Report: Annual Report

Project Title: Human Genetic Marker for Resistance to Radiations and Chemicals

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Progress Report:

Research Objective

The major objective of this project is to understand the genetic basis for resistance of humans to radiations and chemicals. In the fission yeast *S. pombe*, a gene called *rad9* plays a key role in promoting resistance to DNA damaging agents and controlling cell cycle progression after radiation or chemical exposure. This investigation focuses on the characterization of a human homologue of this yeast gene, called *HRAD9*, with the long-term goal of developing the gene as a genetic marker to predict inherent susceptibility to the deleterious health effects caused by DNA damage. The aims concern a molecular characterization of *HRAD9* and determination of its role in mediating the cellular response to radiations and chemicals, as well as its potential role in carcinogenesis.

Research Progress and Implications

This report summarizes work after the third year of a three-year project. The focus has been on pursuing the characterization of *HRAD9*, especially in terms of the relationship between its structure and function in human cells. During the current award period there were two major findings, that the gene is a pro-apoptotic element and also that its function in programmed cell death is distinct from its role in regulating cell cycle progression after DNA damage is incurred (i.e., cell cycle checkpoint control). These results are important since they revealed a new role for HRAD9, regulating apoptosis, in addition to its previously known checkpoint control function. This indicates that HRAD9 plays an even more significant role in the cellular response to radiation or chemical exposure than previously thought since the protein participates in two major cellular processes that are activated by DNA damage.

In collaboration with Dr. Hong-Gang Wang's group (Moffitt Cancer Center, Tampa, FL), we found that amino acids 15 through 30 in the N-terminal region of the HRAD9 protein are highly homologous to the BH3 domain of pro-apoptotic BCL-2 family members. In addition, we found via co-immunoprecipitation and two-hybrid assays that HRAD9 can bind the anti-apoptotic proteins BCL-2 and BCL-x_L only when the BH3-domain is intact. Furthermore, overexpression of *HRAD9* causes apoptosis in many cell lines, and a mutant form of the protein containing an altered BH3-like region is incapable of inducing programmed cell death. Co-overexpression of the anti-apoptotic protein BCL-2 with HRAD9 does not result in apoptosis, suggesting that the process is controlled at least in part by the relative amounts of each of these proteins. These findings indicate that HRAD9 plays an important role in regulating apoptosis, in addition to its previously described checkpoint control and other radioresistance-promoting functions.

In another study we found, again using co-immunoprecipitation and two-hybrid assays, that HRAD9 physically interacts with two other human checkpoint control proteins, HRAD1 and HHUS1. Furthermore, detailed analyses of those interactions using partially deleted forms of each protein indicated that HRAD1 and HHUS1 bind to the C-terminal region of HRAD9. Therefore, these results suggest that HRAD9 protein has at least two functional domains, one in the N-terminal region that regulates apoptosis and the other in the C-terminal region that governs cell cycle checkpoint responses. The current hypothesis that is being pursued to explain these results is that HRAD9 helps the cell essentially "decide" whether to undergo suicide or delay cycling to enhance repair and promote survival after DNA damage is incurred. Furthermore, it is predicted that the set of proteins bound to HRAD9 will ultimately control which functional domain of the protein is active. These results are very important since apoptosis and checkpoint control are two major cellular responses to radiation or chemical exposure, and the evidence indicates that HRAD9 modulates and coordinates both of these processes. It is likely that through the regulation of these two pathways HRAD9 determines the severity of the effects caused by DNA damage.

Planned Activities

For the remaining award period, experiments addressing the dual role of HRAD9 in the cellular response to DNA damage will be performed. In addition, more data will be collected to attempt to establish a firm link between *HRAD9* polymorphisms and cancer, in particular cervical cancer where previous experiments have suggested that the two are associated.

Information Access

Komatsu, K., T. Miyashita, H. Hang, K.M. Hopkins, W. Zheng, S. Cuddeback, M. Yamada, H.B. Lieberman, and H.-G. Wang. (2000) Human homologue of *S. pombe* Rad9 interacts with Bcl-2/Bcl-xL and promotes apoptosis. Nature Cell Biol. 2:1-6.

Hang, H., and H.B. Lieberman. (2000) Physical interactions among human checkpoint control proteins HUS1p, RAD1p and RAD9p, and implications for the regulation of cell cycle progression. Genomics 65:24-33.