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## Melanoma Spawns Tumors with Deadly Efficiency

By examining the tumor-promoting properties of individual cancer cells, Howard Hughes Medical Institute scientists have found that melanoma cells spawn new tumors with deadly efficiency. The new studies show that at least one of every four melanoma cells has the capacity to seed the development of new tumors.

HHMI investigator Sean Morrison and his colleagues at the University of Michigan Medical School report their findings in the December 4, 2008, issue of the journal *Nature*. Elsa Quintana and Mark Shackleton, postdoctoral fellows in the Morrison laboratory, performed the experiments, and Timothy Johnson, director of the University of Michigan's Multidisciplinary Melanoma Program, provided melanoma samples with patients' consent.

For years, cancer researchers have debated whether most cancer cells are equally likely to cause new tumors. One prevailing hypothesis among cancer researchers is that only cancer stem cells -- a tiny subset of highly proliferative cells -- can actually give rise to tumors. As recently as January 2008, a prominent report in *Nature* estimated that as few as one in a million melanoma cells is capable of prompting tumor growth.

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Indeed, researchers have put much effort into devising new ways to identify cancer stem cells by virtue of distinctive molecular markers that may separate them from other cancer cells. Once identified, cancer stem cells may be targeted by new cancer drugs that prevent tumor formation by hitting the source of the cancer. Some have even suggested that cancer might be more effectively treated using therapies that are designed to target small subpopulations of cancer stem cells, rather than trying to eliminate all cancer cells.

A leader in stem cell research, Morrison said he has an open mind about the potential existence of cancer stem cells. But he also believes that additional work is required to detect tumorigenic human cancer cells and to assess the frequency of those cells in different types of cancer. "Identification of the full spectrum of cells with the potential to drive the growth and progression of human cancers will be required to develop more effective new treatments," he said.

Scientists normally begin experiments to assess how efficient cancer cells are at forming tumors by first injecting large numbers of cancer cells into mice with weakened immune systems. After that has been done, the researchers count how many tumors grow in the mice. They can then compare the number of tumors formed with the number of cells injected and arrive at a rough estimate of what percentage of cancer cells were able to form new tumors.

According to Morrison, researchers knew that the mice typically used for these experiments, known as NOD/SCID mice, were not perfect models. Even though they have weakened immune systems, the mice retain some defensive immune cells that can suppress the growth of human cells.

But, said Morrison, "it was thought that the assays only moderately under-estimated the frequency of cancer cells with the potential to form tumors. People thought improving the mice might improve sensitivity tenfold or a hundredfold, but they thought 'why bother doing the work to go from one in a million to one in ten thousand, since that would not change the conclusion that only rare cancer cells have the potential to proliferate extensively?'" explained Morrison.

Morrison thought it was important to continue to challenge the existing model, so his team identified multiple improvements in the assay, including transplanting human melanoma cells into more severely immune-compromised mice, called NOD/SCID IL2R $\gamma^{\text{null}}$ . These mice are missing the gene for a receptor protein required for immune cells known as natural killer cells to function properly. The team found that 250,000 times as many melanoma cells formed tumors in this modified assay as compared to the standard NOD/SCID mice.

The team tested the melanoma cells by transplanting batches of cells and - more importantly - individual melanoma cells. For batches of melanoma cells, they found that about 25 percent of the cells formed tumors. About 27 percent of the single melanoma cells formed tumors when transplanted into the NOD/SCID IL2R $\gamma^{\text{null}}$  mice.

"As far as we know, this is the first time anyone has been able to show that individual cells from human cancers can efficiently form tumors. The result provides direct support to the idea that many cancer cells are capable of proliferating extensively and forming tumors," noted Morrison. The finding

suggests that targeting just a small subset of melanoma cells will not be enough to stop the cancer from growing.

"We think the underestimation of tumor-causing cells is a general problem in many cancers, not just specific to melanoma," says Morrison. "Researchers in the field need to go back to optimize the assays they have been using and to re-evaluate the evidence underlying cancer stem cell models."

An important premise of the cancer stem cell model is that the cancer stem cells that cause tumors are distinguishable at the molecular level from other cancer cells. This individuality is an important property that scientists would theoretically be able to exploit in designing new therapies to target only tumor-causing cells. But when Morrison's team looked at 50 different molecular markers found on the surface of the melanoma cells, it could find no differences between the tumor-causing cells and those that did not spawn tumors.

"The simplest interpretation is that most melanoma cells have a 25 percent chance of forming a tumor...and that melanoma does not contain intrinsically distinct types of tumorigenic and non-tumorigenic cancer cells," said Morrison, "but it is impossible to prove that because somebody could come along and discover that the fifty-first marker does distinguish cancer stem cells from normal cancer cells."

Morrison acknowledges that it is possible that cancer stem cells are simply more common in certain cancers. But, he said, "as is often the case, the truth is probably somewhere in between the two prevailing theories.

"We expect that some cancers will follow a cancer stem cell model, while other cancers will not," he said. "Many cancers will be like melanoma, a good old-fashioned cancer in which every cell is bad."

The approaches used in the current study should help researchers analyze other cancers to more accurately estimate the frequency of cells with tumorigenic potential, as well as to study how individual cancer cells change over time. "The ability to study tumorigenesis from single human cancer cells opens new doors," Morrison said. "This does make things a lot more complicated, but cancer is a resourceful disease. To defeat it we must do the work to understand its complexity."