Targeting Mitochondria Emerges as Therapeutic Strategy

For most cancer researchers, mitochondria are an afterthought. “I was brought kicking and screaming into mitochondria [research],” said Gerard Hockenbery, Ph.D., of the Fred Hutchinson Cancer Research Center in Seattle. The 1996 discovery that the protein cytochrome c came from mitochondria, “said David Hockenbery, Ph.D., at the 2005 annual meeting of the American Association for Cancer Research. Evan began looking at mitochondria after linking the myc oncogene to apoptosis, or programmed cell death. Others are starting to find their own paths. “All roads lead to the mitochondria,” Evan added.

“It wasn’t too long ago, at the major programmed cell death meetings, there would be almost no mention of mitochondria,” said David Hockenbery, Ph.D., of the Fred Hutchinson Cancer Research Center in Seattle. The 1996 discovery that the protein cytochrome c acted to trigger apoptosis forced people to look at mitochondria, because that’s where cytochrome c came from. “So something had to be happening at the mitochondrial level,” said Hockenbery. “Since then, the field has just sort of exploded.”

The biggest sign of that explosion has been the arrival of drugs that target prosurvival BCL-2 family members. These proteins localize to mitochondria and prevent cytochrome c release and apoptosis. At least three BCL-2 inhibitors are now in the clinic, with several others in preclinical development. (See table, p. 1801.) Meanwhile, other compounds acting on mitochondria are showing promise against cancer. Finally, new discoveries are linking energy production inside and outside mitochondria to cancer, revealing new drug targets.

Live and Let Die

The BCL-2 story began in 1984 when Carlo Croce, M.D., and his group at the University of Pennsylvania in Philadelphia discovered the BCL-2 gene at the translocation breakpoint for follicular lymphoma. (BCL-2 stands for “B-cell lymphoma–leukemia gene 2.”) Four years later, David Vaux, Ph.D., and Stanley Korsmeyer, M.D., separately showed that the protein, which binds to the outer mitochondrial membrane, functions to block cell death. In the early 1990s, John Reed, M.D., Ph.D., then at Penn, demonstrated that antisense oligonucleotides targeted to BCL-2 mRNA could kill B-cell lymphoma cells in vitro.

At first, drug companies avoided BCL-2 because any small-molecule inhibitor would have to block a protein–protein interaction—a formidable task. BCL-2 and a related protein, BCL-xl, function by binding to prodeath BCL-2 family members, especially Bax and Bak, and preventing them from inducing apoptosis. No one imagined how to block that coupling until 1996, when a group led by Steve Fesik, Ph.D., at Abbott Laboratories in Abbott Park, Ill., determined the crystal structure of BCL-xl, revealing an intriguing groove in the protein where the proapoptotic proteins bound. If small-molecule mimetic agents could be designed to fit the groove, reasoned Fesik and others, it should be possible to find an effective BCL-2–BCL-xl inhibitor.

That theory is now being tested. GeminX, a Montreal biotechnology company, has a compound in phase I clinical trials that inhibits all five prosurvival BCL-2 family members. Abbott’s compound, found together with Idun Pharmaceuticals (now part of Pfizer), is in late preclinical development. In animal models, “we’re seeing frank regression of tumors,” said Fesik in a July conference call with reporters. Reed, now director of the Burnham Institute, and medicinal chemist Shaomeng Wang, Ph.D., at the University of Michigan in Ann Arbor, have each found compounds that fit BCL-2’s pocket. They’re based on gossypol, a natural product derived from a Chinese herbal medicine. Wang’s compound, licensed to San Diego–based biotech company Ascenta, is in phase I studies.

There’s hope that these drugs will kill cancer cells and not normal cells, because, in theory, cancer cells are highly stressed and poised on the brink of programmed cell death, with only abnormally high levels of BCL-2 and BCL-xl keeping them alive. “If you inhibit them, the cancer cells will die because they’re so dependent on these BCL-2 family members,” said Fesik. “These cells are relatively sick cells, unlike the normal cells, and are destined to die.” Normal cells lack an active death program, the theory goes, so losing BCL-2 function shouldn’t matter.

But no one yet knows if this optimism is justified. “We’ll just have to see how that plays out, if that really accounts for the differences between normal cells and cancer cells,” said Hockenbery. Some normal cells also have high levels of BCL-2, Hockenbery pointed out, and defects in knockout mice show that prosurvival BCL-2 family genes are essential for some normal tissues. “That’s … the grain of salt you have to take with this,” he said.

Efficacy is the other issue. Patients taking Genasense, Genta’s anti–BCL-2 antisense drug, had more remissions than those receiving chemotherapy alone in phase III trials first in malignant melanoma and then in chronic lymphocytic leukemia (CLL). But the differences weren’t dramatic. (Genta withdrew its melanoma application to the U.S. Food and Drug Administration but plans to apply for CLL.) “That doesn’t dissuade me at all from targeting BCL-2,” said Anthony Letai, M.D., Ph.D., of the Dana-Farber Cancer Institute in Boston. Letai thinks antisense may just be the wrong approach. “We just don’t know about how well it’s lowering
BCL-2 levels,” he said. The smallmolecule strategy is “a much more promising venture.”

**Myriad of Mechanisms**

The small-molecule BCL-2 inhibitors now surging through drug company pipelines are classic examples of so-called rational drug design: Identify a target, discover its function, solve the structure, and craft compounds to fit. In contrast, there are several drugs in development that kill cancer cells but only later were found to target mitochondria. These include drugs that activate the permeability transition pore, a protein complex that spans the inner and outer membranes. Changing its conformation can release cytochrome c and trigger apoptosis. Other compounds inhibit mitochondrial ATP synthase, the enzyme that actually makes ATP (adenosine triphosphate), the cell’s energy source, thus starving the cell of energy. Still others block the peripheral benzodiazepine receptor, which is involved in cholesterol transport into mitochondria. Finally, there are toxins that seem to be absorbed by mitochondria in cancer cells but not normal cells.

Compared with a rational approach with preselected targets, “we’ll have to see if picking out compounds that seem to have a mitochondrial focus is better or worse as an approach,” said Hockenbery.

One such promising compound is imexon, which was studied in a small European clinical trial in the late 1970s but was then shelved. Beginning in the mid-1990s, researchers at the Arizona Cancer Center in Tucson discovered that imexon binds to and sequesters molecules in mitochondria involved in antioxidant activity, thus making cells much more sensitive to the oxygen free radicals released as part of ATP generation in the mitochondria and causing apoptosis. “This is a novel mechanism,” said Robert Dorr, Ph.D., chief scientific officer of AmpliMed, which is testing imexon in several phase I studies. “It’s not a story that people have heard before.”

**Starving Tumors To Kill Them**

The mitochondrion, of course, is not just the site of the cell’s apoptosis decision. It’s the cell’s power plant, generating energy in the form of ATP from pyruvate, an intermediate product of glucose metabolism, and oxygen—a process called respiration. Many researchers think respiration is unrelated to apoptosis and consider the mitochondrion just a convenient place to hold proteins like cytochrome c for eventual release, triggering cell death. “Probably more than half the field would feel that way,” said Hockenbery.

But other scientists are now finding links between mitochondrial physiology and apoptosis. For example, Craig Thompson, M.D., and his colleagues at Penn have shown that upregulation of the intracellular kinase Akt leads to increased glucose uptake and a shift toward ATP production by glycolysis, the anaerobic metabolism of glucose, even in the presence of oxygen. This conversion to aerobic glycolysis, the Warburg effect, is seen in about two-thirds of tumors, according to Thompson. Several companies are now developing drugs designed to interfere with glycolysis as a way to stop such tumors from growing. (See News, Vol. 96, No. 24, p. 1805.)

Thompson has now identified a new metabolic target in cancers. In the October issue of Cancer Cell, he described a novel metabolic cycle that enables cancer cells to continue burning glucose for energy, bypassing normal feedback mechanisms. The same cycle allows tumors to spin glucose into fatty acids for the synthesis of lipids, which are crucial for tumor growth in several ways. “He definitely links [tumor] anaerobic metabolism with the synthesis of fatty acids, and that’s entirely new,” said Massimo Loda, M.D., an associate professor of pathology at the Dana-Farber Cancer Institute. “It’s quite wonderful, actually.”

**New Metabolic Target**

The new pathway may have a point of vulnerability. Normally the molecule citrate, transported from the mitochondrion and built up in the cytosol, inhibits glycolysis, turning off glucose metabolism. That process would shut down tumor cells that depend on glycolysis. But Thompson’s group found that Akt-expressing tumor cells show increased phosphorylation and activation of ATP citrate lyase (ACL), the enzyme that cleaves citrate when it leaves the mitochondrion. By keeping citrate from building up in the cytosol, activated ACL keeps citrate from shutting down glycolysis. “As long as that enzyme is working to capacity,” Thompson observed, “glycolysis can run out of check.” And tumor cells can keep growing.

Loda says ACL may be “the first metabolic enzyme that can act as an oncogene.” Will drugging it work against cancer? “We think that [ACL] might be a very good target,” Thompson said. His group used small interfering RNA and small-molecule inhibitors to knock down the enzyme in tumor cell lines and mouse xenografts. The result: 80%–90% growth inhibition in cells. “That’s how much of the fatty acid synthesis is impaired when you block that pathway,” explained Thompson.

ACL is only one potential target. As scientists focus more closely on tumor cell mitochondrial bioenergetics, others are sure to emerge. In most cancer cells, “there’s a unique form of metabolism going on in the mitochondria,” Thompson pointed out. “If you look at the whole cycle plus the downstream consequences … there are a number of steps that might be targetable by new therapeutic approaches.” —Ken Garber