MedicalXpress

Seven-year study pays off with 'most detailed' picture of head and neck cancer stem cells to date

September 15 2016

Cancer stem cells resist therapy and are a major cause of relapse, long after the bulk of a tumor has been killed. A University of Colorado Cancer Center study published in the *Journal of the National Cancer Institute* provides the most comprehensive picture to date of head and neck cancer stem cells, identifying genetic pathways that cancer stem cells hijack to promote tumor growth and visualizing the process of "asymmetric division" that allows a stem cell to create tumor tissue cells while retaining its own stem-like profile. The study is the result of seven years of research and innovation, including the development of novel techniques that allowed researchers to identify, harvest, and grow these elusive stem cells into populations large enough to study. This major body of work provides specific targets for the development of new cancer therapeutics.

"We wanted to determine the relationships between key genetic alterations and how head and neck cancer stem cells harness those alterations to drive initiation and growth," says CU Cancer Center investigator Antonio Jimeno, MD, PhD, the Daniel and Janet Mordecai endowed professor for cancer stem cell research, director of the University of Colorado School of Medicine's Head and Neck Cancer Clinical Research Program, and the paper's senior author. The current project was performed in collaboration with the Gates Center for Regenerative Medicine of which Dr. Jimeno is a faculty member. Jimeno started his work with cancer stem cells as a post-doc at Johns

Hopkins University, but as he explains, "I focused on head and neck cancer stem cells because there has been an increase in head and neck cancer incidence of about fifty percent over the past ten years in the U.S. and we need to better understand what is at the root of this disease."

Previously, a major challenge in characterizing cancer stem cells has been gathering a cell population large enough to study.

"There is a lot of 'noise' in cells and you need a lot of them because with only a few cells, it's impossible to tell which of these genetic differences are meaningful features of cancer stem cells and which are just genetic noise," says first author Stephen Keysar, PhD, research assistant professor in the Jimeno lab.

To solve this problem, the group first gathered tumor samples from a larger number of head and neck cancer patients – 10 patients in all – more than in any previous study. These samples represented both tumors associated with alcohol and tobacco use and tumors caused by the human papilloma virus (HPV).

"It is important to always remember that we were able to make a difference thanks to the generosity of our patients, who enabled us to work with representative cancer models," Jimeno says.

These tumors were then grown in mice. Subsequently, the group undertook the painstaking process of isolating enough cells for genetic studies and one-by-one transplanting these patient-derived tumor samples onto new mice to study how cancer stem cells initiate tumor growth.

"Sometimes it took a year just to get enough cells to study," Keysar says.

"Antonio is a great example of perseverance," says Dennis Roop, PhD,

director of the Gates Center and also an investigator at the CU Cancer Center and the individual whom Jimeno credits with "much of the philosophy behind this work." "Antonio was submitting all these grants, and the reviewers were saying, 'There's no way you can do this; there's no way you'll get enough cells to characterize.' He simply found ways to prove them wrong."

This included leveraging private research funding, primarily from the Gates Center for Regenerative Medicine, the Daniel and Janet Mordecai Foundation and the Peter and Rhondda Grant Fund.

"Private funding allowed Antonio to do the groundwork and develop the techniques that eventually made his proposals to the NIH so compelling that he was able to get support. In the case of those of us who are driven to do what we do, you just find a way to get these things accomplished. This is a great example of how bridge funding from the private sector can move research forward," Roop says.

Here is what the group found:

- First, head and neck cancer stem cells are, in fact, distinct from the rapidly dividing cells that form the bulk of tumors, and there is little difference between cancer stem cells in HPV- and HPV+ cancers. Both are marked by CD44 expression and aldehyde activity, and both use the key pathway PI3K to drive their survival, growth and resistance to anti-cancer therapies.
- The group found that the PI3K pathway, which is the most common alteration in head and neck cancer, then deploys SOX2, a transcription factor, to activate programs that modulate 'stemness' within the cell's nucleus. For example, SOX2 was found to control aldehyde activity, which is a common cancer stem cell marker and a well-known driver of cancer stem-cell-mediated tumor growth.

"In normal cells, PI3K is used as a sensor for energy," Jimeno explains. "For a cancer cell to act cancerous, it needs metabolic flexibility – it needs to be able to over-use energy – and so this 'energy sensor' is a pathway it wants to hijack. After chemo, PI3K helps the cell shut down and weather the storm. Then when the chemo is gone, PI3K helps cancer stem cells start back up again."

Chemotherapies kill rapidly-dividing cells. PI3K shuts down a cancer stem cell's metabolism, placing the cell in a dormant state. This gives cancer stem cells the ability to evade the trap of chemotherapy.

So what happens when you remove this ability? When the group eliminated SOX2 in mouse models of head and neck cancer, tumors became sensitive to therapies that previously had failed. But when the group amplified SOX2, tumors became even more resistant.

"This molecular thread from PI3K to SOX2 to aldehyde was responsible for all the features that define cancer stem cells," Keysar says. Further, "Since SOX2-expressing cells fully behave like cancer stem cells, we now have a new laboratory tool to study cancer stem cell biology and therapeutics."

The work also allowed the group to witness an event of the stem cell cycle that had, at best, been only partially characterized in head and neck cancer.

"It was like the snow leopard of the Himalayas," Jimeno says. "We knew it existed because of the tracks, but no one had taken a picture of it – that is, until someone patiently perched on a frozen ridge for two years with a camera. We did just that."

The event Jimeno refers to is "asymmetric division" of cancer stem cells. When a normal cell divides, it creates two identical copies of itself.

However, if stem cells divided symmetrically, it would result in two stem cells but no differentiated cells, or two differentiated cells with the loss of the original stem cell. In either case, symmetrically dividing stem cells would not be able to promote tumor growth while also retaining their stemness.

The group was able to document that when cancer stem cells divide, "they don't divide into two of the same," Jimeno says. "One cell retains a stem profile, and the other goes a step beyond into differentiation."

Overall, this seven-year line of inquiry offered three major advances: it characterized head and neck cancer stem cells; it documented asymmetric division in head and neck cancer stem cells; and it identified genetic mechanisms that allow these cancer stem cells to grow and resist therapy. Importantly, identifying these genetic mechanisms of resistance may also help researchers and doctors overcome it.

"SOX2 and aldehyde inhibitors are now under exploration, and we've also done trials of early PI3K inhibitors here at CU Cancer Center," Jimeno says.

"This has been an excellent example of team science," Roop says. "You have Antonio – a brilliant young clinician-scientist – leading a group that includes basic scientists, pathologists, bio-informaticians and statisticians, and their expertise can combine to attack a problem in a way that no individual would be able to do on their own. This work will provide the basis for the development of new therapeutic strategies."

Provided by University of Colorado at Boulder