

« 3-D cultures of individual cells from breast tissue produce hollow spheres of cells, shown here in green, which mimic the “acini” that characterize normal breast tissue. The red color marks cells in the center of the spheres that are undergoing a crucial, normal process called programmed cell death.

» CSHL Assistant Professor Senthil Muthuswamy



3-D

Breast Cancer Research

» The cells of our bodies live in a complex three-dimensional world in which many of them can tell up from down, back from front, right from left, inside from outside, near from far, or neighbor from neighbor. They do this by interpreting information from a vast assortment of biochemical signposts that tell them—provided they’re “listening”—to live or die, to grow or stop growing, or to move or stop moving.

One hallmark of cancer is that cells lose their ability to detect or respond properly to such live/die, grow/don’t grow, and move/don’t move signals from their environment. As a result, cancer cells live and grow when they shouldn’t and go where they don’t belong in the body. The latter two-step process, called invasion and metastasis, is particularly insidious because when tumors stay put, cancer therapies are fre-

quently more successful than when tumor cells invade tissues locally and subsequently metastasize to other locations.

Large numbers of the genes and processes involved in invasion and metastasis will continue to be discovered by researchers who profile cancer cell DNA in the absence of any cells at all (see page 3), by those who are pioneering the development of powerful animal models of cancer at CSHL, and by still others who use traditional cell cultures growing along the two-dimensional surface of the familiar Petri dish. Nevertheless, scientists are beginning to appreciate how a method for culturing cells in 3-D might fill an important niche between traditional 2-D cultures and whole animal models.

Enter CSHL Assistant Professor Senthil Muthuswamy, who came to the Laboratory in 2001 after completing postdoctoral studies at ARIAD Pharmaceuticals and Harvard Medical School. By culturing cells from healthy human breast tissue in a way that mimics their natural three-dimensional environment, and by then triggering the cells to take on properties associated with early, pre-invasive breast lesions, Senthil is discovering vital clues about how early-stage breast lesions might be treated before they advance to an invasive stage.

Healthy breast tissue contains ducts or tubelike structures through which milk flows. Connected to the ducts are lobular structures that bear many individual units called acini (“a-sign-aye,” singular: acinus), which secrete milk into the ducts and are arranged like a cluster of grapes. Each acinus is a hollow sphere of milk-producing cells with a characteristic archi-

✎ In breast tissue, acini are arranged like a cluster of grapes into structures called lobules. The formation of normal lobules, and the development of breast cancer, result from proper or improper communication between acini and the cells and molecules that surround acini called stromal cells and the extracellular matrix. Little is known about this communication process and how it goes wrong in breast cancer. As a first step toward exploring the process, Senthil’s lab has found that adding stromal cells to the 3-D culture system (below, left) triggers the formation of structures that resemble lobules (below, right).

tecture owing to cells that “know” to stop growing and stop moving lest they crowd their neighbors or fill in the hollow center of the sphere.

In the earliest stages of breast cancer, however, a cell on the surface of an acinus can lose its sense of place and begin to grow out of control. The resulting unrestrained cell proliferation leads to the disruption of the normal architecture of the acinus, which then produces far too many cells. At this stage, the acinus might still be enclosed in a saclike structure called a basement membrane. But if the lesion progresses to an invasive stage, the basement membrane is disrupted and proliferating cells invade local tissues. From there, the cancer can

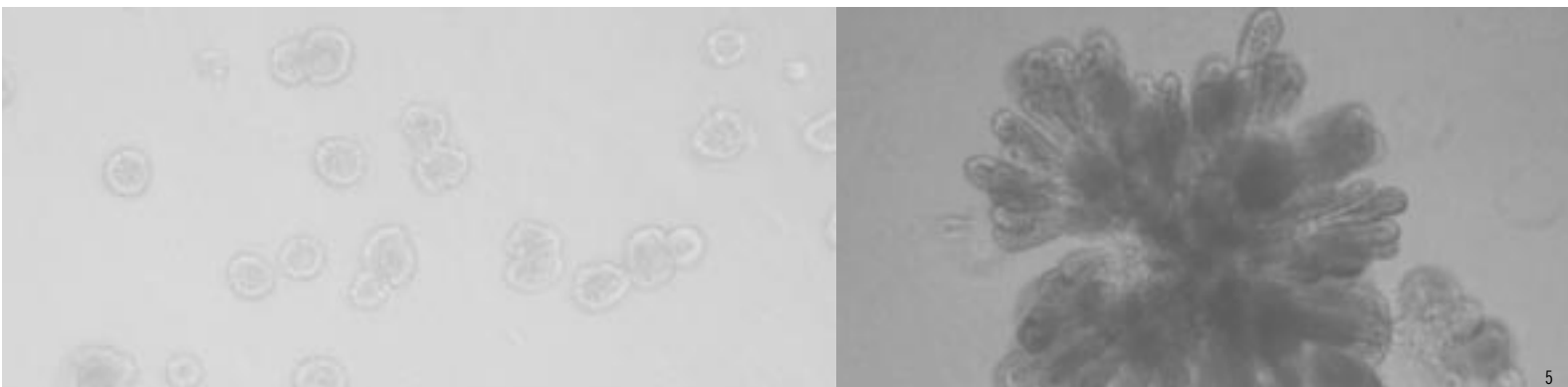
Senthil has shown that a protein encoded by the cyclin E oncogene is primarily responsible along with ErbB2 and ErbB3 for the development of invasive breast cancer

spread to the lymph nodes, after which metastasis to other tissues and organs may follow.

To study breast cancer, Senthil and his colleagues culture individual cells from acini, in 3-D, by seeding them within an artificial growth medium formulated to resemble their natural surroundings. When cultured in this way, the cells proliferate (during a fourteen-day incubation period) and form hollow spheres with an architecture remarkably similar to acini in breast tissue. Senthil can test the effect of genes believed or known to be involved in breast cancer by experimentally inducing or inhibiting the function of the genes at any time before, during, or after the incubation period.

In their first series of experiments, Senthil and his colleagues are teasing apart the role in breast cancer of four related proteins, called epidermal growth factor receptors. One such receptor, encoded by the ErbB2 oncogene, is frequently elevated in the most common form of breast cancer and is correlated with poor clinical prognosis, but is also the target of a potentially effective therapy called Herceptin (an antibody that

Reveals Oncogene Roles



binds to ErbB2 receptor and blocks its growth-promoting effects). However, the existence of other epidermal growth factor receptors (ErbB1, ErbB3, and ErbB4), not to mention the ability of these proteins to function both as “homodimers” (e.g. ErbB1/ErbB1, ErbB2/ErbB2) and as “heterodimers” (e.g. ErbB1/ErbB2, ErbB2/ErbB3) has complicated the diagnosis and treatment of breast cancer.

Senthil now has evidence that ErbB3 cooperates with ErbB2 to stimulate cell proliferation and to prevent cells from undergoing the programmed cell death that he and his colleagues suspect normally hollows out the acinus. Thus, instead of being hollow, the acinus fills with cells, which can then become invasive. Indeed, Senthil’s lab has also found that the activation of both ErbB2 and ErbB3 triggers local tissue invasion by stimulating the secretion of enzymes (matrix metalloproteases) that break down the barriers that normally act to seal the spaces between cells and prevent cells from moving where they shouldn’t. As a result, a combination therapy targeted at both ErbB2 and ErbB3 (or at ErbB2, ErbB3 plus cyclin E: see below) might be a good way to block the development of invasive breast cancer.

Using a similar approach, Senthil’s lab has recently resolved the roles of two other oncogenes, suspected from studies with 2-D cultures, to be involved in breast cancer. In a nice demonstration of the power of the 3-D cell culture system to provide

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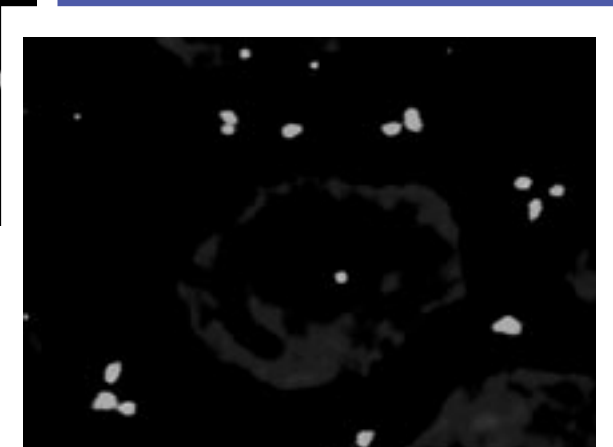
Close-up, cross section view of an experimental acinus produced by 3-D culture. Image depicts normal tissue architecture (with DNA marked in dark blue and “Golgi apparatus” marked in light blue/green) that is disrupted in breast cancer.

more accurate information than traditional 2-D cultures, Senthil and his colleagues have shown that a protein encoded by the cyclin E oncogene, but not a related protein called cyclin D, is primarily responsible along with ErbB2 and ErbB3 for the development of invasive breast cancer.

Senthil’s work has begun to reveal new molecules and processes that might be targeted with therapies to prevent invasive, metastatic breast cancer. Like the vast majority of other solid tumor types, breast cancer arises from “epithelial” cells. Therefore, Senthil’s findings may well impact the diagnosis and treatment of many different kinds of cancer. **Peter W. Sherwood**



“Tripartite junctions” or places where three cells touch each other have a unique and important way of organizing themselves in normal breast tissue (left). This architecture is disrupted in breast cancer tissue (right), a process that Senthil’s lab is beginning to understand with the hope of improving breast cancer diagnosis and treatment.



Grassroots Organizations Propel Breast Cancer Research

» Although government funding for cancer research is generous and supports a substantial portion of the Laboratory's breast cancer research, grassroots funding provided by local and national breast cancer advocacy groups also spurs our greatest discoveries. Since 1990, CSHL has been chosen to receive the funds raised by the following organizations, all hopeful that through our combined efforts, breast cancer can be eliminated:

✂ **1 in 9, the Long Island Breast Cancer Action Coalition**, is among the Laboratory's most avid and generous supporters. For more than a decade, this vibrant group has held fundraising events to support its cancer research grant to CSHL. Through its extraordinary efforts, 1 in 9 has raised more than \$1 million to date. This support has been put to very good use in the laboratory of Michael Wigler, the chief investigator funded by 1 in 9 (see page 3). As 1 in 9 has grown, it has raised breast cancer awareness throughout Long Island and beyond, it has exponentially increased its funding to the Laboratory, it has encouraged other grassroots organizations in their efforts to fund cancer research, and it has spearheaded legislative campaigns to increase state and federal funding for breast cancer research.

✂ In the last three years, the **MIRACLE Foundation**, founded by the late Michael Tenaglia, has donated \$200,000 to support the research of Mike Wigler and others at CSHL. The MIRACLE Foundation's contributions have already made a substantial impact on breast, ovarian, pancreatic, and brain cancer research at CSHL, and on studies of leukemia and lymphoma.

✂ **Long Islanders Against Breast Cancer (L.I.A.B.C.)** is an all-volunteer organization based in Dix Hills/Melville. In its first three years of existence, led by a board of only 16 volunteers, L.I.A.B.C. has raised more than \$800,000 to support CSHL breast cancer research. L.I.A.B.C. brings a new group of supporters to the Laboratory, all of whom are hopeful that funding research will bring meaningful answers for breast cancer.

✂ **F.A.C.T.-Find A Cure Today** is a new fund-raising group based in Lloyd Harbor/Huntington. Working in conjunction with the American Cancer Society, F.A.C.T. has raised significant funds to support Senthil Muthuswamy's research (see page 4).

✂ A group of Floral Park and Stewart Manor residents who in 1999 lost their dear friend and family member, Elizabeth McFarland, to breast cancer have banded together to help fight this disease by holding an annual event called **Liz's Day**. From Liz's Day proceeds over the past five years, CSHL has received more than \$150,000 to provide for the purchase of equipment essential to Mike Wigler's research.

✂ **The Huntington Breast Cancer Coalition** has supported the Laboratory for nearly a decade. Through collaboration with several other Long Island groups, the Huntington Breast Cancer Coalition has encouraged fundraisers throughout Suffolk County to support CSHL cancer research and continues its efforts in education and awareness throughout Huntington Township.

✂ **Glen Cove C.A.R.E.S.**, a fundraising group based in Glen Cove and other North Shore communities, has donated more than \$25,000 to support Masaaki Hamaguchi's research. All proceeds from their annual gala support research and other patient-related causes.

✂ **The Judi Shesh Memorial Foundation** has worked to raise funds for breast cancer awareness, support, and research for more than five years and made its first gift to Cold Spring Harbor Laboratory in 2003. Through its annual walk/run event, the foundation raises significant funds for breast cancer support on Long Island, and has already made plans to fund further research at CSHL.

✂ **Breast Cancer Coalitions** in Babylon, Manhasset, Great Neck, Long Beach, Plainview-Old Bethpage, and West Islip also support research at CSHL, as do several other community-based advocacy groups.

By funding research that has and will continue to yield vital discoveries concerning the diagnosis and treatment of breast cancer, and by providing patients the information and support they need to battle and survive the disease, grassroots organizations including those named above are bringing us ever-closer to the day when breast cancer mortality will be a thing of the past. **Jeffrey J. Picarello**

