Part V

Molecular Genetics

Can Cancer Tumors Be Starved to Death?

One of the most exciting recent developments in the war against cancer is the report that it might be possible to starve cancer tumors to death. Many laboratories have begun to look into this possibility, although it's not yet clear that the approach will actually work to cure cancer. One of the most exciting and frustrating things about watching a developing science story like this one is that you can't flip ahead and read the ending—in the real world of research, you never know how things are going to turn out.

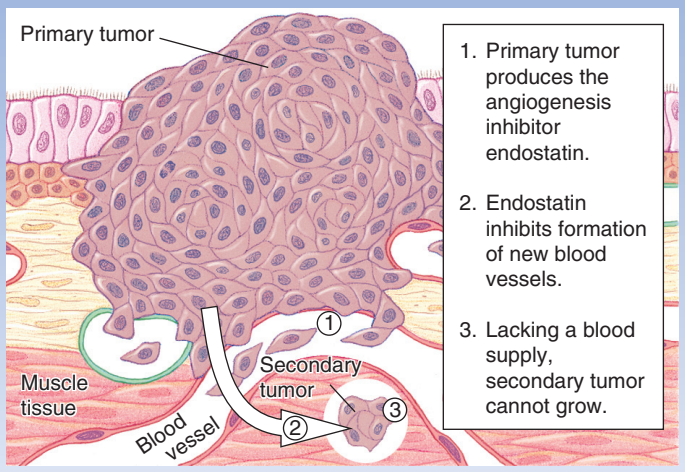
This story starts when a Harvard University researcher, Dr. Judah Folkman, followed up on a familiar observation made by many oncologists (cancer specialists), that removal of a primary tumor often leads to more rapid growth of secondary tumors. "Perhaps," Folkman reasoned, "the primary tumor is producing some substance that inhibits the growth of the other tumors." Such a substance could be a powerful weapon against cancer.

Folkman set out to see if he could isolate a chemical from primary tumors that inhibited the growth of secondary ones. Three years ago he announced he had found two. He called them angiostatin and endostatin.

To understand how these two proteins work, put yourself in the place of a tumor. To grow, a tumor must obtain from the body's blood supply all the food and nutrients it needs to make more cancer cells. To facilitate this necessary grocery shopping, tumors leak out substances into the surrounding tissues that encourage angiogenesis, the formation of small blood vessels. This call for more blood vessels insures an ever-greater flow of blood to the tumor as it grows larger.

When examined, Folkman's two cancer inhibitors turned out to be angiogenesis inhibitors. Angiostatin and endostatin kill a tumor by cutting off its blood supply. This may sound like an unlikely approach to curing cancer, but think about it—the cells of a growing tumor require a plentiful supply of food and nutrients to fuel their production of new cancer cells. Cut this off, and the tumor cells die, literally starving to death.

By producing factors like angiostatin and endostatin, the primary tumor holds back the growth of any competing tumors, allowing the primary tumor to hog the available resources for its own use (see above).

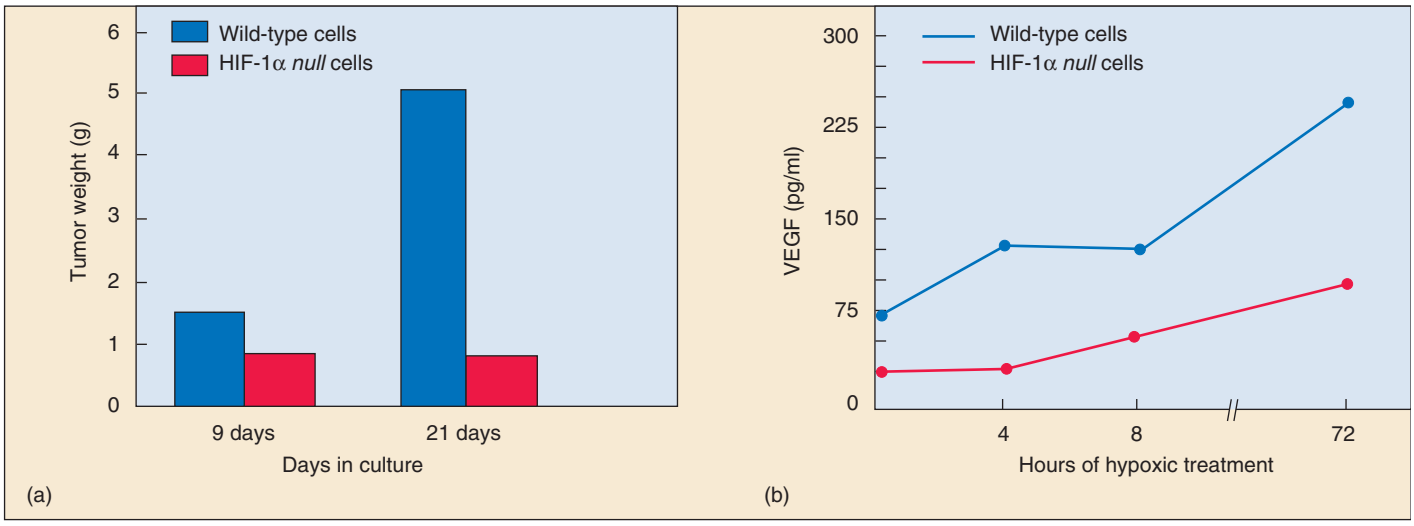


How primary tumors kill off the competition. Tumors require an ample blood supply to fuel their growth. The growth of new blood vessels is called angiogenesis. Inhibiting angiogenesis offers a possible way to block tumor growth.

In laboratory tests the angiogenesis inhibitors caused tumors in mice to regress to microscopic size, a result that electrified researchers all over the world. Other scientists were soon trying to replicate this exciting result. Some have succeeded, others not. Five major laboratories have isolated their own angiogenesis inhibitors and published findings of antitumor activity. The National Cancer Institute is proceeding with tests of angiostatin and other angiogenesis inhibitors in humans. Preliminary results are encouraging. While not a cure-all for all cancers, angiogenesis inhibitors seem very effective against some, particularly solid-tumor cancers.

Gaining a better understanding of how tumors induce angiogenesis has become a high priority of cancer research. One promising line of research concerns hypoxia. As a solid tumor grows and outstrips its blood supply, its interior becomes hypoxic (oxygen depleted). In response to hypoxia, it appears that genes are turned on that promote survival under low oxygen pressure, including ones that increase blood flow to the tumor by promoting angiogenesis. Understanding this process may give important clues as to how angiogenesis inhibitors work to inhibit tumor growth.

So how does a lowering of oxygen pressure within a tumor promote blood vessel formation? Dr. Randall Johnson of the University of California, San Diego, is studying one important response by a tumor to hypoxia—the induction of a gene-specific transcription factor (that is, a protein that activates the transcription of a particular gene) that promotes angiogenesis. Called HIF-1, for *hypoxia inducible factor-1*, this transcription factor appears to induce the transcription of genes necessary for blood vessel formation.



Tumor growth in HIF-1α *null* cells and wild-type cells. (*a*) The size of tumors formed by the HIF-1α *null* cells were significantly smaller compared to those formed by wild-type cells. (*b*) HIF-1α *null* cells had significantly lower levels of VEGF protein production under hypoxic conditions compared to wild-type cells. VEGF promotes the formation of capillaries.

The Experiment

In order to examine the involvement of the hypoxia-inducible transcriptional factor (HIF-1) in angiogenesis, Johnson and his co-workers were faced with the problem that HIF-1 has many other effects on cell growth. To get a clear look at its role in angiogenesis, the researchers turned to embryonic stem cells. Embryonic stem cells are cells harvested from early embryos, before they have differentiated, while they are still capable of unlimited division. Because such stem cells have the capacity to form tumors (teratocarcinomas) when injected into certain kinds of mice, they offer a good natural laboratory in which to study how HIF-1 might influence cancer growth. The research team first prepared a mutant HIF-1 embryonic stem cell line in which the function of the transcription factor encoded by HIF-1 was completely destroyed or *null*.

The researchers then grew these HIF-1 *null* stem cells under hypoxic conditions. If HIF-1 genes indeed foster tumor growth in normal cells by promoting angiogenesis, then it would be expected that these *null* cells would be unable to promote tumor growth in this way.

The researchers tested the ability of *null* cells to promote tumor growth by injecting HIF-1α *null* cells into laboratory mice, and in control experiments injecting wild-type stem cells. The injected cells were allowed to grow and form tumors in both *null* and control host animals. The tumors that formed were then examined and measured for differences.

To get a closer look at what was really going on, the *null* and wild-type cells were compared in their ability to actually form new blood vessels. This was done by examining levels of mRNA of a growth factor that plays a key role in the formation and growth of blood vessels. This factor is a protein called vascular endothelial growth factor (VEGF). The levels of VEGF mRNA in the cells were determined by hybridizing cDNA VEGF probes to mRNA isolated from tumors, and measuring in each instance how much tumor mRNA bound to the cDNA probe. In parallel studies, antibodies were used to determine levels of VEGF protein.

The Results

The researchers found that the *null* cells were greatly compromised in their ability to form tumors compared to the wild-type cells with the effects becoming more significant over time (see graph *a* above). Tumors were five times larger in wild-type cells than in the HIF-1 *null* cells after 21 days. Clearly knocking out HIF-1 retards tumor growth significantly.

This decrease in the size of tumors produced by *null* cells is further supported by the results of the VEGF protein analysis (see graph *b* above). Levels of the protein VEGF rise in wild-type cells under conditions of hypoxia, increasing the immediate availability of oxygen to the tumor by promoting capillary formation. The researchers found levels of VEGF protein were lower in *null* cell tumors, and responded to hypoxia at a lower rate.

Both the decrease in tumor size and the lower level of VEGF in the HIF-1 *null* cells supports the hypothesis that HIF-1 plays an essential role in promoting angiogenesis in a tumor, responding to a hypoxic condition by increasing the levels of VEGF.

Do the angiogenesis inhibitors like angiostatin, being tested as cancer cures, in fact act by inhibiting VEGF? The sorts of experiments being carried out in Johnson's laboratory, and in many other cancer centers, should soon cast light on this still-murky question.