# Change in Cancer Gene Pinpointed

## *Just one small change can convert a normal cellular gene into one capable of causing cancerous transformation*

Efforts to understand how cancer originates were given a nudge a year or two ago when investigators found that a number of human and animal cancer cells contain genes that could be transferred into cultured cells, which consequently underwent cancerous transformation. Since then much effort has been directed toward determining the precise difference between the cancer genes and the normal cellular genes to which they were found to be related.

The answer is now in for one of the cancer genes, that found in a line of human bladder carcinoma cells, and its cellular counterpart. Robert Weinberg of the Massachusetts Institute of Technology (MIT) says, "The conclusion is that the only functional difference between the two genes depends on a single nucleotide change."

Moreover, increased production of the product of the altered gene is not necessary for transformation, a surprising finding in view of evidence suggesting that cancer results from increased expression of a normal gene, perhaps one involved in growth control or cellular differentiation. Here, at least, it is an altered gene product that appears to be at fault, in a manner as yet unclear.

To identify the gene change, Weinberg, with Clifford Tabin and Scott Bradley of MIT, collaborated with Ravi Dhar, Esther Chang, and Douglas Lowy of the National Cancer Institute (NCI), and Edward Scolnick, who recently moved from NCI to Merck Laboratories in Westpoint, Pennsylvania, in a series of what they call "mix-and-match" experiments.* In these experiments, the investigators systematically exchanged segments of the proto-oncogene and corresponding segments of the transforming gene. (Proto-oncogenes are normal cellular genes that have the potential to transform when appropriately activated.) The recombinant genes were then transferred into cultured NIH3T3 cells to see which had transforming capabilities. Introduction of a specific 350-nucleotide-long segment into the nontransforming gene caused its activation. The reciprocal experiment inactivated the oncogene.

Meanwhile, Mariano Barbacid and E. Premkumar Reddy of NCI had embarked on a similar series of experiments.† They identified a 930-nucleotide segment of the bladder oncogene as crucial for transformation. When both groups compared the crucial segments from the bladder oncogene and the proto-oncogene, they found that the sequences differed in the same nucleotide. A guanine was replaced by a thymine base. As a result, the twelfth amino acid residue from the amino terminal end of the protein product of the gene is converted from the normally present glycine to valine in the oncogene. Michael Wigler and his colleagues at Cold Spring Harbor Laboratories have also found this single change to be essential for transformation. There are other nucleotide differences between the two genes, but only the one matters for transformation.

The Weinberg group has compared expression of the oncogene in bladder carcinoma cells with that of the proto-oncogene in normal bladder epithelial cells. They found the proto-oncogene and the oncogene to be expressed in roughly comparable amounts at the level of both RNA and protein. In gene transfer experiments, only the oncogene transformed the recipient cells, but the normal gene product was, if anything, made in greater quantities.

Wigler reports similar findings in transfer experiments. "The NIH3T3 cells are expressing enormous amounts of normal protein and are not particularly transformed, but the same cells making much less of the altered protein are. The transforming protein is at least 100 times more potent in inducing the transforming phenotype."

This result was unexpected as other work has shown that increased expression of a proto-oncogene may cause transformation. For example, the oncogenes carried by the animal cancer viruses are actually cellular genes that the viruses have picked up during the course of infection. In cells infected by these natural recombinant viruses, more of the genes' products are made under the control of the viral regulatory elements than when they are under cellular control, and transformation results.

Moreover, the bladder oncogene was shown last spring to be closely related to the *ras* (for rat sarcoma) gene of Harvey sarcoma virus and its mouse counterpart, the *bas* (for BALB/c mouse sarcoma) gene. Lowy, Scolnick, and their coworkers have shown that the transforming potential of the cellular homolog of the *ras* gene can be activated by tying it to a viral regulatory sequence, which results in increased formation of its protein product. But increased expression does not appear to be a factor in transformation by the bladder oncogene. There may be two ways of activating the *ras* proto-oncogene—by increasing its expression or by introducing into it a single nucleotide change.

How this change causes transformation is unclear. The consequent alteration in the gene product may make it more efficient at doing what it normally does or it may acquire a new function. Resolving this issue will not be easy. Although Lowy and Scolnick have identified the protein products of the *ras* gene family, what they do remains a mystery.

Also surprising is the fact that a single nucleotide change can confer transforming capabilities on a gene. The development of human cancer naturally involves several steps and latent periods of 20 years or more. Barbacid says of the current work, "We have at best only a portion of a complex picture."

One possibility is that the change seen in the bladder oncogene is just one of a sequence of changes, all of which are needed for transformation. The gene may work when transferred into NIH3T3 because these cells are partially transformed, and thus particularly susceptible to transformation by some genes, although clearly not by all.

There remains the nagging question of whether the bladder oncogene does more than just evoke transformation in a laboratory assay, whether it in fact causes bladder cancer in real life. Although this is not yet known for certain, its viral homolog, the *ras* gene, is quite capable of causing cancers in the animals infected by appropriate viruses. Activation of the cellular proto-oncogene, whether as a result of increased gene expression or of an altered product, may do the same in humans.—**JEAN L. MARX**

*The experiments were described at a workshop on "Oncogenes: Evaluation of Basic Findings and Clinical Potential," which was held on 2 and 3 September at Roswell Park Memorial Institute and at a Science Writers' Seminar held at the National Institutes of Health on 14 October. A paper coauthored by C. J. Tabin, S. M. Bradley, C. I. Bargmann, R. A. Weinberg, A. G. Papageorge, E. M. Scolnick, R. Dhar, D. R. Lowy, and E. H. Chang appears in the 11 November issue of *Nature*.

†The paper coauthored by E. P. Reddy, R. K. Reynolds, E. Santos, and M. Barbacid is also in the 11 November issue of *Nature*.


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