Inducing Indigestion: Companies Embrace Autophagy Inhibitors

By Ken Garber

Autophagy, the process of “self-eating” within cells, has been controversial in cancer since it was first linked to the disease in 1999. Because autophagy can either suppress or promote tumors, depending on the context, the field has been marked by both confusion and unwillingness by drug companies to get involved in therapies.

But that’s changed. A consensus is emerging that it’s the tumor promotion role of autophagy that’s important in established tumors, leading to more than a dozen ongoing clinical trials of a commercially available autophagy inhibitor. And drug companies are now rushing to start their own autophagy programs, actively looking for new and better compounds. “It’s been a total turnaround,” said Eileen White, Ph.D., an autophagy researcher at the Cancer Institute of New Jersey in New Brunswick.

Although hopes are high for such drugs, doubts persist about the wisdom of inhibiting autophagy, at least in some situations. “Autophagy is, as is commonly said, a double-edged sword,” said Daniel Klionsky, Ph.D., an autophagy researcher at the University of Michigan in Ann Arbor. Evidence for widespread activated autophagy in human tumors, as opposed to cell lines, is growing but still frustratingly deficient. And the field awaits its first unambiguous clinical success. But autophagy is now a hot field for translational cancer research.

**Tumor Suppressor to Promoter**

Autophagy, first reported in 1963, is a remarkable process common to all eukaryotes. In autophagy, a membrane called the phagophore forms, elongates, and encircles damaged and unneeded proteins and organelles in the cytoplasm. This new organelle, the autophagosome, later merges with (and deposits its contents into) the lysosome, an organelle that breaks down cellular debris. Autophagy functions as a garbage disposal for cells, which use the breakdown products to fuel energy production and to replenish building blocks for proteins and other essential molecules.

In 1999, Beth Levine, M.D., at Columbia University in New York showed that beclin-1, a protein required for autophagy induction, functions as a tumor suppressor gene. Between 40% and 75% of human breast, ovarian, and prostate cancers show loss of one copy of beclin-1, suggesting that autophagy plays an important role in preventing these tumors.

But whereas autophagy clearly protects against at least some tumors, it can promote others. About 6 years ago, White’s group found that tumor cells lacking the cellular machinery for apoptosis, or programmed cell death, could survive long periods when starved of nutrients—although how they survived was a mystery. In 2006, White reported that autophagy was the survival mechanism and that inhibiting autophagy killed the cells. This and other accumulating evidence suggested that tumor cells use autophagy to survive conditions of metabolic stress, such as hypoxia, common in tumors. Gradually the field embraced the tumor-promoting function of autophagy.

The dominant view now is that autophagy suppresses growth early in tumor development but promotes growth later. “The field is now fully on board with that,” said Ravi K. Amaravadi, M.D., an oncologist at the University of Pennsylvania in Philadelphia. “Once tumors are established, especially in the tumor microenvironment, and especially in the context of cancer therapy, autophagy is a tumor-promoting mechanism,” he said.

Exactly how autophagy promotes tumors, though, has been a mystery. Two reports published this year in *Genes & Development* offer some insight. In the first study, White’s group introduced the Ras oncogene into cells with normal autophagy and defective autophagy. The autophagy-competent cells, put into mice, developed into much faster-growing, more aggressive tumors than the autophagy-deficient cells. The autophagy-deficient cells displayed damaged mitochondria and diminished oxidative energy production, or respiration (which occurs in mitochondria), suggesting a major role of autophagy in supporting energy metabolism. On the basis of these experiments, White thinks that autophagy is promoting tumor growth by both freeing up the components of cellular respiration and disposing of defective mitochondria.

That’s the same conclusion that Alec Kimmelman, M.D., Ph.D., at the
Dana-Farber Cancer Institute in Boston, reached in the second Genes & Development article, published online in March. Kimmelman’s group blocked autophagy in pancreatic cancer cells, slowing cell proliferation and decreasing oxygen consumption. Adding a key energy production intermediate to these autophagy-defective cells led to renewed growth. Autophagy, Kimmelman concluded, allows tumor cells to grow by maintaining energy production.

Kimmelman also found abundant markers of autophagy on primary human pancreatic tumor samples, markers that were absent in early precursor lesions, “meaning that autophagy was activated late in the process of malignant transformation,” he said. This finding, too, fits the current model of autophagy in cancer.

**Lagging Science?**

The biggest surprise in both studies was that Ras-transformed cells showed high levels of autophagy even with abundant nutrients. (Normal cells show little, if any, autophagy under such conditions.) This finding suggests that Ras itself, not external stress, causes the autophagy, through an unknown mechanism. White thinks that Ras-triggered cell growth itself is a stressor requiring the autophagy response and that such tumor cells are in a state of “autophagy addiction.”

The implication is that targeting autophagy in Ras-driven tumors (about one-fourth of all tumors) should be especially effective.

That hypothesis underlies a phase II Dana-Farber clinical trial in pancreatic cancer. (Ras drives about 80% of pancreatic tumors.) Patients in whom standard chemotherapy treatment failed receive single-agent hydroxychloroquine (HCQ), an antimalarial drug that inhibits autophagy at the lysosome stage. HCQ, because it’s cheap and relatively nontoxic, has become the autophagy inhibitor of choice for clinical trials. At least 16 phase I and phase II trials, most in solid tumors using HCQ with other treatments, are under way. Companies involved include Pfizer, Novartis, Millennium Pharmaceuticals, and Merck.

However, concern remains that the science is lagging. Studies implicating autophagy in tumor promotion have come mainly from cell lines, not tumor tissue. “There is very limited information in animal models and from primary human tumor samples,” said David Gewirtz, Ph.D., who studies experimental cancer therapeutics at Virginia Commonwealth University in Richmond. For technical reasons, however, it’s very hard to track autophagic flux—the autophagy process in human tumor samples. And static markers of autophagy can be misleading. “There aren’t many assays that can be used to follow autophagy in mammalian cells,” said Klionsky. “In vivo, there’s almost nothing.”

But researchers are now starting to venture beyond cell lines. Immunohistochemistry (staining tumor cells with antibodies to highlight individual proteins) is now feasible, although tracking autophagic flux remains challenging. And, Amaravadi’s group has developed a semiquantitative approach for looking at autophagy in tumor samples by using electron microscopy. The team recently tracked autophagy this way in human metastatic melanoma samples. “We found these astronautically high levels of autophagy, better than we could ever create in the lab,” Amaravadi said. “The higher the level of autophagy in your tumor, the worse you did as a patient.” As for animal models, White’s group has crossed genetically engineered mouse cancer models with autophagy conditional-mutant mice, enabling future in vivo studies of autophagy and cancer.

Another concern with autophagy inhibitors is that they could actually protect tumors from chemotherapy, since certain chemotherapy drugs may kill tumor cells by inducing autophagic cell death. Still, that’s not a reason not to go forward with such trials, said White. “The evidence for autophagic cell death is still very, very weak,” she said. “Until someone explains what it is, and demonstrates that it can occur in a physiological condition in vivo, I think conclusions that you can get autophagic cell death are going to be hard to substantiate.”

Gewirtz, though, considers autophagic cell death real and believes that some chemotherapy drugs induce it in patients. “There’s extensive preclinical literature, with a variety of agents, showing that autophagy can kill tumor cells,” he said. He acknowledged that in vivo evidence for this phenomenon is scant but pointed out that the same is true for autophagy’s protective role in chemotherapy.

**New Trials, New Drugs**

Meanwhile, HCQ trials are proliferating, partly thanks to a 2006 randomized, placebo-controlled glioblastoma clinical trial published in the Annals of Internal Medicine. Researchers at the National Institute for Neurology and Neurosurgery in Mexico City added chloroquine (a more toxic version of HCQ) to standard chemotherapy and radiation after surgery. This approach resulted in a median overall survival time of 24 months for the chloroquine-treated group versus 11 months for control subjects. Because of low patient numbers, the results did not reach statistical significance, but news of the trial spread broadly, prompting many oncologists worldwide to give chloroquine or HCQ to their glioblastoma patients. Amaravadi advises against doing so except in clinical trials, owing to combination therapy’s potential toxic effects. The National Cancer Institute is sponsoring a phase I/II trial of HCQ with temozolomide (Temodar) and radiation in glioblastoma.

Another important combination trial, at Penn, is a phase I study of HCQ plus temsirolimus (Tesoril), an mTOR inhibitor. Temsirolimus is approved for renal cell carcinoma, but responding patients inevitably relapse. Clinical trials of mTOR inhibitors in general have been disappointing, since mTOR, a kinase that basically acts as a nutrient sensor, is an important driver of cell growth. Because mTOR is a negative regulator of autophagy, mTOR inhibitors could be increasing autophagy and paradoxically keeping tumor cells alive.

Adding an autophagy inhibitor should overcome this resistance. In animal models, mTOR inhibitors mostly block tumor cell growth but don’t kill the cells outright, and HCQ shows similar effects. “But if you put the two together, there’s this massive synergy and you get cancer cell death,” said White.

This potential to overcome resistance to kinase inhibitors drives much of the drug company interest in autophagy, White added. For example, virtually all major pharmaceutical companies are developing PI3 kinase inhibitors for cancer. And these drugs, like mTOR inhibitors, enhance autophagy, so companies see the logic of combination treatment.

But HCQ is not the ideal autophagy inhibitor. Amaravadi, monitoring drug effects in the glioblastoma clinical trial, found that the drug did not always inhibit autophagy. “So it may be that HCQ needs to be at very high doses to block autophagy,
and that might not be possible with many anticancer regimens in combination,” Amaravadi said.

White agreed that better autophagy inhibitors are needed. “All of us in the field don’t think that [HCQ] is the be-all and end-all,” she said. “We are using it now because we can, and we’re hoping that preliminary data look encouraging and that we will learn from this approach. But there will be better drugs.” In fact, many drug companies and some academic groups are now screening for specific small-molecule autophagy inhibitors. Several autophagy-related proteins are considered good targets for potential drugs. With drug companies now fully committed, the hypothesis that autophagy protects established tumors will soon be tested in the only setting that really matters: people with cancer.

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