The 23-Million-Dollar Quest Pays Off

Researchers at Harvard Medical School have isolated and characterized a human tumor protein called angiogenin that stimulates the growth of blood vessels. In addition, they have cloned the gene that encodes the protein and determined its nucleotide sequence. The results, which were published in a series of three papers in the 24 September issue of *Biochemistry*, provide the first direct proof for the existence of a specific protein that participates in blood vessel formation.

The findings also open the way to a better understanding of the development both of normal organs and of solid tumors, including breast, lung, and colon cancers, which are the major malignancies afflicting human populations. Ultimately, the work may provide practical benefits, such as ways to stimulate blood vessel growth therapeutically, to facilitate wound healing, for example, and also new methods for detecting and treating cancers.

The isolation of angiogenin and its gene is the culmination of a quest that began about 10 years ago when Monsanto Company agreed to give Harvard Medical School some $23 million in endowment and research moneys over a 12-year period (*Science*, 25 February 1977, p. 759). The funds were to support work by Harvard's Judah Folkman and Bert Vallee on the then elusive "tumor angiogenesis factor" (TAF).

Earlier work by Folkman had suggested that cancer cells produce TAF, which serves to foster the growth of blood vessels into developing tumors. Without TAF, the hypothesis went, solid cancers would not have an adequate blood supply and could therefore not grow. However, efforts to purify TAF and characterize it biochemically had largely met with frustration, which raised doubts about its existence.

In the new effort, biochemist Vallee, who had long been interested in organ development, was to direct a major endeavor aimed at pinning down TAF at last. Monsanto Company, in exchange for its support for the research, was to receive patent rights to the agent—if it indeed existed. The company would also gain a great deal of experience in cell culture and other techniques crucial to the then incipient science of biotechnology.

Originally Vallee and his colleagues attempted to isolate TAF from the same rat carcinoma cells used by Folkman for his investigations. They made some progress but decided about 4 or 5 years ago to switch to human tumor cells, largely because there seemed to be greater potential for clinical application if a human angiogenesis factor could be isolated, according to James Riordan of the Vallee group. The cells they used were derived from a human colon cancer, coincidentally of the same type for which President Reagan was recently treated.

The Harvard workers eventually isolated about 1 milligram of pure human angiogenin from some 2000 liters of the fluid in which the cancer cells were grown. The protein is extremely potent in eliciting blood vessel growth; quantities in the femto- to picomolar range are sufficient. "The results essentially confirm the belief that there are molecules of this type capable of inducing organ development," Riordan reports.

The molecule appears not to be the same as the TAF studied by Folkman, however. Angiogenin contains 123 amino acids, has a molecular weight of 14,400, and is secreted by cancer cells into the culture medium. In contrast, partially purified TAF was obtained from cell nuclei and appeared to have a molecular weight of 100,000, although the smaller protein might have been present in this preparation.

Once Riordan, Vallee, and their colleagues determined the amino acid sequence of angiogenin, probes for detecting and cloning the gene could be constructed. The Harvard workers collaborated on the cloning work with Kotoku Kurachi and Earl Davie of the University of Washington. The angiogenin gene proved to be one of a small number of mammalian genes that lack introns, sequences that are edited out of the corresponding messenger RNA and are not represented in the protein structure. The significance of the angiogenin gene's lack of introns is unclear.

Somewhat surprisingly, the amino acid sequence of angiogenin turned out to be 35 percent identical to that of ribonuclease, an enzyme that breaks down nucleic acids. In particular, the amino acids that are needed for the catalytic activity of the enzyme are maintained in angiogenin. Conservation of the structure implies that angiogenin might also have some kind of hydrolytic activity, the investigators speculate, but this remains to be demonstrated. The Harvard workers have not been able to detect ribonuclease activity in angiogenin, nor does the enzyme appear to promote blood vessel growth. "I don't know of a similar situation where molecules have that much similarity but have such different functions," Riordan notes.

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With the cloned angiogenin gene in hand, it should be possible to produce ample quantities of the material for studying blood vessel formation. Results already indicate that the protein is all that is needed to initiate the process. Its activity is probably not limited to cancer cells. The gene is expressed in normal fetal liver, for example. Moreover, angiogenin may be necessary to maintain blood vessels even after they are formed. An implant of the protein in rabbit corneas causes the ingrowth of blood vessels, which deteriorate when the implant is removed.

A major reason for interest in angiogenic materials is the possibility that they might find therapeutic application. Because they are secreted by solid tumors, they might, for example, serve as the basis for a test for detecting the cancers. They might also be useful in cancer therapy. An agent that blocks angiogenin activity is potentially capable of preventing the growth of solid tumors, although such agents also have the potential to produce severe side effects if angiogenin has a normal role in maintaining blood vessels. All these possibilities remain to be explored, but at the very least the work of the Vallee group makes the experiments possible.—**JEAN L. MARX**