

Insights Into the Origins of Breast Cancer and Its Putative Stem Cells



Kornelia Polyak,
M.D., Ph.D.

Study questions “cancer stem cell” hypothesis in breast cancer. But scientists identify promising cellular targets for new therapies.

A Dana-Farber Cancer Institute study challenges the hypothesis that “cancer stem cells”—a small number of self-renewing cells within a tumor—are responsible for breast cancer progression and recurrence, and that wiping out these cells alone could cure the disease.

Instead, the scientists report in the March issue of *Cancer Cell* that they have identified two genetically distinct populations of cancer cells in samples of human breast tumors—one of the types being a cell recently proposed by other scientists to be a true breast cancer stem cell.

“If the breast cancer cells were all coming from a single cancer stem cell, you might be able to cure the disease with just one drug,” said Kornelia Polyak, M.D., Ph.D., of Dana-Farber, senior author of the paper. “But our findings suggest that the tumor cells come from a ‘stem-like’ progenitor cell, and then diverge genetically, so I think you have to treat both cell types.”

The results suggest that both cell types, and probably others, are involved in the development of breast cancer. While analyzing the genetics of each cell type, the researchers discovered that the proposed “cancer stem cells” were driven by an activated molecular pathway that makes them resemble normal stem cells. Women whose breast tumors are largely made up of these “stem-like” cells are at higher risk of recurrences.

On the positive side, the abnormal activated pathway in these cells, known as the TGF- β 1 signaling pathway, can be blocked by experimental drugs now entering clinical trials, said Polyak, who is also an associate professor at Harvard Medical School. Such inhibitors, in combination with other therapies, may improve the prognosis in breast cancers fueled by these cells. Dr. Polyak is also an Associate Editor of *Cancer Biology & Therapy*.

According to a longstanding cancer model, known as “clonal evolution,” tumors arise from normal cells that mutate and generate abnormal offspring that also mutate, forming a mass of genetically varied cancer cells. However, there has been a new wave of interest in an alternative explanation—that tumors are initiated and driven by a single, abnormal type of adult stem cell found in, for example, breast tissue, resulting in a population of genetically identical tumor cells. Moreover, several pathways and genes required for normal stem cell function are activated in cancer cells and play essential roles in the development of tumors.

According to the cancer stem cell hypothesis, the few self-renewing stem cells that fuel the cancer are difficult to kill, and their persistence may explain why tumors so often recur following successful therapy.

In 2003, scientists purified what they proposed were breast cancer stem cells from patients’ tumors. The distinctive molecule, or marker,

on the cells’ surface, known as CD44+, was identical to the marker on normal breast cells. When injected into mice lacking an immune system, the CD44+ cells demonstrated the ability to initiate breast tumors. The scientists also found closely related cells with a CD24+ marker and suggested that they were offspring of CD44+ cells.

The team led by Polyak and Michail Shipitsin, also of Dana-Farber and HMS, used gene activity analysis to clarify the relationship of the two cell types. They generated gene libraries from CD24+ and CD44+ cells purified from normal mammary epithelium and fluids within the chest, and from primary invasive tumor samples collected from breast cancer patients.

The findings, the scientists reported, fit more closely with the clonal model than the cancer stem cell hypothesis. That is, the CD24+ cells were very similar to the CD44+ cells, but not always genetically identical which they would have been if the CD44+ cells were true stem cells and the CD24+ their offspring.

“Although CD44+ cells appear to express many stem cell markers, the genetic difference between CD24+ and CD44+ cells within a tumor questions the validity of the cancer stem cell hypothesis in breast cancer, and suggests clonal evolution involving intra-tumoral heterogeneity as an alternative explanation,” the authors wrote.

Moreover, the Polyak team found that the CD44+ cells, but not the CD24+ cells were driven by the activated TGF- β 1 pathway. For that reason, they said, “tumors composed of mostly CD44+ cells may have worse clinical behavior than tumors mainly composed of CD24+ cells, and these patients may benefit from therapy targeting the TGF- β 1 pathway.”

Other authors of the paper are from Harvard Medical School; Johns Hopkins University School of Medicine; GeneGo Inc.; the Vavilov Institute for General Genetics, Moscow; Harvard School of Public Health; Brigham and Women’s Hospital, and Beth Israel Deaconess Medical Center. This work was supported by Novartis Pharmaceuticals, Inc., the National Institutes of Health, and the US Department of Defense.

Dana-Farber Cancer Institute (www.dana-farber.org) is a principal teaching affiliate of the Harvard Medical School and is among the leading cancer research and care centers in the United States. It is a founding member of the Dana-Farber/Harvard Cancer Center (DF/HCC), designated a comprehensive cancer center by the National Cancer Institute.

For more information, contact: Bill Schaller and Richard Saltus; Tel.: 617.632.4090.

Transglutaminase as a Mediator of Drug Resistance

An overexpressed protein protects human pancreatic cancer cells from being forced to devour themselves, removing one of the body’s natural defenses against out-of-control cell growth, researchers at the University of Texas MD Anderson Cancer Center report in the March, 2007 issue of *Molecular Cancer Research*.

The protein tissue transglutaminase, known by the abbreviation TG2, previously has been found by researchers at MD Anderson and elsewhere to be overexpressed in a variety of drug-resistant cancer cells and in cancer that has spread from its original organ (metastasized).

Biomarkers May Predict Propensity for Angiogenesis in Ovarian Cancer

"In general, you rarely see overexpression of TG2 in a normal cell," says Kapil Mehta, Ph.D., professor in the MD Anderson Department of Experimental Therapeutics, who began 10 years ago studying TG2 as an inflammatory protein.

Mehta and colleagues in the past year have connected TG2 overexpression to drug-resistant and metastatic breast cancer, pancreatic cancer and melanoma.

Expression of TG2 is tightly regulated in a healthy cell, Mehta says, and is temporarily increased in response to certain hormones or stress factors. "However, constitutive expression of this protein in a cancer cell helps confer protection from stress-induced cell death," Mehta says. "We are developing TG2 as a pharmaceutical target and are now working with a mouse model to that end."

The mechanisms by which TG2 might promote drug-resistance and metastasis have remained elusive, the researchers note. In this paper, the MD Anderson team shows in lab experiments that inhibiting the protein in pancreatic cancer cells leads to a form of programmed cell suicide called autophagy, or self-digestion.

TG2 was inhibited in two separate ways. First, the researchers blocked another protein known to activate TG2. Secondly, they also directly targeted TG2 with a tiny molecule known as small interfering RNA tailored to shut down expression of the protein.

In both cases, the result was a drastic reduction of TG2 expression (up to 94 percent) and telltale signs of autophagy in the cancer cells, which became riddled with cavities called vacuoles.

When autophagy occurs, a double membrane forms around a cell organ, or organelle. This autophagosome then merges with a digestive organelle called a lysosome and everything inside is consumed, leaving the vacuole and a residue of digested material. If enough of this happens, the cell dies.

Gabriel Lopez-Berestein, M.D., professor of experimental therapeutics and study co-author, notes that the research also shows that the self-consuming cell death prevented by TG2 is independent of a prominent molecular pathway also known to regulate autophagy called the mammalian target of rapamycin.

"Targeting TG2, or its activating protein PKC, or both, presents a novel and potentially effective approach to treating patients with pancreatic cancer," Lopez-Berestein said. Research in the mouse model remains in the early stages, the researchers caution.

The researchers also show that the TG2 pathway also is separate from another, better known, form of programmed cell death called apoptosis.

Apoptosis, like autophagy, is a normal biological defense mechanism that systematically destroys defective cells by forcing them to kill themselves. In apoptosis, the cells die via damage to their nucleus and DNA, with other cellular organelles preserved. Autophagy kills by degrading those other organelles while sparing the nucleus.

Mehta's lab reported in a Cancer Research paper in September, 2006 that TG2 overexpression also activates a protein called nuclear factor-kB known to play a role in regulating cell growth, metastasis and apoptosis. This pathway, Mehta explained, could make TG2 an attractive target for other forms of cancer as well.

Co-authors with Mehta and Lopez-Berestein are: co-first authors Ugur Akar, Ph.D., and Bulent Ozpolat, M.D., Ph.D., and Jansina Fok, all of the Department of Experimental Therapeutics, and Yasuko Kondo, M.D., Ph.D., of the M. D. Anderson Department of Neurosurgery.

Funding for this research was provided by the National Cancer Institute of the National Institutes of Health.

Researchers identify ovarian cancer biomarkers Finding could be first step toward new screening tool, treatment target

Researchers have identified markers unique to the cells of blood vessels running through ovarian tumors. The finding, while preliminary, could one day improve screening, diagnosis and treatment for this disease.

The team of researchers from the University of Michigan, University of Pennsylvania, and universities in Greece and Italy used a laser technique to isolate blood vessel cells from 21 ovarian tumors and four normal ovarian tissue samples. From there, they were able to determine which genes the vascular cells expressed.

The results identified more than 70 markers that were present in large amounts in the blood vessels of cancer tissue but not in the vessels of normal tissues. The researchers went on to study in detail 12 markers that had not previously been linked to tumor blood vessels. The study appears in the March 1, 2007 issue of the *Journal of Clinical Oncology*.

"Some of these genes, depending on how highly expressed they were in the tumor vasculature, were also prognostic of a patient's survival. We suspect when these genes are highly expressed it may be a sign of a tumor that's able to grow blood vessels more efficiently, and therefore is more aggressive. This may help us down the road in treatment decisions," says lead study author Ronald Buckanovich, M.D., Ph.D., assistant professor of internal medicine and obstetrics and gynecology at the University of Michigan Medical School. Buckanovich was at the University of Pennsylvania when he conducted this research.

The study analyzed the largest number of samples to date in tumor vasculature, or blood vessel, profiling. While many of the genes identified in this analysis have been shown previously to be involved in tumor vasculatures for other cancer types, several of the markers appear to be new.

In addition, the researchers were able to determine that some of the markers present in large amounts in ovarian tumors were not expressed by normal ovaries or other healthy organs. The researchers also found these markers were not present in normal reproductive tissues that experience blood vessel growth, such as the placenta or endometrium. This suggests that the markers are specific to tumors and would not be mistaken for normal blood vessel growth in women of reproductive age.

If the markers do prove to be specific to ovarian tumors, researchers believe that could be a new avenue to develop drugs that would target the blood vessels and strangle the tumor.

Biomarkers are also seen in other cancer types as a potential screening tool. A new way of detecting ovarian cancer could make a significant dent in this disease, where 70 percent of patients are diagnosed after the tumor has grown large or spread. There are few or no symptoms early in the disease and no effective screening tests. Early diagnosis is crucial, marking the difference between a 95 percent survival rate for cancers found at the earliest stage and 20 percent survival among patients diagnosed with advanced disease.

"All the things we could hope for are present with this approach: It has potential for diagnosis, imaging, treatment and prognosis. It needs more work and much more confirmation, but our early results are promising," Buckanovich says.

Continued research will look at developing antibodies and methods to detect these novel proteins. "In some cases, these are genes that many people have never worked on before," Buckanovich says.

The American Cancer Society estimates 22,430 women will be diagnosed with ovarian cancer this year and 15,280 women will die from it.

The research is very preliminary at this point. Any potential screening or treatment benefit would be many years in the future. For information about currently available therapies, call the Cancer AnswerLine at 800-865-1125 or visit their web page.

In addition to Buckanovich, study authors were Dimitra Sasaroli, Anne O'Brien-Jenkins, Jeffrey Botbyl, Rachel Hammond, Lance A. Liotta, Phyllis A. Gimotty and George Coukos, all from the University of Pennsylvania; Dionysios Katsaros of the University of Turin in Italy; and Raphael Sandaltzopoulos of the Democritus University of Thrace in Greece.

Funding for the study was from the National Institutes of Health, a National Cancer Institute Specialized Program of Research Excellence (SPORE) grant, the U.S. Army Medical Research and Materiel Command Grant, the Marcia and Philip Rothblum Foundation and the Ovarian Cancer Research Fund. The laser-capture microdissection facility was supported by the Fannie Rippel Foundation.

Splice Variant of a Growth Factor Receptor Promotes Ligand-Independent Growth

New research from the University of Miami Sylvester Comprehensive Cancer Center shows that a protein receptor activated by a growth hormone plays a key role in the growth of a variety of cancers. This could have a major impact on cancers that are fueled by hormones, like most breast and prostate cancers. Nobel laureate Andrew V. Schally, Ph.D., M.D., M.S., a professor in the Department of Pathology and the Division of Hematology-Oncology at the University of Miami Leonard M. Miller School of Medicine, has long been a leader in the study of hormone-related cancers. He and his colleagues showed that a splice variant (SV1) of a hormone receptor stimulated breast cancer cells in the laboratory. The research was published in the March 12, 2007 edition of the *Proceedings of the National Academy of Sciences*.

SV1 is a hormone growth factor receptor—a protein molecule which "receives" and responds to growth hormone-releasing hormone (GHRH). Previous research showed that both the growth hormone-releasing hormone and the variant receptor of protein, SV1, played some role in a variety of malignancies, including prostate, pancreatic, kidney, breast, ovarian, and bone cancer. In a normal setting, GHRH binds to receptors in the pituitary gland and stimulates the release of growth hormone, which induces growth in normal tissues. How GHRH attached to and stimulated cancer cells wasn't clearly understood.

In this study the researchers used a breast cancer cell line that did not have either the full-length receptor for GHRH (GHRHR) or the splice variant of the hormone receptor SV1. When they exposed those cells to GHRH they did not respond, which was expected. In subsequent tests, they forced the expression of both the full

receptor and the SV1 in a line of breast cancer cells. In both cases, the sensitivity to GHRH was restored and the malignant cells were activated to divide and grow—in these experiments, at about double the rate of the control cells.

But the most important finding was that cells with the SV1, the splice variant, began to grow even without the added ligand growth hormone-releasing hormone, GHRH. In fact, in the absence of GHRH, the SV1 cells were five times more likely to stimulate tumor cell growth than the control cells—cells without either receptor. The cells with the full GHRHR receptor showed very little activity without the addition of the growth hormone. SV1 clearly plays an independent role in the stimulation of cancer growth.

"This paper demonstrates the physiological or pathological importance of GHRH," said Schally, who also holds an appointment at the Miami Veterans Affairs Medical Center. "You must do this before you can proceed to working on therapies for these cancers because you have to show that it's important and also try to elucidate the mechanism so you can attack it."

GHRH, the growth hormone-releasing hormone, besides its role in controlling the release of growth hormone from the pituitary, may stimulate not only the initial cell to which it attaches but also surrounding tumor cells. Understanding how it, and the protein splice variant receptor, work may create new opportunities to block their roles in hormone-responsive cancer. Dr. Schally is a world leader in targeting hormone-related cancers and is largely responsible for the field of hormone ablation for the treatment of prostate and other cancers.

Schally came to the UM Miller School of Medicine and the Miami VA in September of 2005 from Tulane University and the New Orleans VA. "Our work was the first to identify this new growth factor which is present in a variety of cancers," Schally said. "We're the only ones who discovered this receptor of GHRH." He worked with colleagues from the University of Athens Medical School in Greece and, in fact, one of the Greek co-authors, Nektarios Barabutis, Ph.D., is now with Dr. Schally in Miami continuing the work.

"Here in Miami we want to produce new, more powerful antagonistic analogs of GHRH so we're more able to battle these hormone-dependent cancers," said Schally, who has developed a number of hormone analogs, which can be powerful therapies against cancer. "So this paper is in line with that work." The work is considered important enough that Dr. Schally has been invited by the journal *Nature Clinical Practice* to write a review on the clinical implications of GHRH, to be published this fall.

UM/Sylvester opened in 1992 to provide comprehensive cancer services and today serves as the hub for cancer-related research, diagnosis, and treatment at the University of Miami Leonard M. Miller School of Medicine. UM/Sylvester handles nearly 1,600 inpatient admissions annually, performs 3,000 surgical procedures, and treats 3,000 new cancer patients. All UM/Sylvester physicians are on the faculty of the Miller School of Medicine, South Florida's only academic medical center. In addition, UM/Sylvester physicians and scientists are engaged in more than 250 clinical trials and receive more than \$30 million annually in research grants. UM/Sylvester at Deerfield Beach opened in 2003 to better meet the needs of residents of Broward and Palm Beach counties. This 10,000 square-foot facility at I-95 and S.W. 10th Street offers appointments with physicians from six cancer specialties, complementary therapies from the Courtelis Center, and education and outreach events. <http://www.sylvester.org>.

For more information, contact: Kelly Kauffhold; Tel.: 305.243.5184; Email: kkauffhold@med.miami.edu

Timeless/Tipin Complex Protects From UV Damage

This study was supported by the National Institute of Environmental Health Sciences and the National Cancer Institute.

For more information, contact: Les Lang; Tel.: 919.843.9687; Email: llang@med.unc.edu/ Dianne Shaw; Tel.: 919.966.7834; Email: dgs@med.unc.edu

New research from the University of North Carolina at Chapel Hill School of Medicine has identified two proteins that may help protect against skin cancer.

The study, which appeared in the online edition of the journal *Molecular & Cellular Biology*, indicates that two proteins, named Timeless and Tipin, form a complex that regulates the rate at which DNA is replicated after exposure to ultraviolet radiation.

Ultraviolet radiation in sunlight damages the DNA in skin cells. If left unrepaired by the cell, this damage can turn into mutations that lead to cancer. Before cells divide, they must replicate, or copy, their DNA to form new daughter cells. If damage in the DNA is discovered even after the cell has given a “go-ahead” to replicate its DNA, the Timeless/Tipin complex sends a signal throughout the nucleus of the cell to slow the rate of replication. This slowdown may give the cell additional time to repair its DNA and potentially save itself from becoming cancerous or from dying in response to ultraviolet radiation.

“What we discovered here was that the cell can send out an additional SOS and slow DNA replication even after it has begun,” said Dr. William Kaufmann, a professor of pathology and laboratory medicine and a member of the UNC Lineberger Comprehensive Cancer Center and Center for Environmental Health and Susceptibility.

“We’ve known for 25 years that a cell can stop DNA replication from even starting when it detects damage in its own DNA—this gives the DNA repair mechanisms in the cell the time to find and repair the damage,” he said.

Using an innovative new technique to visualize the replication of DNA strands exposed to ultraviolet radiation, Kaufmann and his co-authors noted a slowdown in DNA replication when Timeless and Tipin were present in the cell. Building blocks for DNA were labeled with fluorescent molecules so that tracks of newly synthesized DNA could be observed under the microscope and their lengths measured.

Though the study specifically examined only the Timless/Tipin response to ultraviolet radiation, Kaufmann speculates that this response may be relevant to other types of DNA damage as well—including those used as treatments for cancer.

“This protective response may make some cells more resistant to certain types of cancer therapies which work by inducing the cancer cell to die. If the cell, even if it is a cancer cell, is given this additional time to recover from treatment, it may be able to survive it, much to the detriment of the patient.” Kaufmann said.

Ultraviolet radiation in sunlight causes at least one million cases of skin cancer in the U.S. annually and greater than fifty thousand cases of melanoma.

UNC co-authors on the study include Dr. Keziban Ünsal-Kaçmaz and Dr. Aziz Sancar of the department of biochemistry and biophysics; Dr. Paul Chastain and Dr. Marila Cordeiro-Stone of the department of pathology and laboratory medicine; and Dr. Ping Ping Qu of the department of biostatistics in the School of Public Health. Co-author Dr. Parviz Minoos is from the University of Southern California School of Medicine.