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Revealing New Clues About the Spread of Colon Cancer

Researchers have discovered a gene that appears to allow colon tumors to spread to other parts of the body, a process called metastasis. The gene codes for an enzyme that may be central to the metastatic process, suggesting the possibility that the enzyme could be targeted by drugs to block the spread of colon cancers. Metastasis is the primary cause of death from colon cancer.

In an article published online in the October 12, 2001, edition of *Science*, Howard Hughes Medical Institute investigator [Bert Vogelstein](#) and colleagues at the Johns Hopkins University Oncology Center reported identifying the gene, called *PRL-3*.

The researchers identified *PRL-3* after developing a profile of gene expression in cells they microdissected from cancers that had metastasized to the liver. The genetic profile was developed using SAGE (serial analysis of gene expression), a technique invented by the researchers to determine the level of expression of genes. In SAGE, the enzyme reverse transcriptase is used to produce complementary DNA from the messenger RNA (mRNA) derived from cells under study. The DNA is then snipped at a defined position, creating a unique identifier "tag" that corresponds to a single gene. The researchers can then analyze the number of unique tags present in their sample and deduce how much mRNA exists for each gene -- a measure of gene activity.

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Vogelstein said that the group's initial analyses of gene expression in the metastatic tissue yielded confusing results. "When we initially took metastatic lesions from patients, purified the RNA, and then looked at their gene expression profiles, we found many of the [gene] transcripts were clearly derived from non-neoplastic cells," he said. "The problem is that tumors are composed of multiple cell types; not just the neoplastic cells. Liver metastases from colon cancers contain a supporting stroma tissue, as well as inflammatory cells and normal liver cells," he said.

The researchers devised a purification procedure that enabled them to isolate only the metastatic cells. This technique involved separating cells based on their attraction to an antibody specific for colorectal epithelial cells in liver tissue, which constitute the metastatic cancer cells. "Once we did this separation," said Vogelstein, "the gene expression patterns became much clearer and more reliable."

SAGE analysis of these isolated cells revealed 38 gene transcripts that appeared to be enriched in the metastatic cells, indicating that they were switched on during metastasis. Although all the genes were elevated in some of the metastatic lesions compared to normal colorectal epithelium and non-metastatic cancers, only one, called *PRL-3*, was consistently overexpressed.

"We were surprised and pleased that several of the transcripts were overexpressed in many metastases," said Vogelstein. "And it was particularly striking that this one gene, *PRL-3*, appeared to be quite consistently overexpressed."

Vogelstein and his colleagues were especially interested in *PRL-3* because it codes for an enzyme. "Most genes implicated in solid tumors are tumor suppressor genes whose activities are switched off during tumor formation. The proteins encoded by these genes are not good targets for potential drug therapies because drugs generally inhibit enzymatic activities. You cannot inhibit an activity that is not present in the tumor cell because it has been inactivated. On the other hand, there aren't many enzymes that have been shown to be overexpressed in cancers in a pathogenic way and related to metastasis," said Vogelstein. *PRL-3* codes for a tyrosine phosphatase, an enzyme that likely controls the activity of other proteins by removing a phosphate from them.

When the scientists quantified the level of *PRL-3* expression in normal, non-metastatic and metastatic tissues, they found clear differences. "This gene was expressed at very low or undetectable levels in normal colon epithelium," said Vogelstein. "It was expressed at low levels in the early stages of colorectal neoplasia. And its expression was clearly much higher in metastatic lesions from the liver."

Importantly, the scientists were also able to compare *PRL-3* expression in normal epithelium, primary cancers and metastatic lesions from the same patients. Such comparison eliminates the potentially misleading differences when tissues from different patients are compared. "In each of the six cases studied, *PRL-3* expression was quite a bit higher in the metastases than in the primary tumor," said Vogelstein.

The scientists also discovered that the higher levels of *PRL-3* expression they measured were associated in a few cases with a process known as gene amplification -- in which overexpression of a gene is caused by a large increase in the number of copies of the gene. Gene amplification is a characteristic mechanism by which the overexpression of growth-regulating genes occurs in human cancers.

"The discovery that this gene was not only overexpressed but also amplified provided very strong evidence for causality of this gene in the metastatic process," said Vogelstein.

According to Vogelstein, the scientists will now search for the biochemical and physiologic roles of *PRL-3* in colon cancer metastasis. One of the goals of future studies will be to launch a search for molecules that inhibit the phosphatase function of *PRL-3* and to discover whether this inhibition thwarts metastasis.