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Human Genetic Marker for Resistance to Radiations and Chemicals

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Research Objective

The broad objective of the project is to understand the molecular basis for the response of cells to radiations and chemicals, with the pragmatic goal of being able to identify human subpopulations that are exceptionally sensitive to DNA damaging agents. The project focuses on HRAD9, a human orthologue of the fission yeast Schizosaccharomyces pombe gene rad9. S. pombe rad9::ura4+ mutant cells are highly sensitive to ionizing radiation, UV and many chemicals, such as the DNA synthesis inhibitor hydroxyurea. They also lack the ability to delay cycling transiently in S phase or in G2 following a block in DNA replication or after incurring DNA damage, respectively -i.e., they lack checkpoint controls. The attempt by mutant cells to progress through mitosis in the absence of fully intact DNA accounts at least in part for their sensitivity to DNA damaging agents. Cells bearing rad9::ura4+ also aberrantly regulate UVDE, an enzyme that participates in a secondary DNA excision repair pathway. The key role played by S. pombe rad9 in promoting resistance to chemicals and radiations suggests that the evolutionarily conserved human cognate also has important functions in mammals. The first set of aims in this proposal centers on characterizing the structure and expression of HRAD9, to assess structure/function relationships and potentially link protein activity to a specific tissue. The next set of aims focuses on determining the role of HRAD9 in radio/chemoresponsiveness and cancer.

Research Progress and Implications

This report summarizes work performed after 2 years of a 3 year project. Progress has been made towards completion of all of the specific aims. In collaboration with Dr. Scott Davey at Queen's University (Ontario, Canada), human genomic DNA clones bearing homology to HRAD9 have been isolated and the DNA sequence of the genomic version of the gene has been partially determined. Twelve kbp of relevant sequence has been obtained. This genomic DNA contains 3/4 of the previously determined cDNA sequence and about half the coding region. Six introns and six exons have been identified to date. To aid in the identification of evolutionarily conserved regions of HRAD9, and therefore domains of the human protein likely to be important for function, the mouse version of the gene has been isolated. Mrad9 encodes a 389 amino acid long, 42,032 Dalton protein that is 27% identical and 56% similar to S. pombe Rad9p, and 82% identical and 88% similar to HRAD9 at the amino acid level. Expression of the Mrad9 cDNA in S. pombe rad9::ura4+ cells restores nearly wild-type levels of hydroxyurea resistance and early S phase checkpoint control to mutant fission yeast cells. However, UV resistance is only minimally restored and mutant cells remain sensitive to gamma radiation. Mrad9 genomic DNA was isolated from a mouse 129/SvEv library. The Mrad9 gene was localized to a 15kbp genomic DNA fragment, and contains 10 exons separated by 9 introns. These studies demonstrate the existence of a murine orthologue of S. pombe rad9+, and underscore at least the partial evolutionary conservation of rad9+-dependent checkpoint control mechanisms. Comparison of the amino acid sequences from yeast and mammalian Rad9 proteins reveals several short regions found in all versions of the protein, and these sequences are serving as targets for in vitro mutagenesis to define functional domains.

Northern blot analysis has been performed to determine whether there is tissue-specific expression of HRAD9. RNA from 16 different human tissues was probed with HRAD9, and expression was found 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.

However, HRAD9 RNA was most abundant in the liver and detected at the lowest levels in the lung.

Several strategies are being pursued to eliminate or reduce HRAD9 protein levels in human cells to define the biological function of the gene. Interestingly, multiple attempts using anti-sense RNA resulted in overexpression of the gene. Northern blot analysis revealed that HRAD9 RNA was markedly abundant in anti-sense producing versus mock treated human cells, suggesting that HRAD9 may be regulated by a novel feedback mechanism. Preliminary studies of S. pombe rad9 indicate that alteration of one region of the protein may produce a dominant-negative mutation. As an alternative strategy to make human HRAD9 mutant cells, this conserved region will be altered in the gene, and subsequently tested for dominant-negative activity in a human genetic background. Previously, we localized HRAD9 to human chromosome 11q13.1-13.2. 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 on the role of S. pombe rad9 in cell cycle control, which would be consistent with a tumor suppressor gene. Consequently, the status of HRAD9 in control and cervical carcinoma cells and tissues was examined. PCR was used to amplify regions of the HRAD9 gene, or RT-PCR was employed to generate HRAD9 cDNA from samples. Amplified DNA was then examined by SSCP and limited DNA sequence analysis to identify mutations. Using this strategy, the same polymorphism, a T to C transition, was detected 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). Furthermore, this polymorphism was found in 7 of 7 primary cervical carcinoma biopsies. Significantly, two nontumorigenic human cell lines and blood samples from 17 unrelated patients having 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, but the polymorphism has the potential to act as a genetic marker for predisposition to the disease.

Planned Activities

All of the specific aims described in the original application will be pursued essentially as indicated. These goals include determining the entire sequence of the genomic version of HRAD9, looking at the structure and expression of HRAD9 in normal and cancer cells and tissues, examining the biological consequences of reducing or eliminating HRAD9 expression, and establishing whether HRAD9 expression is regulated.

Other Access To Information

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. (In press).