Pancreatic cancer: stressed to death

By Michael J. Haas, Senior Writer

U.S. and EU researchers have shown that the generic malaria drug chloroquine can treat pancreatic cancer by inhibiting autophagy,[1] a process by which cells degrade their own cytosolic components to prolong cell survival. The team already has a chloroquine analog in a Phase II pancreatic cancer trial sponsored by **Dana-Farber Cancer Institute**.

Autophagy is a normal cellular response to nutrient starvation and other cellular stress, but its functions in cancer are complex and not well understood. Indeed, prior studies have shown that promoting autophagy increased the efficacy of targeted therapies in colon and breast cancer cell lines[2] and of gene therapies in primary human brain cancer cells,[3] whereas inhibiting autophagy synergized with chemotherapy to treat lymphoma in mice.[4]

In 2008, a Japanese group reported that one component of the autophagy system—microtubule-associated protein 1 light chain 3α (MAP1LC3A; LC3A; LC3)—was upregulated in primary pancreatic tumors and correlated with increased tumor size and poor patient outcomes.[5]

Those findings prompted a group led by Alec Kimmelman, assistant professor of radiation oncology at Dana-Farber and **Harvard Medical School**, to postulate that pancreatic tumors depend on autophagy for survival and that autophagy inhibitors could treat the disease.

Indeed, the team confirmed that autophagic activity was greater in primary pancreatic tumor cells and human pancreatic cancer cell lines than in breast cancer, lung cancer and normal pancreatic cell lines.

Next, the team found that chloroquine—a known autophagy inhibitor—decreased the proliferation and growth of pancreatic cancer cell lines compared with those of breast and lung cancer cell lines.

The team obtained similar results *in vitro* with another autophagy inhibitor, bafilomycin A1, or siRNA knockdown of *ATG5 autophagy related 5 homolog* (*ATG5*). Bafilomycin A1 targets a vacuolar H^+ ATPase that plays a role in acidification of autophagolysosomes. ATG5 plays a key role in the formation of autophagosomes, which are double-walled vesicles that sequester proteins and organelles for degradation.

Lastly, the team showed that chloroquine lowered tumor growth and increased survival in four mouse models of pancreatic cancer compared with in a xenograft mouse model of lung cancer.

Because chloroquine and its analogs have been used in malaria and autoimmune indications for years, the findings provide a rationale for beginning clinical testing of those drugs to treat pancreatic cancer, the team wrote in its report in *Genes & Development*.

The team included researchers from the **Boston University School of Medicine**, **Massachusetts General Hospital Cancer Center**, **Helmholtz Center Munich**, **Technical University Munich**, **San Raffaele Scientific Institute** and **University of Milan**.

"It is becoming more and more clear to cancer researchers that targeting cell stress pathways can enhance the antitumor effects of other therapies," said Michael Palladino, SVP, CTO and cofounder of cancer company **Nereus Pharmaceuticals Inc.**

The new findings, he said, show that "pancreatic cancer requires autophagy for survival and that inhibiting it increases oxidative damage and cellular stress."

Stefan Rehnmark, CEO of cancer company **Axcentua Pharmaceuticals AB**, agreed that treating pancreatic cancer with chloroquine "seems very feasible, as the authors have shown the effects of autophagy on the cancer at the genetic and cellular level, as well as *in vivo*."

He noted that the team's results were in a *K-Ras*–driven model of pancreatic cancer. Thus, said Rehnmark, the data are especially relevant because activating *K-Ras* mutations are frequently found in human pancreatic and other cancers.

Clues from the clinic

Kimmelman's team has started a Phase II trial of the chloroquine analog hydroxychloroquine to treat metastatic pancreatic cancer patients who have progressed on one or two courses of first-line chemotherapy.

Hydroxychloroquine, a generic analog of chloroquine, is approved to treat or prevent malaria and to treat rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

> **"All of these cell stress pathways are slightly different, which opens up opportunities for combination therapies to target more than one of them."**
>
> ***—Michael Palladino, Nereus Pharmaceuticals Inc.***

The doses used in the trial are comparable to those used to treat patients with autoimmune disorders and are in the range of doses the team used for its *in vitro* experiments, but Kimmelman said it is unclear whether those doses will inhibit autophagy in human pancreatic tumors.

Thus the trial will monitor markers of autophagy such as levels of LC3 and p62 (sequestosome 1; SQSTM1) in peripheral white blood cells. Although such markers will not specifically indicate whether autophagy is inhibited in the tumor, "they will let us know whether the blood levels of hydroxychloroquine are sufficient to inhibit autophagy *in vivo*" and thereby allow the researchers to infer the drug's effects on the tumor cells as well, he said.

The team is also examining how inhibiting autophagy alters metabolism in pancreatic cancer cells and whether other tumor types or subtypes depend on autophagy for survival.

Rehnmark said he would be interested to see chloroquine or its analogs combined with gemcitabine—the standard of care for pancreatic cancer—as a first-line therapy in the clinic.

Besides gemcitabine, Palladino also wanted to see chloroquine or its analogs combined with histone deacetylase (HDAC) inhibitors and proteasome inhibitors—both of which induce cellular stress by different mechanisms than autophagy inhibitors.

"All of these cell stress pathways are slightly different, which opens up opportunities for combination therapies to target more than one of them," he said.

Palladino did caution that the timing for administering agents that induce cellular stress in tumors would be critical because the various pathways do not function entirely independently of one another. Thus, studies that explore how the cell stress systems interact would reveal how to best exploit them therapeutically, he said. "That is a lot of work to do, but it will probably be necessary to attack aggressive cancers like pancreatic cancer."

"It would also be interesting to follow the effects of chloroquine on metastasis as this is the primary killer in human pancreatic cancer patients," added Michael-Robin Witt, CSO of Axcentua.

The findings are unpatented and are available for licensing or partnering, Kimmelman said.

Kimmelman's team isn't the only one pursuing hydroxychloroquine's autophagy inhibition in oncology. Last week, **University of Glasgow** researchers disclosed that they are testing the molecule in nine patients with chronic myelogenous leukemia (CML) that is resistant to standard therapy with a tyrosine kinase inhibitor.

Eli Lilly and Co. markets the nucleoside analog Gemzar gemcitabine to treat pancreatic and other cancers.

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