New Drugs Target Hypoxia Response in Tumors

We breathe air that is 21% oxygen, but some of our tissues get by on 7%, and many tumor cells on 1% or less. Tumors cope with very low oxygen concentration, or hypoxia, in several ways. The best-studied mechanism is activation of a transcription factor, hypoxia-inducible factor 1 (HIF-1), which has emerged as a promising target for cancer therapy. The first clinical trials of HIF inhibitors are poised to begin, but unanswered questions of HIF biology continue to loom large.

Tumor hypoxia was first described a half-century ago, when R.H. Thomlinson and Louis Gray, Ph.D., of the Mount Vernon Hospital in England, showed that solid tumors contain zones of very low oxygen and that this hypoxia compromised radiation therapy. Abnormal or poor blood vessel growth, a fundamental hallmark of tumors (and one that angiogenesis-inhibiting drugs seek to exploit), was later shown to be the cause. Over the last few decades, though, hypoxia has come to be seen not just as a by-product of tumor growth but as a driver. “The argument used to be, is hypoxia and tumorigenesis the chicken or the egg?” said Amato Giaccia, Ph.D., of Stanford University.

“Which comes first? Is hypoxia driving tumorigenesis?” That debate is now almost moot. “The evidence now is becoming pretty convincing that hypoxia, due to regulation of a variety of transcription factors and pathways, is able to accelerate and modify tumor evolution,” said Giaccia.

Tumors respond very aggressively to hypoxia. In 1990, Richard Hill, Ph.D., of the Ontario Cancer Institute in Toronto, showed that hypoxia and reoxygenation could increase the metastatic potential of tumor cells. In 1996, Giaccia showed that the hypoxia microenvironment could select for tumors, because cells that lacked p53 overran p53 wild-type cells in an experimental hypoxic environment. Those two papers “set the stage,” said Giaccia. “Not only was hypoxia bad for therapy, but hypoxia could also affect malignant progression and metastasis.”

HIF-1 soon dominated the field. The protein was identified separately by Gregg Semenza, M.D., Ph.D., of the Johns Hopkins Medical School in Baltimore, and Peter Ratcliffe, M.D., of the University of Oxford in England in 1992 as a transcription factor that bound to the erythropoietin gene. Two years later, Semenza showed that HIF-1 upregulated virtually all the glycolytic enzymes, enabling the tumor cell to generate energy through glycolysis in a low-oxygen environment, or anaerobic glycolysis. In 1995, Semenza cloned HIF-1, and the following year he demonstrated that VEGF, or vascular endothelial growth factor, was an HIF-1–regulated gene. That discovery tied HIF-1 directly to tumor angiogenesis.

Over the last decade, the list of genes turned on by HIF-1 has steadily grown.
Semenza estimates that as much as 5% of the genome can be HIF-1 regulated, although that number varies by cell type. These genes, said Semenza, are “all the things you need to make a cancer cell.”

Tumor Promoter or Suppressor?

Hypoxia “impacts on virtually everything we can think about: genomic instability, glycolysis, angiogenesis, motility,” said M. Celeste Simon, Ph.D., of the University of Pennsylvania.

“Cells acquire a very metastatic phenotype because of HIF targets.”

Semenza, speaking at the April annual meeting of the American Association for Cancer Research (AACR), summarized data from several groups showing that HIF-1 overexpression in human tumor samples is associated with a higher rate of patient mortality. The sheer number and lethality of HIF-1-regulated genes makes a cause-and-effect relationship between HIF-1 expression and tumor aggressiveness plausible, if not yet provable. But Semenza cautioned that “the regulation of these genes is not due just to the HIF-1” and that “not all of the genes that are regulated by HIF-1 will be induced in every tumor.” The code that determines which HIF-1 target genes will be up-regulated in any particular cancer, Semenza added, remains unknown.

Adding to the complexity is the fact that HIF-1, in certain situations, can be a tumor suppressor as well as a tumor promoter. For example, HIF-1 can turn on proapoptotic genes and can upregulate genes such as p21 and p27 that inhibit cell cycle progression. “There’s probably very tight regulation of HIF,” said Giaccia. “Too little or too much of it, depending on the cell type, depending on the circumstances, can either accelerate or inhibit tumor growth.” That means HIF inhibitors are likely to work against only certain tumors. “Part of the challenge is to understand which cancers will be amenable to a small-molecule inhibitor of HIF-1,” Semenza said.

Finding Drugs

Finding specific HIF-1 inhibitors hasn’t been easy, because it is a transcription factor and therefore not a conventional “druggable” target.

But several government and company efforts seem to have borne fruit. Giovanni Melillo, M.D., of the National Cancer Institute, has been screening for HIF-1 inhibitors since 1999. His first real hits were the camptothecins—well-known topoisomerase I inhibitors that damage DNA.

Initially disappointed at not finding new compounds, Melillo’s group later discovered that the camptothecins inhibited HIF-1 by a novel mechanism involving blockage of protein accumulation. “For the HIF-1 effect, we showed that DNA damage is not required,” said Melillo. At small doses given daily, the drug blocked tumor growth and angiogenesis in mouse xenograft models.

A phase I human trial of topotecan, an FDA-approved semisynthetic camptothecin analogue, given over an extended time course, is already open for patient enrollment. Topotecan thus should be the first HIF-1 inhibitor to be tried in humans. Only patients expressing high levels of HIF-1α in their tumors will qualify, and inhibition of HIF-1α level will be the trial’s primary endpoint. Glycolytic activity and angiogenesis will also be monitored.

Ideally topotecan, given this way, will have little of the toxicity associated with the standard cytotoxic dose. A drug lead found in a later screen, NSC 644221, also seems to have a cytostatic effect. “Giving the drug over a more prolonged period of time … seems to be required to have a sustained inhibition of HIF-1α signaling,” said Melillo.

The second HIF-1 inhibitor likely to enter the clinic is PX-478, from ProlX Pharmaceuticals in Tucson, Ariz. Garth Powis, Ph.D., and his colleagues at the Arizona Cancer Center identified PX-478 in 2001 in a screen for HIF-1 inhibition. PX-478 has shown impressive activity in a variety of xenograft animal models. Tellingly, tumor HIF-1 levels correlated closely with antitumor effects. “Those with high HIFs, we got cures,” said ProlX CEO Lynn Kirkpatrick, Ph.D. “Those with low, we got less. That information is pretty convincing that it’s a target-related effect that we’ve seen.” The mechanism of HIF-1 inhibition, however, remains unclear, so it’s still possible that the drug has other targets. ProlX expects to begin phase I clinical trials late this year or early next year.

Meanwhile, the Seoul National University College of Medicine in Korea is in the lead-optimization stage with YC-1, another small-molecule HIF-1 inhibitor, together with Seoul-based biotech company BizBiotech. Originally developed as an anticoagulant, the drug has shown activity in xenograft models. (See article, Vol. 95, No. 7, p. 516.) In addition, other known anticancer agents, including rapamycin and the heat shock protein 90 inhibitor 17-AAG, have been shown to inhibit HIF-1.

Novel approaches targeting HIF-1 are also being tried. Corgentech, a South San Francisco-based biotech company, is developing decoy oligonucleotides against HIF-1. At the 2005 AACR meeting, Corgentech reported antitumor effects in combination with the VEGF inhibitor bevacizumab (Avastin). Giaccia, meanwhile, is screening for agents that selectively kill cells with elevated HIF, as opposed to halting their growth, as direct HIF inhibitors generally do. “We’re not interested in inhibiting HIF; we’re interested in killing those cells,” said Giaccia. Hypoxic tumor cells, in the absence of HIF, “ultimately may find a way to grow in an HIF-independent
manner,” said Giaccia, hence the need for cell killing.

**Lingering Doubts**

Whether HIF-targeted drugs will have serious side effects in humans is not yet known. Since erythropoietin is a probable HIF-1 target gene, one worry is that prolonged inhibition will lead to oxygen deprivation in normal tissues. Bone marrow cells might also be vulnerable because they exist at naturally low oxygen levels and thus might express HIF-1. But at normal oxygen levels, HIF-1 is rapidly degraded, with a half-life of about 5 minutes, suggesting a very low base level of activity. That means side effects should be minimal. Nevertheless, “in that period of time where it’s rapidly turned over, is it doing something that’s important?” asked Giaccia. “Maybe HIF plays a role in normal tissues that is independent of hypoxia.”

Another question is whether HIF-1 is always the right hypoxia target. HIF-2, cloned in 1997, was originally thought to be an HIF-1 isoform restricted to endothelial cells, but it’s now known to activate a different set of target genes in a variety of tissues. Work by Bill Kaelin, M.D., of Harvard University and Richard Klausner, M.D., then at the NCI, showed that HIF-2, not HIF-1, was responsible for renal cell carcinoma growth in animals. Blocking HIF-1 did not shrink tumors in that model.

Despite the renal cell results, “most of the evidence so far actually favors HIF-1 as the major factor important in tumor progression,” said Melillo. That’s the mainstream view, which Simon thinks may be wrong. “There are going to be situations where HIF-1 is a tumor promoter, but I think there’s going to be even more situations where HIF-1 is a tumor suppressor, and it’s HIF-2 that’s going to be a tumor promoter,” she said. Simon is now undertaking experiments, using conditional knockouts of HIF-1 and HIF-2 in mice, designed to see which HIF is more important in a variety of physiologic tumors.

Answers to all these questions should also begin arriving once HIF-targeted drugs complete the first clinical trials. “We know very little about the role that HIF plays in the clinic, in patients, or how to inhibit, what’s the best way to do that,” said Melillo. “We’re just approaching that arena right now.”

—Ken Garber