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New Finding Re-opens the Book on Colon Cancer Stem Cells

Not all cancer cells are equal. Over the past 10 years, researchers have come to believe that cancer stem cells are scattered thinly through tumors, and that they must be destroyed to halt tumor growth. If even the smallest number of cancer stem cells remains, tumors can come roaring back and sometimes spread to distant organs. But the challenge for researchers has been how to find a marker that is unique to the cancer stem cells.

New research by Howard Hughes Medical Institute (HHMI) scientists underscores just how difficult it is to identify unique markers of cancer-initiating cells. The experiments show that a protein previously thought to identify only colon cancer stem cells is actually prevalent throughout primary colon tumors but absent in some cells that initiate metastatic colon cancer.

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— **Shahin Rafii**

Shahin Rafii, an HHMI investigator at Weill Medical College of Cornell University, Sergey Shmelkov, and Jason Butler, who are both postdoctoral fellows in Rafii's lab, and their Weill-Cornell colleagues published their findings May 23, 2008, in the *Journal of Clinical Investigation*. The group collaborated with researchers from Memorial Sloan-Kettering Cancer Center and Regeneron Pharmaceuticals.

Cancer biologists believe that cancer stem cells, like other adult stem cells, can both divide into new stem cells and differentiate into more specialized cells. So, Rafii says, if you don't get rid of those few cancer stem cells—even if you kill [the other] 95 percent of the tumor—then the cancer is going to continue to grow. He says these cells may also be the only part of a tumor

that is able to invade surrounding tissue and spread (metastasize) to other parts of the body.

There has been a lot of excitement in identifying and characterizing cancer stem cells, says Rafii. However, he says, the methods that scientists currently use to purify cancer-initiating cells can give variable results. A better approach is needed, he says.

For this, many researchers have looked to the varied proteins that cover the cells' surfaces. These molecules are useful markers for the identification and purification of many different types of cells, and researchers have searched for one that is found only on cancer stem cells. In early 2007, other researchers reported that colon cancer cells with a protein on their surface called CD133—which has been used for several years to identify normal stem cells and cancer stem cells in other tissues—appeared to be the main initiators of colon cancer.

However, these studies were primarily performed using commercial antibodies that may not recognize the full gamut of CD133 surface expression. Antibodies are molecules of the immune system that recognize specific molecules on cells, and allow the body to tell the difference between its own tissues and invading pathogens. Rafii thought the technique might have missed large numbers of cells expressing the protein.

He says that is a problem, because cancer stem cells are thought to comprise just a small fraction of tumors. So one would expect that if indeed CD133 is a colon cancer stem cell marker, then only a subset of tumor cells should express it, says Rafii. That's what the previous studies had found.

But Rafii and his group, who had significant prior expertise working with pathways that regulate CD133 expression, thought that the molecule might actually be more widely expressed, and therefore not an accurate marker for stem cells. Rafii decided to verify the results using a more sensitive genetically based method.

The group created a mouse in which they replaced the gene encoding CD133 with a reporter gene called *lacZ*. When treated with a stain, cells expressing *lacZ* turn blue, in this case allowing the team to see which cells were expressing CD133.

Unexpectedly, they discovered that CD133 on the surface of the majority of healthy intestinal cells—not just stem cells, but also in mature cells of the intestinal lining.

This finding prompted us to explore the actual contribution of CD133-expressing cells in tumor initiation and growth, says Rafii. The team examined human colon tumors and discovered that most of the tumors' cells express CD133. Their findings were similar when they used their genetically engineered mice to look for CD133 in tumors.

To do this, they crossed mice that are genetically prone to developing colon cancer with mice engineered to express *lacZ* in every CD133-expressing cell. To our surprise, we found that CD133 was expressed in virtually all of the primary colon tumor cells.

The group also looked at secondary, or metastatic, tumors, and discovered that whereas almost all primary colon tumor cells expressed CD133, not all metastatic cells did. Surprisingly, CD133-negative cells in metastatic human colon cancer to the liver were also capable of tumor initiation, says Rafii. In fact, tumors generated by cells *not* expressing CD133 tended to grow more aggressively than those originating from cells that did express the protein.

Thus, our study sets forth the concept that the origins of primary and metastatic tumors are decidedly not the same, and we must broaden our thinking beyond CD133-positive cells when it comes to the investigation of metastatic colon cancer to the liver, says Rafii.

As far as continuing the search for cancer stem cell markers, Rafii thinks other strategies may be more effective. While surface markers can make it easy to identify cells, they actually have little to do with the functional aspects of a cancer stem cell, says Rafii. So, rather than focusing on the surface markers, [we might be able to] identify markers that make the cells functionally unique—such as chemotherapy resistance. According to Rafii, that may be a better way to identify the true cancer-initiating cells that actually make treating advanced and metastatic cancers so difficult.