Scientists discover marker to identify, attack breast cancer stem cells

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Breast cancer stem cells wear a cell surface protein that is part nametag and part bull's eye, identifying them as potent tumor-generating cells and flagging their vulnerability to a drug, researchers at The University of Texas MD Anderson Cancer Center report online in *Journal of Clinical Investigation*.

"We've discovered the first single marker for breast cancer stem cells and also found that it's targetable with a small molecule drug that inhibits an enzyme crucial to its synthesis," said co-senior author Michael Andreeff, M.D., Ph.D., professor in MD Anderson's Departments of Leukemia and Stem Cell Transplantation and Cellular Therapy.

Andreeff and colleagues are refining the drug as a potential targeted therapy for breast cancer stem cells, which are thought to be crucial to therapy resistance, disease progression and spread to other organs.

"It's been difficult to identify cancer stem cells in solid tumors," Andreeff said. "And nobody has managed to target these cells very well."

The marker is the cell surface protein ganglioside GD2. The drug is triptolide, an experimental drug that Andreeff has used in preclinical leukemia research. The team found triptolide blocks expression of GD3 synthase, which is essential to GD2production.

Triptolide stymied cancer growth in cell line experiments and resulted in smaller tumors and prolonged survival in mouse experiments. Drug

development for human trials probably will take several years.

Cancer stem cells are similar to normal stem cells

Research in several types of cancer has shown cancer stem cells are a small subpopulation of cancer cells that are capable of long-term self-renewal and generation of new tumors. More recent research shows they resist treatment and promote metastasis.

Cancer stem cells are similar to normal stem cells that renew specialized tissues. The breast cancer findings grew out of Andreeff's long-term research in mesenchymal stem cells, which can divide into one copy of themselves and one differentiated copy of a bone, muscle, fat or cartilage cell.

Andreeff has shown these mobile mesenchymal stem cells home to wounds, including tumors, making them potential carriers of cancer therapy.

An important cellular transition also comes into play.

Co-senior author Sendurai Mani, Ph.D., assistant professor in MD Anderson's Department of Molecular Pathology and Co-Director of the Metastasis Research Center, is an expert on epithelial-to-mesenchymal transition (EMT). About 85 percent of all solid tumors start in the lining of an organ, called the epithelium. Mani and colleagues at MIT showed that epithelial cells can be induced to take on stem cell properties by forcing them to undergo EMT.

"This change from stationary epithelial cells to the mobile mesenchymal stem cells is an important step in metastasis," Mani said.

Andreeff and Mani in 2010 discovered that human mammary epithelial

cells that undergo epithelial-to-mesenchymal transition act similarly to human bone-marrow-derived mesenchymal stem cells. They can home in to wounds and differentiate into the same cell types.

GD2 separates cancer stem cells from other tumor cells

In the current project, the researchers hypothesized that the cell markers expressed on the surface of mesenchymal stem cells would also be expressed on the surface of breast cancer stem cells.

They found that GD2 expression, one such mesenchymal stem cell marker, divided the breast cancer cell lines into two distinct groups: about 4.5 percent of cells were GD2-positive and about 92.7 percent were GD2-negative.

GD2-positive breast cancer cells:

- Form twice as many mammospheres, a clumping of cells considered an indicator of tumor-forming capacity, as compared to GD2-negative cells. And the spheres were three times as large.
- Migrate four times as fast as GD2-negative cells.
- Form five times as many tumors when 10 cells of each type are transplanted into mice.

GD2-positive cells also have general cancer stem cell marker

A known combination marker of cancer stem cells is high expression of CD44 and low expression of CD24 surface proteins. The researchers found 85 percent of GD2-positive breast cancer cells were CD44 high/CD24 low, while only 1 percent of GD2-negative cells shared that

characteristic.

An analysis of 12 human breast cancer tumors found an even higher correlation of 95.5 percent between GD2+ cells and CD44 high/CD24 low status.

Comparing gene expression between GD2+ cells and CD44 high/CD24 low cells revealed 100 percent correlation in the expression of 231 genes.

GD2+ cells had greater expression of genes involved in migration, invasion and epithelial-mesenchymal transition than GD2- cells. They also had a nine-fold increase in GD3 synthase, a key enzyme in the eventual synthesis of GD2.

Further experiments showed that:

- Inducing EMT raised the percentage of GD2+ cells in two breast cancer cell lines.
- Knocking down GD3 synthase cut the percentage of GD2+ cells by more than half.
- Mice injected with 1 million breast cancer cells having a small interfering RNA that blocked GD3 synthase never developed tumors even after eight weeks, while all of the control mice with active GD3S developed tumors.

Triptolide stymies tumor growth, extends survival

The researchers then used triptolide, a known inhibitor of GD3 synthase, to treat immune-deficient mice injected with breast cancer cells. Of the mice treated, 50 percent did not develop breast cancer and the other half had smaller tumors than the control mice. The treated mice also lived

longer than the controls.

GD2's function in cancer stem cells remains unclear. "As GD2 is an immune suppressant, it would be needed by cancer stem cells to counter immune cells during metastases," said first author Venkata Lokesh Battula, Ph.D., of MD Anderson's Department of Leukemia. "Inhibition of GD2 expression in cancer cells may enhance the inherent ability of immune cells to kill cancer cells."

Provided by University of Texas M. D. Anderson Cancer Center