ROS overload

By Joanne Kotz, Senior Editor

Boston researchers have identified a molecule that kills tumors by taking the already high level of reactive oxygen species in cancer cells and upping it further. The molecule had efficacy in a wide range of mouse models of cancer,[1] and the scientists have formed **Canthera Therapeutics Inc.** to develop drug-like analogs and identify genetic biomarkers for predicting patient response.

The discovery of the reactive oxygen species (ROS)-boosting molecule started unexpectedly with a cell-based screen for compounds that could modulate p53 activity.

p53 is a tumor suppressor protein that is inactivated in many cancers. To look for small molecules that could increase the activity of p53, a team led by Anna Mandinova, Sam Lee and Stuart Schreiber conducted a screen for compounds that could activate p53-dependent gene expression in cells. The top hit from this screen was piperlongumine, a plant-derived natural product.

Mandinova is a faculty member and Lee is associate director of the Cutaneous Biology Research Center at **Massachusetts General Hospital**. Schreiber is a founding member of the **Broad Institute of MIT and Harvard** and a professor in the chemistry and chemical biology department at **Harvard University**.

In a panel of cancer cell lines, piperlongumine induced apoptosis, whereas vehicle did not. Surprisingly, the researchers found the compound induced cell death regardless of whether the cancer cell contained wild-type or mutant p53.

"We were looking for a p53 activator," said Lee. "We thought that we had found a p53-specific activator, but then we saw that piperlongumine could act independently of p53." Thus, the team next wanted to determine how the compound was killing cancer cells independently of p53 mutational status.

In an *in vitro* proteomic assay, piperlongumine bound a handful of proteins involved in the cellular response to oxidative stress, including glutathione S-transferase π1 (GSTP1) and carbonyl reductase 1 (CBR1).

This led the team to look at the effect of the molecule on oxidative stress in cells. In human cancer cells, but not in normal cells, piperlongumine increased ROS levels.

Compared with normal cells, many cancer cells have high levels of ROS. Excessive levels of ROS cause oxidative stress, which triggers apoptosis if left unchecked. To avoid apoptosis, cancer cells adapt to oxidative stress conditions by increasing the expression of antioxidant proteins.[2]

Collectively, the team's results suggest piperlongumine acts by blocking this antioxidant pathway in cancer cells.

Finally, the team tested the activity of the molecule *in vivo*. In mouse models of melanoma and bladder, breast and lung cancer, piperlongumine lowered tumor growth compared with vehicle, with no evidence of acute toxicity.

Data were published in *Nature*.

Dialing up ROS

Mandinova, Lee and Michael Foley, director of the Chemical Biology Platform at the Broad Institute, formed Canthera in 2009 to pursue these results.

"Piperlongumine is nearly insoluble. We had a tough time doing the *in vivo* experiments," said Mandinova. Canthera has developed analogs that are "substantially more water soluble, with better bioavailability and tolerability," she added.

"The antitumor effects achieved with piperlongumine are really intriguing, as the results to date suggest the promising selectivity of the agent for killing tumor versus normal cells," said Paul Workman, deputy CEO and director of the **Cancer Research UK** Cancer Therapeutics Unit at **The Institute of Cancer Research**.

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> ***—Paul Workman, The Institute of Cancer Research***

"There is pretty good evidence in the paper that the agent is exploiting a vulnerability in the homeostatic control of ROS, which cancer cells seem to become susceptible to following transformation by oncogenes. The mechanism of action is a really intriguing and potentially very exciting one. As the authors state, there has been work in this general area of redox control before with agents that, for example, deplete glutathione, but this ROS effect is different, and the new paper will reactivate and encourage interest," noted Workman.

"This is a nice study, as the authors have demonstrated the value of phenotypic-based screening for identifying novel mechanisms in oncology," said Ray Tabibiazar, president and CEO of **Ruga Corp.**, which targets tumor adaptation pathways including cancer metabolism and endoplasmic reticulum stress. He noted that "the *in vivo* data look very promising. Piperlongumine shows robust tumor growth inhibition."

"Targeting stress responses is a critical novel approach for cancer therapeutics," said Tabibiazar. In tumors, stress responses are upregulated either by stresses from the microenvironment or as a result of stress induced by oncogenic mutations that increase the cell's metabolism.

This increased stress can be exploited therapeutically. For example, Velcade bortezomib leads to cancer-selective effects because the tumor is already under significant stress, and the increased protein load caused by inhibiting the proteasome "pushes them over the edge and leads to tumor cell killing," he added.

Velcade is marketed in the U.S. by **Takeda Pharmaceutical Co. Ltd.**'s **Millennium Pharmaceuticals Inc.** subsidiary to treat multiple myeloma (MM) and mantle cell lymphoma (MCL).

One compound with a similar mechanism of action is elesclomol, a mitochondrial metabolism inhibitor that increases ROS levels to trigger apoptosis in cancer cells. **Synta Pharmaceuticals Corp.** has elesclomol in Phase II trials for ovarian cancer and anticipates initiating a Phase II trial in non–small cell lung cancer (NSCLC) in 2012. The compound is in Phase I studies for acute myeloid leukemia (AML).

"ROS are a byproduct of cancer cell metabolism and a key element of cancer cell signaling," said Safi Bahcall, president and CEO of Synta. "This paper moves the ROS induction field forward with another compound that selectively elevates ROS to trigger apoptosis preferentially in cancer cells versus normal cells."

Targets and markers

The critical next steps will be better understanding piperlongumine's mechanism of action and determining markers that predict response to the molecule.

"From a development standpoint, the lack of a precise mechanism of action and a marker of activity will make it challenging to optimize the molecule and establish a pharmacokinetic/pharmacodynamic relationship," said Tabibiazar.

"It will be really essential to identify the precise molecular target of piperlongumine," agreed Workman. He noted that he also would like to see a more extensive analysis of piperlongumine in much larger panels of hundreds of cancer cell lines as well as more extensive studies in various *in vivo* mouse models "as a means to identify the most sensitive tumors and their genotypes."

Because there are so many proteins involved, one of the challenges in targeting broad pathways in cancer cell metabolism is that it may be trickier to find genetic biomarkers, said Bahcall. He noted that for the trials of elesclomol, Synta is selecting patients based on the activity of specific enzymes in the serum that provide a marker for mitochondrial metabolic activity.

The Boston team is now looking to further define the compound's mechanism of action and to decipher the genetic factors that determine piperlongumine response. "We are determining the genetic features of human cancers that correlate with hypersensitivity to this compound with the hope of identifying the optimal patient population," said Schreiber.

Initially, the researchers have seen some increased sensitivity to the compound in cancers characterized by mutations in *β-catenin* (*CTNNB1*), said Mandinova.

More than 95% of colon cancers, as well as a subset of lung cancers, have *β-catenin* mutations. Thus, these could be initial patient populations to target, added Lee.

Mandinova said Massachusetts General Hospital has filed one patent application covering piperlongumine for cancer indications, and the IP is licensed by Canthera. The company has filed an additional patent application covering composition of matter for piperlongumine analogs.

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