Oncogenes Amplified in Cancer Cells

Some cancer cells contain extra copies of specific oncogenes, including the myc *gene and its relatives. Does this contribute to the development of cancers?*

The circumstantial evidence implicating oncogenes—genes that cause the malignant transformation of cells—in the genesis of naturally occurring human cancers has been building rapidly in the past few years. A recent case in point is the discovery by several investigators that some cancer cells contain many extra copies of particular oncogenes. The result implies that the oncogene amplification played a role in the development of the cancers, possibly because it resulted in excess production of the gene products. Even if the amplification is not one of the initial steps by which cells become cancerous it may at least contribute to the progression of cancers to a more highly malignant form.

Many tumor cells show evidence of gene amplification. "This is a much more common feature of tumors, particularly of solid tumors, than was once thought," points out J. Michael Bishop of the University of California School of Medicine in San Francisco. Gross chromosomal abnormalities that are indicative of gene amplification are being found in tumor cells with increasing frequency. The abnormalities include homogeneously staining regions (HSR's), which can occur on chromosomes that are otherwise normal in appearance, and extremely small pieces of chromosomes called double minutes, large numbers of which may be present in each cell. Moreover, gene amplification may also occur in cells that do not have such visible changes.

Bishop and his San Francisco colleagues, Kay Alitalo, Manfred Schwab, and Harold Varmus, began looking for oncogene amplification in cancer cells as part of their efforts to determine what activates the transforming potential of the genes. So far there are 20 or so oncogenes, most of which were originally identified in viruses that cause cancers in laboratory animals. The oncogenes are not themselves of viral origin, however. They are cellular genes that were picked up by the viruses during the course of infection.

Because the structures of the cellular counterparts of the viral oncogenes are very closely conserved throughout evolutionary history, the supposition is that the cellular genes have fundamental functions in regulating normal cell division or differentiation. The question then is what causes these genes to become oncogenic and produce the uncontrolled growth and aberrant differentiation seen in tumor cells. There are two possible answers to the question, which are not mutually exclusive. The gene product may be made in excessive amounts or at the wrong time or the gene may be altered in some way so that an abnormal product is made. Establishing which of these possibilities is the important one for a given oncogene has been difficult.

To determine whether oncogene expression is elevated in cancer cells, a comparison must be made with the corresponding normal cells, which may not be available. And if there is an abnormality, it is not easy to determine whether this is primary or secondary, Bishop notes. "If the expression is elevated, it could mean that the increased expression is part of the malignant phenotype."

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Instead of looking randomly for increased oncogene expression, the San Francisco group turned to tumor cells that already showed evidence of gene amplification. In other situations the product of the amplified gene is made in increased amounts. For example, according to Robert Schimke of Stanford University, amplification of a gene coding for an enzyme that inactivates a chemotherapeutic drug results in increased production of the enzyme by cancer cells. Consequently, the cells become resistant to the drug.

In addition, Robert Gallo and his colleagues at the National Cancer Institute (NCI) and Stephen Collins of the Veterans Administration Hospital in Seattle and Mark Groudine of the Fred Hutchinson Cancer Research Center, also in Seattle, had already shown that the *myc* oncogene, which is so called because it was identified in the virus MC29, is amplified in a line of cultured human leukemia cells. Expression of the gene in the cells is very high (as measured by production of the corresponding messenger RNA). The Gallo group found that the primary tumor cells from which the cultured line was derived also carried extra copies of the gene, indicating that the amplification had occurred in the patient and was not an artifact of culturing.

Schwab and his colleagues concentrated in their early stages of their work on neuroblastomas, tumors of nerve cells that frequently carry HSR's and double-minute chromosomes. "There we got lucky," Bishop says, "because the amplified oncogene we found had not been previously discovered, but was related to *myc*." If the amplified gene, which they designated N-*myc* because it was first identified in the neuroblastoma cells, had not been related to a known oncogene for which the workers had a probe, it would have escaped detection.

At about the same time, two other groups—one including Peter Melera and his colleagues at Memorial Sloan-Kettering Cancer Center and the other including Samuel Latt of Harvard Medical School, Frederick Alt of Columbia University College of Physicians and Surgeons, and their colleagues—also found evidence of gene amplification in neuroblastoma cells. The amplified genes turned out to be either identical, or at least very similar, to the N-*myc* gene identified by the San Francisco workers. "The genes are very closely related, but there may be subtle differences in sequences," Latt says. His group has also bestowed the designation N-*myc* on the amplified gene.

The N-*myc* amplification appears to be a frequent occurrence in neuroblastoma cells. Among them, the various groups have screened some 20 lines of cultured cells. Almost all have had extra copies of the gene. In addition, the San Francisco workers have found the N-*myc* gene to be amplified in four of six primary neuroblastoma tumors that were surgically removed from patients who had not yet received any chemotherapy.

One of the few neuroblastoma lines that did not contain extra N-*myc* copies has been found by other investigators to contain a newly discovered member of the *ras* gene family in its activated form.

Activation of the *ras* gene may be an alternative to N-*myc* amplification in the development of a neuroblastoma.

There is also the possibility that two or more oncogenes may work together to cause cancer (*Science*, 11 November, p. 602). Investigators are now trying to determine whether the neuroblastoma and other types of tumor cells in which amplified genes have been identified carry additional activated oncogenes.

Lung cancer cells are among the other types of tumor cells having amplified oncogenes, according to John Minna and his colleagues at NCI and the Naval Medical Center in Bethesda, Maryland. They have evidence suggesting that *myc* gene amplification is correlated with the expression of a more malignant form of small cell carcinoma of the lung (SCLC).

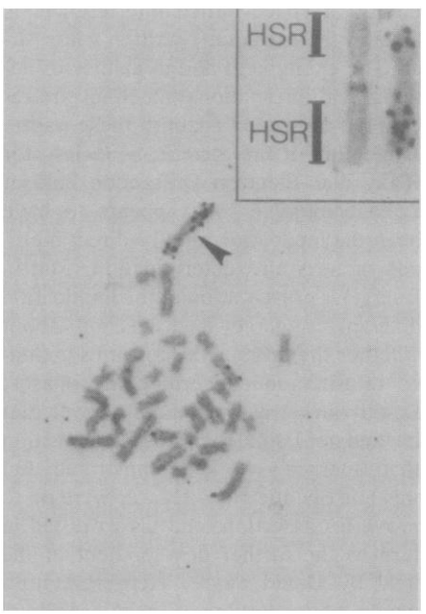
SCLC accounts for about 25 percent of all lung cancers. Tumor cells that are taken from about two-thirds of the patients and grown in culture are of the classic type, having features that suggest a greater degree of differentiation than cells taken from the remaining one-third. This second type of cell, which is given the designation "variant," grows more rapidly in culture and is more resistant to radiation than the other type. Patients who have the variant type of tumor cells have an even worse prognosis than those with classic SCLC.

The Minna group found that 8 of 18 lines of human lung cancer cells contained extra *myc* gene copies. The eight included all five lines of variant SCLC cells, which had from 20 to 75 times as much *myc* DNA as normal cells. Two of the eight nonvariant SCLC lines had lesser degrees of *myc* gene amplification, and the gene was also amplified in one of five lines of lung cancer that were not of the SCLC type. "We suggest that a sizable number of lung cancers have progression that results from selective amplification of the *myc* gene," Minna says.

Although the Minna group has not yet looked at primary lung tumors, they see the amplification in cultured cells at the earliest time tested, indicating that the change had already occurred in the patient. How early amplification might occur in the development of SCLC or other cancers is unclear. It might be a relatively late change by which the less aggressive SCLC cells convert to the more malignant variant. Alternatively, the variant cells with their extra *myc* gene copies may be a subpopulation in the original tumor that subsequently outgrows the less aggressive cells. Tumors generally consist of heterogeneous populations of cells.

The San Francisco group has also found an amplified *myc* gene in a line of human neuroendocrine tumor cells. But members of the *myc* gene family are not the only oncogenes that may be amplified. According to the San Francisco workers, a line of human colon carcinoma cells has extra copies of the *myb* gene, which may be distantly related to *myc*, and a line of cells derived from a mouse tumor of the adrenal cortex carries multiple copies of a *ras* gene. In addition, Collins and Groudine have found the *abl* oncogene to be amplified in a line of chronic myelogenous leukemia (CML) cells.

There may be a relation between oncogene amplification and translocation of the genes to abnormal chromosomal locations. Tumor cells from more than 90 percent of CML patients carry an aberrant chromosome, the Philadelphia chromosome, formed by the attachment of a portion of chromosome 22 to chromosome 9. Recently a group of investigators from the Netherlands, England, and the United States showed that in such cells the *abl* gene has been moved from its normal location on chromosome 9 to chromosome 22, proving that there is, as suspected, a reciprocal exchange of material between the two chromosomes.



Location of an amplified* myc *gene

The micrograph shows some of the chromosomes of a neuroendocrine tumor cell after they have been exposed to a radioactively labeled probe for the myc *gene. The arrowhead points to a marker chromosome, which bears homogeneously staining regions and, in this micrograph, several dark grains indicating binding of the probe. The inset compares the labeled chromosome with the corresponding unlabeled chromosome and shows that the locations of the* myc *gene copies correspond to those of the HSR's. [Reprinted with permission from K. Alitalo* et al, Proc. Natl. Acad. Sci. U.S.A. ***80****, 1707 (1983)]*

The CML line used by Collins and Groudine carries a Philadelphia chromosome and they observed that an antibody chain gene, which is normally located on chromosome 22, is amplified to the same extent as the *abl* gene, suggesting that the two may have been amplified together as a consequence of the translocation. Moreover, Latt, Alt, and their colleagues and the San Francisco group have mapped the N-*myc* gene (or genes) to chromosome 2, but the amplified gene copies are found in the HSR regions of other chromosomes or in the double minutes. Melera says, "The general consensus is that in neuroblastoma cells translocation seems to be associated with amplification."

All the groups have found that amplification of the genes is accompanied by their increased expression as determined by increased production of the corresponding messenger RNA's. "The level of expression is concordant with the amplification," Bishop says. This suggests, but does not prove, that it is the increased expression that is involved in the development of the cancers. Changes in structure have not been ruled out.

In fact, the San Francisco group has evidence that an amplified, altered *myc* gene is expressed in one line of neuroendocrine cells, although work with another line suggests that the alteration is not a prerequisite for expression. Melera and his colleagues also find evidence of altered structure in the amplified N-*myc* gene, although more work is needed to confirm this. Finally, Collins and Groudine suggest that the *abl* gene may be altered in the CML cells. "The cells have a new size of messenger RNA as well as more of it," Groudine says.

More work is needed to determine how extensively amplified oncogenes occur in tumor cells. They may be present in only a small minority. The San Francisco group has screened over 100 cell lines for increased expression of many of the known oncogenes and has found it in only a few. Since increased expression accompanies amplification, this indicates that most of the lines do not have extra copies of the oncogenes examined.

Oncogene amplification does not have to occur in all tumor cells for it to contribute to carcinogenesis. There may be many different routes by which a cell may be transformed to malignancy and amplification may not always be necessary. But its consistent presence in the neuroblastoma and variant SCLC cells suggests that it is important for at least some forms of cancer.—**Jean L. Marx**