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Genomic Screen Captures Genes that Prevent Spread of Cancer

Cancer is deadliest when it metastasizes: spreads beyond its original site and invades distant organs. Just as cells are equipped with tumor suppressor genes to keep incipient cancers from growing, they also have genes that prevent this dangerous spread. These metastasis suppressor genes have proved difficult to find--but that may be changing.

Over the past 20 years, only a dozen or so metastasis suppressor genes have come to light. But now Howard Hughes Medical Institute investigator Michael Green has developed a systematic method for screening the genomes of cancer cells to detect likely metastasis suppressors. Green and his colleagues report the advance in the November 1, 2008, issue of the journal *Genes & Development*.

The group has tested the approach in cell lines developed from mice with metastatic melanoma and identified 22 genes which, when “knocked down” with RNA interference, allowed tumors to metastasize but had no effect on the growth of the original tumors.

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- **Michael R. Green**

One of those 22 genes prevented the spread of melanoma cells, which are highly prone to metastasis. The scientists put that gene through rigorous tests in the lab and validated it as a true suppressor of melanoma metastasis. Further work is needed to validate the remaining 21 candidates, but Green thinks they are highly likely to be suppressors of metastasis for a variety of

cancers.

“Metastasis is an important part of cancer biology, but it is a complex process and a tough problem to study,” says Green, whose lab is at the University of Massachusetts Medical School in Worcester. “The most exciting part of the paper is not necessarily that we have found this one metastasis suppressor but that we’ve developed a general approach that can be used to find others - and we can do it with any type of cancer cell.”

Like tumor-suppressor genes, those that squelch metastasis are most evident when they are damaged or altered in ways that disrupt their protective function. The first metastasis-suppressors were unearthed in the late 1980s. Reduced activity of some of those genes has been found in cancers through microarray studies that measure gene activity in cells. It is still far from clear, however, how the loss of such a gene, or genes, enables a cancer cell to do the things it must when it metastasizes: that is, operate apart from the original tumor; invade normal tissues; survive a long-distance journey through the blood stream; and establish a thriving colony in some target organ.

In devising a functional screen for metastasis-suppressing genes, Green and his colleagues, including first author Stephane Gobeil, exploited two recent technological advances--RNA interference and three-dimensional cell-culture systems.

Traditional cells cultured in sheets on flat plastic dishes fall short of replicating the natural surroundings of cells in the body, with their neighboring cells, fibrous layers, membranes, and adhesion proteins. Newly developed three-dimensional systems are a boon for studying metastasis, says Green. “They are a system that allows you to mimic early events in the metastasis cascade,” he says.

In Green's laboratory, researchers inserted balls of about 1 million cells into collagen, forming a plug that is then coated with an artificial matrix that mimics a cellular basement membrane. Next, the coated plug is embedded in a fibrin matrix within a standard Petri dish. In metastasis assays, cancer cells can be observed as they break off and migrate away from the original cell mass in all directions.

For the experiments, Green chose two mouse melanoma cell lines: One, B16-F0, is only weakly metastatic, while B16-F10 has a high potential for metastasis. The investigators used RNA interference tools--so-called small hairpin RNAs (shRNAs)--to lower the expression of genes in the nucleus of each type of cancer cell. The screen was designed to select for melanoma cells carrying shRNAs that silenced metastasis-suppressing genes, allowing the cells to form satellite colonies. The DNA of those cells was extracted and sequencing identified the shRNAs responsible --and, in turn, the genes themselves.

The screen yielded 78 genes that promoted colony formation from the weakly metastatic B16-F0 line, suggesting their knockdown removed a suppressive function. Next, the scientists administered those 78 shRNAs to the melanoma cells and injected them into the tail veins of mice. Fourteen days later, they examined the rodents' lungs for metastases, and found significant numbers of metastases in mice receiving 22 of the 78 gene-silencing RNAs. Among the 22 were several genes known to play a role in signal transduction, cell cycle regulation, or metabolism/energy pathways.

One gene of interest, *Gas1* (growth arrest-specific 1), is known to govern cell growth. It was markedly down-regulated in the metastatic melanoma cell, said Green. To test this candidate metastasis-suppressor, the researchers injected melanoma cells in which *Gas1* expression had been reduced into the footpads of mice--a more rigorous metastasis assay than the previous tail-vein injection. Those cells generated significantly more metastases in the lungs than cells with normal *Gas1* activity.

The experiments by Green and his colleagues suggest that the *Gas1* gene normally squelches metastasis by killing migrating cancer cells after they have reached their destination. It does so by turning on the programmed cell death, or apoptosis, machinery in melanoma cells, causing them to self-destruct. When *Gas1* is damaged, the tumor cells can escape their death sentence and live to form metastatic colonies of cells.

The scientists are eager to expand their *Gas1* studies to other cancers; it has been previously found to be down-regulated in breast and prostate cancer metastases as well. In addition, Green says, they have plenty of work to do in putting the remaining 21 candidate genes through their paces to see if they are true metastasis-suppressors.