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Genetic Program Builds the Pipeline that Nourishes Tumor Growth

Detailed analysis of the activity levels of thousands of genes from blood vessel cells isolated from normal and cancerous tissue has led to the identification of a genetic program that constructs the pipeline that supplies blood and nutrients to colorectal tumors.

Although the research was done primarily in cells derived from patients with colorectal cancer, the researchers believe that this genetic script may be a common one that runs when tumors need to supply themselves with the factors needed to sustain growth. The findings were reported in the August 18, 2000, issue of the journal *Science* by a research team that included Howard Hughes Medical Institute investigator Bert Vogelstein, Kenneth Kinzler and Brad St. Croix, who are at The Johns Hopkins University Oncology Center. The Hopkins team collaborated on the research with scientists from Duke University Medical Center.

"For close to twenty years we have been studying primary tumors from patients," said Vogelstein. "What's new is that we are now studying the non-neoplastic, or non-cancerous, components in this case the endothelial cells on which tumors depend for their blood supply and nutrients. The idea that these cells are critical to tumor growth was first suggested by Judah Folkman at Harvard," said Vogelstein.

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The researchers started their studies by purifying endothelial cells from normal and cancerous tissue of patients. The researchers next analyzed the amount of messenger RNAs (mRNAs) produced by genes expressed in endothelial cells. The mRNA level of each gene gives the researchers a good

estimate of the activity of each gene.

The scientists used SAGE (serial analysis of gene expression), a technique they invented to determine the activity level of genes. In SAGE, the enzyme, reverse transcriptase, is used to produce complementary DNA from the 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.

The researchers generated about 100,000 tags from endothelial cells in normal and cancerous tissue. These tags corresponded to more than 32,500 unique mRNA transcripts. Many endothelial cell genes showed unique patterns of activity in one or the other type of tissue. The researchers were intrigued by a group of 46 genes, which they called tumor endothelial markers (TEMs), because these genes were elevated tenfold or more in tumor endothelium.

"We were really surprised at the number of differences we found," said Vogelstein. "And, we were delighted in the sense that many of these 46 genes can now become reasonable targets both for research and perhaps for new diagnostics and therapeutics."

The researchers found that at least seven of the 46 genes appear to be involved in forming or extending the scaffolding that cells use in creating new blood vessels. Thus, these genes are likely to be necessary for the growth of new blood vessels produced by tumors, said Vogelstein.

To validate that these genes were, indeed, activated in other types of cancer, the researchers measured the activity of nine of the genes in two other patients with different forms of cancer. Again, they found that these genes were expressed in high levels in tumor cells, but not in normal cells.

The activity levels of a select group of TEMs in cancers of the bone, liver, lungs, pancreas and brain were significantly higher than the activity levels of those genes in normal endothelial cells. "This is a very promising finding because it means that if these genes become therapeutic targets, for example, by creating antibodies against their proteins, those antibodies would not only be potentially useful for colon cancers, but probably for most cancers," said Vogelstein.