Melanoma-initiating cell identified

June 30 2010

Scientists at the Stanford University School of Medicine have identified a cancer-initiating cell in human melanomas. The finding is significant because the existence of such a cell in the aggressive skin cancer has been a source of debate. It may also explain why current immunotherapies are largely unsuccessful in preventing disease recurrence in human patients.

"These cells lack the traditional melanoma cell surface markers targeted by these treatments," said post-doctoral fellow Alexander Boiko, PhD. "Without wiping out the cells at the root of the cancer, the treatment will fail."

Boiko is the first author of the research, which will be published in the July 1 issue of *Nature*. He works in the laboratory of Irving Weissman, MD, the director of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. Weissman is the medical school's Virginia & D.K. Ludwig Professor for Clinical Investigation in Cancer Research and the senior author of the research. He is also a member of the Stanford Cancer Center.

The cancer stem cell theory holds that, like queen bees in a hive, only a subset of cancer cells are at the root of the tumor's growth. These cells can both self-renew (that is, make more of themselves) and differentiate into other tumor cell types.

Any therapy that doesn't wipe out these elite cancer stem, or initiating, cells has no chance of completely eradicating the disease even if it

destroys nearly all other tumor cells. That's why, say proponents, it can be relatively easy to get a patient into remission, but extremely difficult to prevent the cancer stem cells from roaring back and causing a relapse months or years later.

Cancer stem cells were first identified in blood cancers, but have since been identified in a number of solid tumors including bladder, brain, breast and colon cancers. Previous studies in the laboratory of assistant professor of radiation oncology Maximilian Diehn, MD, PhD, in collaboration with the laboratories of Weissman and Stanford colleague Michael Clarke, MD, have indicated that cancer stem cells may be more resistant than other cancer cells to many common treatments like radiation and some chemotherapies. Clarke is the Karel H. and Avice N. Beekhuis Professor in Cancer Biology at the medical school and both Diehn and Clarke are members of the Stanford Cancer Center.

Although a growing body of evidence seems to support the cancer stem cell hypothesis, melanoma has remained a conundrum. A University of Michigan study in 2008 found that as many as one in four melanoma cells could cause cancers in immune compromised mice, suggesting that there may not be a particularly privileged subset of cancer stem cells in this tumor type. Boiko set out to solve the mystery.

"I didn't know if melanoma would in fact have the cancer-initiating cells," said Boiko. "I was completely unbiased, so I was actually sort of surprised to find such a clear-cut answer. It fits exactly what's been discovered in the studies of other solid tumors."

To conduct the study, Boiko analyzed cell surface markers on primary melanoma tumor samples taken directly from patients at the Stanford Cancer Center. In this way, he avoided having to grow the cells for a long period of time in the lab. Continuous culturing, or passage, of cancer cells often gives the cells time to evolve and change in ways that

might not accurately reflect their composition in melanoma patients.

He found that one protein, called CD271, was always expressed on only a fraction of the cells in the human melanoma samples tested: The proportion of cells expressing CD271 varied in the samples from 2.5 to 41 percent of the total cell population; the marker appeared on a mean of 16.7 percent of cells in the samples.

This was interesting because CD271 was previously identified as a marker that identifies a group of cells called the neural crest stem cells. These cells are unique in that they are a multipotent, migratory cell population that becomes many cell types during development including melanocytes (cells responsible for skin pigmentation), bone, smooth muscle, neurons, and cartilage in the head and face.

When Boiko transplanted the melanoma cells from nine human samples into laboratory mice with severely compromised immune systems, he found that the cells expressing CD271 on their surface were much more likely to cause cancers in the recipients than those from the same tumor that didn't express the marker (70 percent versus 7 percent, respectively). And all but one of the newly induced tumors arising from the transplantation of the CD271-positive cells went on to develop a population of a mixture of CD271-expressing and non-expressing cells — indicating that the cells with the marker were both self-renewing and differentiating into other types of tumor cells.

Boiko then collaborated with researchers in the medical school laboratories of professor of surgery Michael Longaker, MD, and assistant professor of surgery George Yang, MD, to further test the tumor initiating properties of the cells expressing CD271. They transplanted normal human skin on to the backs of the immunocompromised mice and injected the skin with the melanoma cells. Only cells expressing CD271 (isolated from melanomas from two

patients) gave rise to tumors and lung metastasis in the mice.

Finally, the researchers looked to see whether the cancer-initiating cells also expressed common cellular antigens currently used for melanoma therapy. They found that melanoma cells expressing CD271 either completely or partially lacked expression of three common therapeutic targets — TYR, MART and MAGE — in 86 percent, 69 percent and 68 percent of melanoma patients, respectively.

"This could be the reason why we often see melanoma patients relapsing and coming back to the clinic," said Boiko. "Our research indicates that it may be more appropriate to also target cells expressing CD271." Such a combination therapy might work to kill both types of cells in the tumor and, hopefully, prevent disease recurrence.

So why do some melanoma tumors seem to have such a large proportion of cancer-initiating cells? Boiko and his colleagues speculate that the answer might lie in the rapidly evolving, aggressive nature of the disease. It's possible that a kind of natural selection among the dividing cancer cells occurs which, in extreme cases selects for one clone, or cell lineage, that for the most part fails to differentiate into non-tumor-initiating cells.

"Extreme care should be taken to identify the origin and the past laboratory history of the cells under study," said Boiko. "Our work was done on primary and minimally passaged patient samples. The variations that we saw in terms of CD271 prevalence in these samples could depend on the stage of the disease the patient was in, and the aggressiveness of that individual cancer."

In addition to Boiko, Weissman, Longaker and Yang, other Stanford researchers involved in the work include post-doctoral fellows Olga Razorenova, PhD, and Daphne Ly, MD; professor of pathology Matt van de Rijn, MD; professor of dermatology Susan Swetter, PhD; associate

professor of surgery Denise Johnson, MD; Paris Butler, MD; otolaryngologist Benzion Joshua, MD; and professor of otolaryngology and neurosurgery Michael Kaplan, MD.

Provided by Stanford University Medical Center