Oncogenes and the Molecular Biology of Cancer

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Gene transfer experiments have made it possible to detect active transforming sequences in the DNA of various human tumor cells. These sequences, the cellular oncogenes, have been found in tumors as diverse as neuroblastomas, leukemias, carcinomas, and sarcomas. They appear to represent molecular determinants that participate in transformation mechanisms that are common to a wide variety of tumors. From this perspective, cancer seems to be a single, unitary disease, and not 100 different diseases, each characterized by a different type of tumor.

Several of these oncogenes have been isolated by molecular cloning (1–4). The most well studied of these is one from a human bladder carcinoma cell line, referred to as T24/EJ. Use of this oncogene in sequence hybridization has revealed a property common to this and the other cellular oncogenes: these oncogenes derive from closely related antecedent genes residing in the normal cellular genome. It appears that these normal genes, often called "proto-oncogenes," become converted into oncogenes via processes of "somatic mutation." This last conclusion remains equivocal, if only because there is little direct evidence to date showing comparison of the oncogene of a tumor with the homologous sequences of closely lying normal tissue.

A variety of mechanisms might be imagined to be responsible for the conversion of a proto-oncogene into an active oncogene. In the case of the bladder carcinoma oncogene, a remarkably simple mechanism was revealed within the last year. Comparison of the bladder carcinoma oncogene with its normal antecedent showed that a single nucleotide alteration, occurring in the protein-encoding portion of the gene, was responsible for the activation. This point mutation affected the twelfth amino acid of the encoded 21,000-dalton protein causing the replacement of a glycine by a valine (5–9). Since little is known about the functioning of this protein, it is impossible at present to explain how such a subtle alteration can have such profound effects on this protein, and in turn, on cellular physiology.

This bladder carcinoma proto-oncogene has homologs in the DNA of all vertebrates. Its evolutionary origins can, however, be traced back much further. Sequence hybridization has found homologs of this and other proto-oncogenes in the DNA of *Drosophila melanogaster* (10). This leads to the realization that these genes are of extremely ancient lineage—their precursors were already present in similar form in the primitive metazoans that served as common ancestors to chordates and arthropods. Such conservation indicates that these genes have served vital, indispensable functions in normal cellular and organismic physiology, and that their role in carcinogenesis represents only an unusual and aberrant diversion from their usual functions.

Because these oncogenes are of truly cellular origin, it is perhaps paradoxical to learn that they were originally discovered, not via gene transfer of cellular DNA, but through their association with transforming retroviruses. Thus, we now know that the bladder carcinoma oncogene is the same oncogene that had previously been described as part of the genome of Harvey rat sarcoma virus. An oncogene found by gene transfer in lung and colon tumors was the same oncogene as that found in Kirsten rat sarcoma virus (11, 12). In the case of both of these viruses, it is known that their oncogenes were derived by acquisition of a proto-oncogene from the genome of the rat. Therefore, a proto-oncogene can become activated in two independent ways: by somatic mutation (in humans) or by a retrovirus (in rodents).

The oncogenes described above are members of the *ras* gene family, originally named because of associations with these rat sarcoma viruses. The gene family has three members that are known to be active in tumors of nonviral etiology: Ha-*ras*, Ki-*ras*, and N-*ras*. Among the human tumors that carry oncogenes, the Ha-*ras* has been found in the above-mentioned bladder carcinoma and in skin carcinomas; the Ki-*ras* in various colon, lung, and pancreatic carcinomas, and several sarcomas; and the N-*ras* in a variety of hematopoietic malignancies, a neuroblastoma, sarcomas, and a colon carcinoma (13, 14).

These data reveal two aspects about the relationship of each oncogene with these various tumors. First, each proto-oncogene can become activated in a variety of tissue types. And second, once activated, the resulting oncogene apparently is able to help transform the cells of these various tissues. There is yet a third related conclusion that comes from these data: a given type of tumor cannot, with any certainty, be traced back to a known oncogene. For example, a colon carcinoma will most likely carry a Ki-*ras* oncogene, but may on occasion be found to carry an active N-*ras* allele within its cells.

Although these *ras* genes appear diverged from one another

This work was presented in a symposium on Activation of Cellular Oncogenes at the Twenty-second Annual Meeting of The American Society for Cell Biology (Baltimore, Maryland, December 1982).

by hundreds of millions of years of evolution, their encoded gene products are remarkably similar, having as much as 90% identity in amino acid sequence (15, 16). This speaks to the importance of their structure in normal cellular physiology. Any substantial deviations from an earlier ancestral sequence has obviously not been tolerated during evolution.

Current research efforts are designed to relate these molecular observations with the biological complexities of the carcinogenic process. One area of interest focuses on the apparent contrast between the results of gene transfer (transfection) and the known multi-step nature of carcinogenesis in vivo. The oncogene transfection has involved use of NIH3T3 mouse fibroblasts, which are converted into tumor cells in a one-step process, and it is difficult to reconcile this observation with the multiple hits thought to be required in the course of natural tumorigenesis.

One resolution of this paradox has come from the realization that the NIH3T3 cells, being established and immortalized in vitro, are themselves highly abnormal. Perhaps they have undergone many predisposing alterations during their adaptation to continuous culture, and are now primed for the final hit—the acquisition of a *ras* oncogene. This idea is vindicated by recent work in our laboratory, which shows that embryo fibroblasts, unlike the extensively passaged NIH3T3 cells, are not transformed into tumor cells by an introduced *ras* oncogene (17).

Such work indicates that a *ras* oncogene requires other cooperating alterations to the cell for the full phenotype of transformation to be realized. One such cooperating alteration is supplied by the establishment/immortalization process. An alternative is provided by other oncogenes. For example, if a *ras* oncogene is introduced together with a second oncogene, such as a *myc* cellular oncogene or polyoma virus large T gene, then transformation of the embryo cells is achieved. This begins to provide a molecular model of multi-step carcinogenesis, following which several of the critical steps involve activation of distinct cellular oncogenes (e.g., *ras*, *myc*). Moreover, it shows that oncogenes are not all functionally identical. Instead, it seems that different oncogenes have distinct and occasionally complementary roles to play in the transformation process.

These results only begin to explain the cancer process. Some basic questions remain unresolved. Why do only 20% of human tumors tested yield oncogenes upon transfection? How many oncogenes need cooperate to create most naturally occurring tumors? And perhaps most intractable, how do the oncogene proteins function to exert such global effects on cellular physiology? We have not even begun to find the answers to these challenging questions.

Received and accepted for publication 23 August 1983.

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