Project ID: **55100**

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

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RESEARCH OBJECTIVE

The major goal of the research project is to define the role of *HRAD9* in the response of cells to radiation or chemical exposure, and to establish this gene as a genetic marker to predict predisposition to the deleterious health effects that may result after exposure to these agents. *HRAD9* is a human homologue of fission yeast *S. pombe rad9*, a gene known to promote radioresistance and chemoresistance, and to regulate cell cycle progression after DNA is damaged or DNA replication is incomplete –i.e., it mediates cell cycle checkpoint control. Therefore, *HRAD9* likely plays an important role in humans to determine the biological consequences of DNA damage.

RESEARCH PROGRESS AND IMPLICATIONS

This report summarizes the work accomplished thus far in the third year of a three year project. These studies have focused on both the human (*HRAD9*) and mouse (*Mrad9*) versions of the *S. pombe rad9* checkpoint control gene, in terms of their structure and function.

In relation to the human gene, coimmunoprecipitation experiments were performed to identify proteins that physically associate with the HRAD9 protein, for the purpose of gaining insight into its function in the context of the whole cell. These investigations indicated that HRAD9 interacts with several tumor suppressor proteins, including p16, p53 and BRCA1, as well as the human checkpoint control proteins HHUS1 and HRAD1. Also, preliminary two-hybrid analyses and studies with the purified proteins suggest that at least HHUS1 and HRAD1 interact directly with HRAD9 and do not just bind to the same large complex of proteins. Since HRAD9 interacts with proteins that participate in carcinogenesis and cell cycle regulation, the results support a role for the protein in these important processes.

Studies have begun to establish the functional significance of these protein-protein interactions. For example, three forms of the HRAD9 protein can be detected by western analysis in most cell lines engineered to overproduce the protein. The protein encoded within *HRAD9* should be approximately 42 kDa, yet the three proteins detected were 50, 58 and 67 kDa in size. Therefore, HRAD9 undergoes extensive post-translational modification, and additional studies revealed that this processing includes phosphorylation. Based on the link between breast cancer and HRAD9 suggested by the ability of this protein to interact with BRCA1, the status of HRAD9 in the breast carcinoma cell line MCF-7 was examined. Western analysis indicated that two of the three processed forms of HRAD9 (the 50 and 67 kDa species) are absent and a 42 kDa protein, corresponding to the predicted size of the unprocessed form is abundant. This aberrant post-translational modification has not been observed in normal human kidney (293T), lung cancer (H1299), astrocytoma (CCF-STTG1), cervical carcinoma (HeLa), or mutant *Abl*, *ATM*, or *p53* derivatives of colorectal carcinoma (RKO) cells. Thus, this lack of appropriate processing is specific for breast cancer. The status of HRAD9 in multiple normal and breast cancer cell populations is being examined to confirm this relationship and to support a role for HRAD9 in this specific type of cancer. The results of this study may therefore establish *HRAD9* as a new genetic element that contributes to breast cancer.

The interaction of HRAD9 with p53, as well as the identification of a BH3-like domain within HRAD9 suggested that the protein might play a role in programmed cell death –i.e., apoptosis. Therefore, several experiments were performed to address this hypothesis. The results indicate that HRAD9 is capable of interacting with multiple anti-

apoptotic proteins and can induce apoptosis when inappropriately expressed. These results therefore indicate that HRAD9 participates in this important cellular response to DNA damage. The specific biochemical events mediated by HRAD9 in programmed cell death are being examined.

In relation to *Mrad9*, we have isolated and determined the DNA sequence of the entire gene and cDNA. Furthermore, we have begun experiments to create a conditional *Mrad9* knockout mouse in order to establish an animal model for assessing the biological impact of *Mrad9* mutations. A targeting construct has been made and introduced into mouse ES cells. Those cells integrating the construct into the inherent location of the gene will be used for morula aggregation experiments as the next step to make mutant mice.

PLANNED ACTIVITIES

Future work will expand the current studies to learn more about the function of *HRAD9* and *Mrad9*. The biological significance of the protein-protein interactions involving HRAD9 identified thus far will be assessed. Studies to establish the prevalence of *HRAD9* polymorphisms in the general population will continue, and the role of these alterations in determining whether individuals are at a high risk for developing deleterious health effects after radiation or chemical exposure will be established. In relation to *Mrad9*, experiments designed to construct a conditional mutant knockout mouse will proceed. The goal for this set of studies is to develop an animal model that will aid in directly assessing the impact of *RAD9* mutations on radioresistance, chemoresistance and the development of cancer after exposure to DNA damaging agents. Together, these studies serve to develop and validate the use of *HRAD9* as a genetic marker for determining whether individuals are hypersensitive to the induction of cancer or other harmful effects by exposure to radiation or chemicals.

INFORMATION ACCESS

Hang, H., S. J. Rauth, K.M. Hopkins, S.K. Davey, and H.B. Lieberman. (1998) Molecular cloning and tissue-specific expression of *Mrad9*, a murine orthologue of the *Schizosaccharomyces pombe rad9*$^{+}$ checkpoint control gene. J. Cell. Physiol. 177:232-240.