Dr. Jeffrey T. Holt during development at 6.5 days when the genes are normally expressed. Because these data strongly suggest that the normal function of BRCA2 is involved in repair of double-strand DNA breaks, researchers in Holt's lab in Nashville decided to see if BRCA2-defective cancer cells are killed more easily than cells with normal BRCA2 when exposed to drugs that induce double-strand breaks.

**Therapy Clue**

Using a BRCA2-defective human pancreatic cancer cell line, the scientists reported in the July 1 issue of this journal that the cell line is both deficient in double-strand break repair and killed more easily by chemotherapy drugs that cause double-strand DNA breaks. Similar results were seen when the cell line was injected into the thighs of nude mice. BRCA2-defective tumors were shrunk significantly by therapies causing double-strand breaks. These results have led Holt and others to see if the results in mice hold up in humans. Several groups have begun studies to see if patients with BRCA2 mutations will have a different response to radiation therapy than those with sporadic cancers.

Another therapeutic approach that may turn out to be important in many more types of tumors, including those that are sporadic, has been proposed by Wen-Hwa Lee, Ph.D., at the University of Texas Health Science Center in San
Antonio Lee sees the most important clinical pay-offs coming from the BRCA2/Rad51 interaction.

In a recent article in Proceedings of the National Academy of Sciences, researchers in Lee's lab identified the portions of BRCA2 protein that associate directly with Rad51. His strategy is to synthesize a small peptide that will block this interaction. Because most breast tumors, as well as other tumors, have an intact BRCA2 protein, a drug that blocks this interaction might leave the tumors more susceptible to damage from radiation or drugs causing double-stranded DNA breaks.

Even though the data for BRCA2 seems to be stronger, some would argue that it's difficult to imagine that BRCA1 and BRCA2 are not both involved in at least some of the same pathways. Lewis Chodosh, M.D., Ph.D., at the University of Pennsylvania Medical Center in Philadelphia, is one of those.

Finding BRCA1's Function: A More Arduous Journey

For BRCA1, the noisiest problem has been where the BRCA1 protein is located in the cell. While most researchers have found the protein in the nucleus of normal cells, some have found evidence of the protein in the cytoplasm of malignant cells, and one group has reported that BRCA1 is present in the cell membrane and in the secretory apparatus. However, in a paper recently submitted to Nature Genetics, Cindy Wilson, Ph.D., from the Division of Hematology Oncology at the University of California, Los Angeles, appears to have settled whatever ambiguity remains.

"It's in the nucleus," said Wilson. "All the antibodies that are specific show distinctive nuclear dot patterns in cells." She compared 20 antibodies from several groups using many tests—western blotting, immunoprecipitation, immunohistochemistry, and cytochemistry on individual cells. Because BRCA1 protein is expressed at very low levels in the nucleus, very clean antibodies are required to see it.

Besides cellular location, another clue to function comes from discovering the proteins that interact with BRCA1. To date, the list has grown quite large, including several enzymes (e.g., helicase, RNA polymerase, and ubiquitin hydrolase) as well as other proteins (e.g., Rad51, p53, BARD1, and CTIP). So far, no clear consensus has emerged about which proteins are important to BRCA1's function.

Adding to the murky picture, is the inability of researchers to produce mice lacking two copies of functional BRCA1 genes, so-called knock-out mice, which frequently provide insights into the function of the missing gene. Mouse embryos lacking BRCA1 protein do not survive.

Given these problems, a paper in the Aug. 14 Science was a first—the first direct functional evidence for BRCA1. Researchers from the Department of Radiation Oncology, University of North Carolina at Chapel Hill, showed that cells deficient in BRCA1 were unable to repair DNA that was damaged by either ionizing radiation (gamma radiation) or hydrogen peroxide. The particular kind of repair that is defective in these cells is called transcription-coupled repair (TCR) — involving machinery that tags along with RNA polymerase and repairs actively transcribing genes.

"Our results make sense with the data that show an association between BRCA1 and RNA polymerase," said Lori Gowen, a UNC graduate student and the paper's first author. "And it is consistent with BRCA1 being a tumor suppressor gene — mutations will accumulate if the gene is involved in repair. But it still doesn't allow us to say whether BRCA1 is a part of the repair machinery or a transcription factor that regulates transcription of genes involved in TCR."

And that seems to be precisely the problem — developing a good system for analyzing BRCA1, either in mice or in human cancer cells. The gene's enormous size suggests that the BRCA1 protein is likely to have several functions, probably involving DNA repair, transcriptional regulation of genes, or others.

Ralph Scully, M.D., Ph.D., a researcher in David Livingston's lab at the Dana-Farber Cancer Institute, Harvard Medical School, Boston, who discovered that BRCA1 associates with Rad51, believes the only way to make progress is to develop a good genetic system.

"We need a tractable genetic system to test out key hypotheses in the field. For example, we need to be able to add back normal BRCA1 to cells lacking functional BRCA1 and show that a specific defect can be reversed," he said.

— Nancy J. Nelson