Oncogene Action Probed

Interest in oncogenes, genes that cause the cancerous transformation of cells, shows no signs of lagging. The "Third Annual Meeting on Oncogenes," which was sponsored by the Frederick Cancer Research Facility of the National Cancer Institute and held on 7 to 11 July, is a recent case in point. Despite the hot and steamy weather, the meeting attracted an overflow crowd of some 500 people to the largely unair-conditioned campus of Hood College in Frederick, Maryland.

Oncogene research is too varied to summarize as a whole, but some trends are evident. The number of oncogenes continues to climb and now stands at about 50, up from 20 or so just 3 or 4 years ago. Evidence is still accumulating in support of the idea that oncogenes are derived from normal growth and developmental control genes that have somehow gone awry. Progress in dissecting the functional and regulatory sequences of the genes is also evident. Still largely lacking, however, is a good understanding of how the genes produce their cellular effects.

New Family of Growth Factor Genes Identified

When a new oncogene is identified these days, there is little doubt that it will prove to be related to one or another of the genes that control cell growth or differentiation. The main question concerns which growth control gene will turn out to be the relative. In keeping with this situation, investigators have found over the past few months that no less than three recently identified oncogenes encode proteins that are structurally similar to the fibroblast growth factors (FGFs).

At very least, the work is defining a new family of growth factors, the full significance of which has yet to be established. At most, it might help to explain the development and progression of the cancers in which the oncogenes were found. These are a diverse group, including a Kaposi's sarcoma, a cancer that is now prominent because it frequently occurs in patients with AIDS (acquired immune deficiency syndrome); human stomach and bladder cancers; and a mouse mammary cancer.

The oncogene from the Kaposi's sarcoma has been isolated and its nucleotide sequence determined by Pasquale Delli Bovi, Claudio Basilico, and their colleagues at New York University School of Medicine. This gene appears to be the same as the *hst* (for human stomach) oncogene, which was previously isolated from two human stomach cancers and also from normal stomach tissue of one of the cancer patients by Masaaki Terada's group at the National Cancer Center Research Institute in Tokyo. The proteins encoded by the *hst* and Kaposi's sarcoma oncogenes both contain 206 amino acids. Their sequences are about 45% identical to that of basic FGF and show a lesser degree of resemblance to the acidic FGF structure.

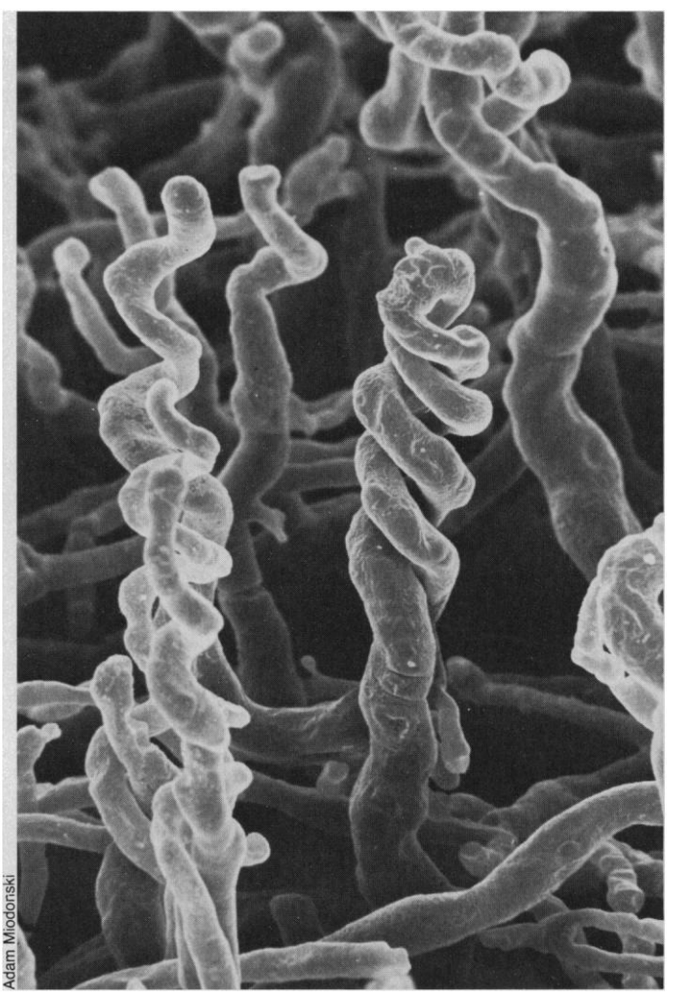
According to Gordon Peters, Clive Dickson, and their colleagues at the Imperial Cancer Research Fund Laboratories in London, *int-2*, an oncogene that was originally detected in mouse mammary cancers caused by the mouse mammary tumor virus (MMTV), also belongs to the FGF family. It, too, resembles basic FGF more closely than acidic FGF. And the third oncogene found to encode an FGF-like protein was isolated from human bladder cancer cells by Xi Zhan and Martin Goldfarb of Columbia University College of Physicians and Surgeons. "It's a remarkable coincidence that three new members of this family were identified at the same time by gene homologies," Goldfarb says. The current total of family members now stands at five, including the two FGF genes themselves.

The results are intriguing in view of findings that the FGFs not only stimulate the division of certain cell types, but are also potent angiogenic agents that foster the growth of new blood vessels. Such new blood vessel formation is essential if solid tumors are to develop to a significant size. Production of the FGFs or related proteins might therefore contribute to the development of solid cancers both because of the proteins' effects on cell division and on angiogenesis.

Basilico cautions, however, that there is as yet no direct evidence that the oncogenes isolated from the Kaposi's sarcoma and the

New blood vessel growth in a human tumor

The growth of solid tumors depends on the formation of new blood vessels to deliver nutrients and remove wastes. The current work raises the possibility that activation of FGF-related oncogenes might contribute to such angiogenesis. For this scanning electron micrograph, the cells of the tumor, a cancer of the larynx, have been removed to reveal the blood vessels. [Arch. Otolaryngol. *106, 321 (1980)*]



Adam Miodonski

other human cancers have anything to do with the etiologies of the cancers in question. All of the oncogenes were detected by their ability to cause the cancerous transformation of mouse fibroblasts growing in culture, but this does not necessarily mean that they have a similar effect in their cells of origin.

Nevertheless, results reported by Robert Gallo of the National Cancer Institute at the III International Congress on AIDS, which was held during the first week of June in Washington, D.C., are consistent with the possibility that activation of FGF or related genes contributes to the development of Kaposi's sarcomas. Gallo and his colleagues find that Kaposi's sarcoma cells produce growth and angiogenic factors. Moreover, when the human tumor cells are transplanted into nude mice, the animals develop typical Kaposi's sarcoma tumors, but of mouse, not human, origin. The results suggest that the human cells are producing something that triggers the growth of the mouse tumors.

An additional indication that angiogenic growth factors might contribute to cancer development comes from Towia Liberman of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, and her colleagues at Meloy Laboratories in Rockville, Maryland, and Springfield, Virginia. They find that human glioma cells express the acidic FGF gene and secrete acidic FGF, or a very similar growth factor. Formation of new blood vessels is a common early sign that gliomas, which are cancers of nervous tissue, are undergoing malignant progression.

Oncogenes are derived from normal cellular genes that undergo some change that either causes them to produce an abnormal product or disrupts their control so that they are expressed inappropriately, making their products in excessive amounts or at the wrong time. The *int-2* gene, for example, is not ordinarily active in adult mouse cells, but is apparently turned on in mammary cells when MMTV sequences that enhance gene expression insert near it in the genome.

The functions of the genes that gave rise to the FGF-related oncogenes are currently unknown, although there is evidence implicating *int-2* as a developmental control gene. Harold Varmus, Gail Martin, and their colleagues at the University of California Medical Center in San Francisco have found that the gene is active in mouse embryos at 7.5 days of gestation, but is essentially turned off by day 8.5 and appears to remain inactive thereafter. "*int-2* is a prototype of a family of oncogenes that encode growth factors that function in some way during embyrogenesis," Peters suggests.

The embyronic functions of *int-2* probably do not include angiogenesis, he notes, because its pattern of expression is not consistent with such a role. The activity of the gene is largely localized to endodermal cells that eventually give rise to internal organs such as those of the digestive and respiratory systems and to certain mesodermal cells.

A great deal generally remains to be learned about the activities of *int-2* and the other newly discovered FGF-related oncogenes, but the family seems certain to attract a great deal of research attention.

ras Oncogene Activated in Human Colon Cancers

Efforts to detect activated oncogenes in human cancers have usually yielded disappointing results. They have been found in only a few percent of the solid tumors that constitute the great majority of human cancers, findings that have cast doubt on the idea that oncogenes might contribute in a major way to the development of those cancers. Now, however, researchers have shown that about 40% of the colon cancers removed during surgery contain active *ras* oncogenes. Morever, the results indicate that the gene activation occurs relatively early in the development of the tumors.

The current results come from Manuel Perucho, Kathleen Forrester, and their colleagues at the State University of New York at Stony Brook and, independently, from Johannes Bos at the State University of Leiden in the Netherlands, Bert Vogelstein at the Johns Hopkins University School of Medicine in Baltimore, and their colleagues.

The *ras* oncogenes, like other oncogenes, are derived from normal cellular genes controlling cell growth and differentiation. Both groups used methods that allow them to detect the specific mutations that convert the normal genes to their oncogenic counterparts. These are simple point mutations that change a single amino acid, usually at position 12, 13, or 61, in the proteins encoded by the genes.

The Stony Brook workers found oncogene-activating *ras* mutations in 26 of the 66 cancers examined and the Leiden–Johns Hopkins group found them in 11 of the 27 colon carcinomas they studied. "Nobody thought that it [the mutation rate] would be as high as 40%," Perucho says. The mutation most frequently seen is in the codon for amino acid 12 of the Ki-*ras* oncogene, which is so called because it was originally identified in the Kirsten sarcoma virus, a mouse cancer virus. The oncogene-activating mutations may be contributing to the formation of malignant tumors by conferring a selective growth advantage on the cells in which they occur.

The use of methods that detect the specific *ras* mutations may account for the difference between the high percentage of colon cancers found to contain the active oncogenes in the current work and the much lower percentages seen in the small number of previous studies. The earlier studies all depended on the "transfection" assay for oncogenes in which tumor cell DNA is transferred into cultured mouse cells to see whether the recipient cells undergo cancerous transformation.

To obtain a positive result with the transfection assay, an intact oncogene must be transferred, but the tumor cell DNA often becomes slightly degraded during tissue handling and DNA extraction and transfer. This could lead to false negative findings, especially with large genes like Ki-*ras*, which is about 45 kilobases long. Partial DNA degradation would be much less likely to interfere with the detection of the short mutated *ras* sequences.

The tissue samples from some of the patients in the current studies included the abnormal but not yet malignant growths called villous adenomas in addition to the frankly cancerous material. Both the Stony Brook and Leiden-Hopkins groups found that the oncogenic *ras* mutations are often present in the villous adenomas as well as in the cancerous lesions, but not in normal tissue from the patients. Although other work had suggested that *ras* activation is a late event in tumor development, these results strongly indicate that it is a relatively early event, occurring before the onset of true malignancy.

The method used by the Stony Brook group to detect the presence of the mutated *ras* genes also enables them to estimate the relative expression of the mutant and normal genes in the cancer cells. "There are no clear differences," Perucho says. "The mutant and normal genes are expressed to the same degree." This implies that the mutants are dominant in that their effects can occur despite the presence of the functional normal counterparts.

The implication is consistent with current views of how *ras* oncogenes transform. The mutations apparently cause changes that maintain the *ras* proteins in a constantly active state, rather than allowing them to be turned off at the appropriate times. Such continuous activity might well prove dominant. ■ JEAN L. MARX

ADDITIONAL READING

J. L. Bos *et al.*, *Nature (London)* **327**, 293 (1987).
K. Forrester *et al.*, *ibid.*, p. 298.