has been performed to determine whether there is tissue-specific expression of *HRAD9*, and also to assess baseline levels of expression in cells not treated with DNA damaging agents. We have probed RNA from 16 different human tissues and found expression to be universal. *HRAD9* RNA was detected in heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon and leukocytes. *HRAD9* RNA was most abundant in the liver and detected at the lowest levels in the lung.

Progress has also been made towards completing the goals described in specific aim 2. We have already constructed plasmids capable of overexpressing *HRAD9*, in either the sense or antisense orientation, and introduced those constructs into human cell lines. We are currently selecting cells receiving the constructs for subsequent characterization, to assess the function of *HRAD9* in human cells overproducing or underproducing the corresponding protein. These experiments will establish the function of *HRAD9* in a human genetic background.

Previously, we found that *HRAD9* resided on human chromosome 11q13.l-13.2. Alterations in this region were found by other investigators to be associated with cervical carcinoma. Therefore, *HRAD9* was considered a candidate for the gene responsible for this type of cancer, based on its map location and also the role of the fission yeast *rad9* gene in cell cycle control, which would be consistent with a gene that functions as a tumor suppressor. Therefore, we have begun to examine the status of *HRAD9* in control and cervical carcinoma cells and tissue samples. PCR was used to amplify regions of the genomic version of *HRAD9*, or RT-PCR was employed to generate large amounts of *HRAD9* cDNA from samples. Amplified DNA was then examined by SSCP and/or DNA restriction enzyme digestion to identify mutations. Using this strategy, we

detected the same polymorphism, a T to C transition, in 3 of 8 independent cervical carcinoma cell lines. This change is in the coding region of the gene, but results in the codon change GGT to GGC, which does not alter the encoded amino acid (i.e., glycine remains in the same position in the protein). Furthermore, this polymorphism was found in 7 out of 7 independent primary cervical carcinoma biopsies. Significantly, two nontumorigenic human cell lines and blood samples from 17 unrelated patients suffering from other types of cancers did not demonstrate this nucleotide change. These results indicate that alterations in *HRAD9* are most likely not responsible for cervical carcinoma. However, our studies have identified a polymorphism that is tightly associated with this type of cancer. Therefore, this result is important because the polymorphism has the potential to act as a genetic marker for predisposition to the disease. In addition, because of the tight association between the polymorphism and cervical carcinoma, *HRAD9* could be used in the future as part of a chromosome walking strategy to isolate the cervical carcinoma gene.

In summary, we have made steady progress in pursuing the specific aims described in the grant proposal. For the remaining award period, experiments will continue to be performed as listed.