UPDATE

High-Throughput Screening Finds Potential Killer of Cancer Stem Cells

By Karen Rowan

The search for agents that target and kill cancer stem cells is on. In August, researchers reported in Cell that high-throughput screening could be used to identify drugs that target these cells, notorious for their resistance to existing treatments and their putative ability to generate new tumors.

Killing cancer stem cells may be the key to preventing cancer’s recurrence, say researchers who have confidence in the cancer stem cell model of carcinogenesis. The model holds that only a specific subset of cancer cells can give rise to new cells or metastases, and some say this explains why cancers can seem to disappear after treatment and then recur with a vengeance. However, there is a debate about how relevant such cells are to cancer treatment and even whether they exist (see related article, News, J. Natl. Cancer Inst. 2009;101: 546–7). Those who believe that the model oversimplifies the complexity of human cancers are wary of this study’s results.

The successful use of high-throughput screening in finding the agents depended on developing a stable, in vitro stem cell culture, said Tamer Onder, Ph.D., who co-led the work with fellow researcher Piyush Gupta, Ph.D. of the Massachusetts Institute of Technology, Cambridge, Mass. “It’s hard to maintain the cancer stem cell state in culture—that’s why it’s been hard to find therapies that target them,” said Onder, now at Children’s Hospital Boston. “Stem cells isolated from patients either do not grow in culture, or when they do, they lose their characteristics.”

Onder said the key to the culture was to induce the cells to undergo an epithelial–mesenchymal transition (EMT), which the team accomplished by inhibiting the cells’ expression of E-cadherin. (Although the link is not completely understood, induction of the EMT activates the same transcription factors that give cancer cells the motility, ability for self-renewal, and resistance to apoptosis that marks cancer stem cells.) The resulting cells displayed three characteristics of cancer stem cells: They could form mammospheres in culture, had two known genetic markers of stem cells (high expression of CD44 and low expression of CD24), and could generate new tumors when injected into mice at a much lower dilution than could the control cells.

About 16,000 compounds were then screened for their ability to kill the stem cells at a greater rate than the control cancer cells. The screen turned up 32 such compounds. The researchers winnowed the results down to the most promising and focused on one called salinomycin. Cancer stem cells treated with salinomycin were much less able to form new tumors when injected into mice. Further, mice previously injected with other human breast cancer cells and treated with salinomycin had tumors that were smaller and contained fewer cancer stem cells than mice treated with a control drug (paclitaxel).

“We were happy to see that the tumors shrank and had fewer cancer stem cells,” said Onder. “This was the proof of principle for us.”

Clever or Too Simple?

“I think this study was very clever,” said Max Wicha, M.D., director of the University of Michigan Comprehensive Cancer Center, Ann Arbor, who worked on the 2003 study in which breast cancer stem cells were first isolated and later cofounded OncoMed, a company developing drugs aimed at cancer stem cells. The most important part of the research, he said, was not the specific drugs that were found but rather the demonstration that it was possible to enrich a culture with cancer stem cells by sending cells through the EMT. Wicha’s group is working on a different way of finding agents that target cancer stem cells. In their approach, genes turned on in cancer stem cells are engineered to express a fluorescent protein, and then single-stranded RNA molecules are screened for their ability to kill the fluorescing cells.

A particularly noteworthy aspect of the current finding, said Wicha, was that nearly all of the 16,000 compounds tested, including existing chemotherapy drugs, left most stem cells intact. Ninety-eight percent of the cells in a tumor may be differentiated cells, and current screens look for compounds that kill most cells in a tumor. The result is that we have developed chemotherapies that are selectively toxic to differentiated cells, but these therapies ultimately fail, he said, because they leave stem cells behind.

The finding that salinomycin is toxic to stem cells also furthers our understanding of cancer stem cell biology, Wicha noted. Salinomycin interferes with normal potassium channel regulation, so further exploration of this mechanism might yield clues to cancer stem cell physiology.

But Kornelia Polyak, M.D., Ph.D., associate professor of medicine at Harvard Medical School in Boston, said that the study may not reflect what happens in people. “A human cancer is much more complex and has many genetic changes,” said Polyak, who believes that cancer cells are more varied in their ability to form new tumors than the cancer stem cell model proposes. And in human tumors, the cancer stem cells and the more differentiated cells are found all together, which could affect how they will react to treatments. “The culture created in this study does not mimic this complexity,” she said.

Polyak added that the work showed that it is possible to develop a screen for drugs
that will work on cells with a particular phenotype. But she would like to have seen testing in more breast cancer cell lines. Most in vitro studies are not predictive in humans, but ones that are use a large panel of samples, she said. And the screening technique that the researchers developed does not reveal the drug’s mechanism of action or the target of the drug within the cell. “You don’t know the side effects, which patients will respond, or why this drug kills the cells,” Salinomycin, she noted, is toxic in people.

Polyak also doubts that killing stem cells would stop tumor growth. “One very important point is that even if you could develop a drug that could target the cancer stem cells, that will not eliminate the cancer,” she said. That’s because each cell’s phenotype—whether it is a cancer stem cell or a more differentiated cell—is plastic and can change.

Onder and Wicha agree that successful cancer treatments will probably involve agents to kill both stem cells and other cancer cells. “Regular cancer cells might convert into cancer stem cells upon therapy or extracellular signals,” said Onder. “Combination treatments would be best.”

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