Cancer: stay hungry

By Lev Osherovich, Senior Writer

A study in the *Proceedings of the National Academy of Sciences* points to a new strategy to protect normal cells from the toxic effects of chemotherapy. The report, from a team at the **University of Southern California**, shows that short-term starvation boosts chemotherapy resistance in normal cells but not in tumors.¹ Academic researchers are starting a clinical trial of the starvation therapy and hope to commercialize their discoveries through a new company they are forming.

The findings have piqued the interest of at least one cancer company, **Threshold Pharmaceuticals Inc.** Charles Hart, director of biology at Threshold, said the *PNAS* paper points to a conserved mechanism in eukaryotes for boosting drug tolerance through dietary restriction.

Chemotherapeutics poison growth-related processes, typically elevated in tumor cells, such as DNA replication and anabolic metabolism. However, toxicity to normal dividing cells is an unavoidable side effect. Thus, there is a need for adjuvants that improve the therapeutic index of chemotherapeutics, which is the relative toxicity to tumors vs. to normal cells.²

Lead author Valter Longo, associate professor of gerontology and Alzheimer's disease research at USC, told *SciBX* that the unexpected cancer finding arose from his lab's primary interest in how organisms fed a calorie-poor, near-starvation diet display longer survival and greater stress resistance than fully fed controls.³

The process of dietary restriction is thought to enhance longevity and stress resistance through signaling pathways that slow down growth in anticipation of lean times ahead. Longo's group and others previously identified some of these pathways in yeast.^{4,5} Among these are the Ras and Akt/S6K pathways, which are often deregulated in tumors.

The team began by testing whether dietary restriction could protect yeast cells from oxidative stress. The researchers used mutant yeast strains that mimic the effects of dietary restriction. When cultured in the presence of the generic chemotherapeutics methyl methanesulfonate or cyclophosphamide, the mutant cells proved more resistant to oxidative stress than the wild-type cells.

Longo then hypothesized that tumor cells do not pick up on starvation cues and would thus gain no benefit from dietary restriction.

"Cancer cells do not need growth factors to grow and they don't obey orders to stop," he said. Exploiting this recalcitrance to boost the therapeutic index of chemotherapy "was the first thing that came to mind," he added.

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University of Southern California**

Longo's team examined how chemotherapeutics affected the survival of primary cultured glial cells compared with the survival of glioma cell lines under conditions of dietary restriction. Growth in low-glucose medium for 24 hours protected the primary cultured cells but not the tumor cells from high doses of cyclophosphamide.

Similar results were seen in mice injected with human neuroblastoma cells. Mice deprived of food for 48 hours tolerated higher doses of etoposide and survived significantly longer than fully fed control mice. Etoposide is a generic alkylating agent that inhibits topoisomerase II.

"This paper is excellent in terms of in the number of different systems tested, from yeast to primary cell culture to mice to human tumor xenografts," Threshold's Hart told *SciBX*. "But to me the *in vivo* animal studies were most dramatic."

Mice that had undergone short-term starvation "could tolerate lethal doses of etoposide," he noted.

Unknown mechanism

What isn't yet known is the mechanism by which starvation induces tolerance to chemotherapy.

Peter DiStefano, CSO of **Elixir Pharmaceuticals Inc.**, told *SciBX* that Longo's findings are hard to interpret without a specific molecular target in hand.

"Short-term starvation is probably triggering a program that improves stress resistance," he said. "I think it's really important, but mechanistically, I just don't know how it works."

DiStefano suggested that in addition to the Ras and Akt/S6K pathways, there might be other stress-related and diet-related mechanisms at play

in Longo's experiment. These could include insulin signaling, adenosine monophosphate kinase, mammalian target of rapamycin and sirtuins.

Dietary restriction-induced chemotherapy resistance in healthy cells could result from modulation of any or all of these pathways, he said.

"We think there are lots of nodes in the network, and nobody has a single smoking gun," said DiStefano. "They're all important."

According to DiStefano, the next step would be to repeat the dietary restriction experiments in mouse models that have disruptions in each of these known pathways. Mutants that do not have improved chemotherapeutic resistance in response to starvation would point to the most relevant biological targets for pharmaceutical development.

Elixir is interested in dietary restriction as a possible approach to lowering blood sugar. Elixir's lead compound is mitiglinide, a potassium channel blocker that stimulates insulin secretion. Mitiglinide, which is marketed in Japan by **Kissei Pharmaceutical Co. Ltd.** as Glufast, is in Phase III testing for type 2 diabetes in the U.S.

Another company, **Sirtris Pharmaceuticals Inc.**, is also developing compounds that mimic dietary restriction—lowering blood glucose levels in hopes of treating type 2 diabetes and, potentially, aging. The lead compound is SRT501, a formulation of resveratrol that agonizes the sirtuin family histone deacetylase SIRT1. SRT501 is in Phase I testing for type 2 diabetes. Sirtris is being acquired by **GlaxoSmithKline plc**.

Neither Elixir nor Sirtris has tested its compounds as chemotherapy adjuvants. Elixir's DiStefano told *SciBX* that without a specific drug target, he doubts that this is something his company "can sink our teeth into."

Threshold's Hart also suggested it will be necessary to test the effect of short-term starvation on the pharmacodynamics and bioavailability of chemotherapeutics.

Hart also wants to know whether short-term starvation improves the therapeutic index of chemotherapeutics that work by different mechanisms than etoposide, such as "antimetabolites, growth factor receptor antagonists, microtubule-targeting agents and kinase inhibitors."

USC's Longo said short-term starvation does protect normal cells from chemotherapeutics that were not reported in the *PNAS* article. "I expect that it will work with the majority but not necessarily all chemo drugs," he said.

Hart said Threshold's 2DG, a nonmetabolizable glucose analog, has been shown to be a pharmacological mimic of dietary restriction. He said 2DG can trigger dietary restriction-like physiological effects,⁶ in addition to killing cancer cells.

The *PNAS* results are "really in our arena," Hart said. 2DG is in Phase I trials to treat solid tumors. Hart added that in Threshold's trial, 2DG is primarily acting as an antiglycolytic agent, not as a mimic of dietary restriction.

Starved for attention

Researchers from both industry and academia told *SciBX* that regardless of the actual mechanism by which short-term starvation improves the therapeutic index of chemotherapies, the approach published in *PNAS* has a clear route to the clinic. They noted that, instead of starving cancer patients, compounds that mimic the effects of dietary restriction could be used in combination with chemotherapy.

"I think this study raises the exciting possibility that short-term fasting could be useful in combination with chemotherapy in people," said Matt Kaerberlein, assistant professor of pathology at the **University of Washington**. "I'm cautiously optimistic about this approach. I'm not sure there are any significant barriers to bringing this to clinical trials."

Longo said the results warrant clinical follow-up. He is collaborating with clinicians to start a 20-patient trial to test the effect of short-term starvation on chemotherapeutic toxicity in blood cells and other

tissues. The trial will be run at the **Norris Comprehensive Cancer Center** at USC and will exclude patients with cachexia, a wasting syndrome.

Cachexia aside, fasting poses a risk to already weak cancer patients, and Longo agreed a pharmacological mimic of dietary restriction is a more attractive option. Moreover, a therapeutic could produce greater levels of protection by homing in on the specific effectors of dietary restriction.

"As you move to the dietary restriction drug targets, you develop more specificity," he said.

Longo said his group has identified two promising drug formulations that "maintain a broad effect against multiple chemotherapeutics," thus mimicking the effects of the starvation treatment. The two drugs are marketed for undisclosed indications, but Longo said USC has patented the use of these formulations as well as short-term starvation as an adjuvant to cancer treatment. The patent also covers the modulation of the genetic pathway that mediates the effect of short-term starvation, according to Longo.

Longo has founded a company, **DSR Pharmaceuticals Inc.**, to commercialize the findings reported in *PNAS*. A forthcoming publication will describe the company's lead compounds and their mechanism of action, he said. Meanwhile, Longo is seeking investors for DSR.

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COMPANIES AND INSTITUTIONS MENTIONED

DSR Pharmaceuticals Inc., Los Angeles, Calif.
Elixir Pharmaceuticals Inc., Cambridge, Mass.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Kissei Pharmaceutical Co. Ltd. (Tokyo:4547), Nagano, Japan
Norris Comprehensive Cancer Center, Los Angeles, Calif.
Sirtris Pharmaceuticals Inc. (NASDAQ:SIRT), Cambridge, Mass.
Threshold Pharmaceuticals Inc. (NASDAQ:THLD), Redwood City, Calif.
University of Southern California, Los Angeles, Calif.
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