More clues revealed in link between normal breast changes and invasive breast cancer

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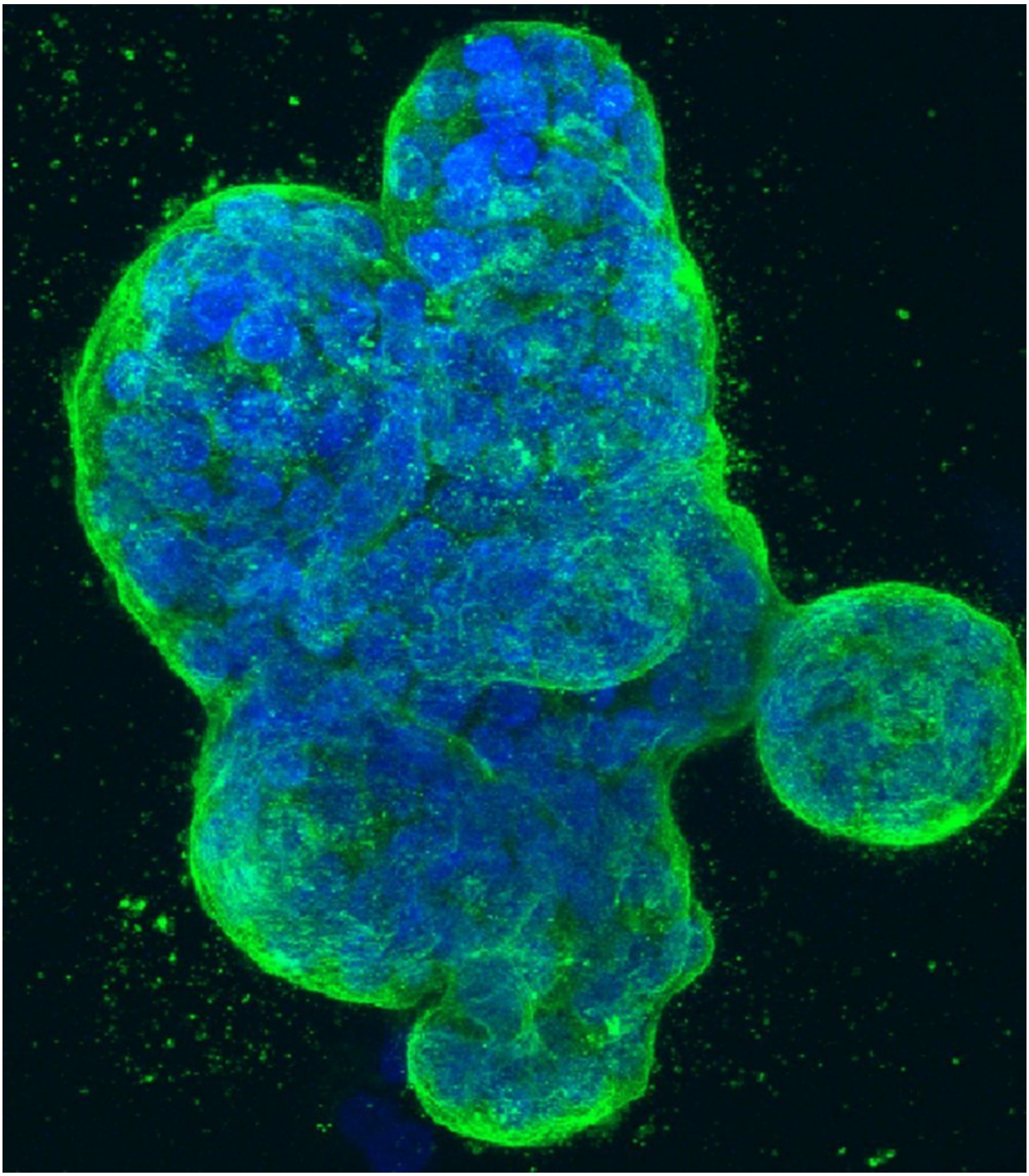

Three-dimensional culture of human breast cancer cells, with DNA stained blue and a protein in the cell surface membrane stained green. Image created in 2014 by Tom Misteli, Ph.D., and Karen Meaburn, Ph.D. at the NIH IRP.

A research team, led by investigators from Georgetown Lombardi Comprehensive Cancer Center, details how a natural and dramatic process—changes in mammary glands to accommodate breastfeeding—uses a molecular process believed to contribute to survival of pre-malignant breast cells.

Their mouse study, published online in *Cell Death Discovery*, shows that a critical switch that operates during breaks in nursing controls whether breast cells that had been providing milk will survive or die. The pro-survival pathway may be an example of a normal pathway that can be co-opted by pre-cancerous cells, including those that could become breast cancer, the researchers say.

If so, the findings may provide a strategy to block a part of the pathway that contributes to cancer, says the study lead author, Anni Wärri, Ph.D., an adjunct professor at Georgetown University Medical Center and the University of Turku in Finland.

"The study, for the first time, identifies the molecular switch—the unfolded protein response (UPR), which activates autophagy—that controls the fate of milk-producing breast cells," she says.

The fact that autophagy, a common cellular housekeeping function, is used to either keep the cells surviving or to mark them for destruction is important in cancer research, because the pro-survival function of autophagy has been seen as key in a number of different tumor types. Investigators conducted this study because the role of autophagy in both breast cancer and in normal mammary gland physiology had not been settled.

"It had not been known how this critical transition between ductal cell survival or death was regulated. Earlier studies had focused on a different pathway—apoptosis, a different form of cell death. We show

that apoptosis pathway is separate from the UPR/autophagy switch, although the processes clearly work together," says the study's senior investigator, Robert Clarke, Ph.D., DSc, co-director of the Breast Cancer Program at Georgetown Lombardi and Dean for Research at Georgetown University Medical Center

The study used mice to study two phases of breast remodeling after lactation—a process known as involution. "Because involution occurs in the same way in all mammals, what is found in mice closely mirrors human female breast physiology," he says.

Clarke adds that this study, in no way, suggests that breastfeeding sets up a mother to develop cancer. "Breastfeeding has been clearly associated with reduced breast cancer risk. That could be because, after breastfeeding is completed, pro-death programming takes over, which may kill abnormal cells."

The two states of involution the researchers studied occur during nursing and weaning, They found that breast cells control this remodeling in opposite ways.

During nursing, breast cells use a pro-survival strategy to maintain ductal lactation during short pauses in milking. This phase is called "reversible" involution because it maintains the milk producing cells to allow milk to be resynthesized once a pup suckles again. But when pups are weaned from the breast, cells flip on a pro-death switch in order to return mammary tissue back to its "normal" non-lactation state through "irreversible" involution.

Before this study, investigators did not know how autophagy is in play during involution and how it is different in the reversible versus irreversible phase of involution.

Researchers found that a buildup in milk protein in the ducts triggers UPR, a natural cellular process, which recognizes that too much protein has been generated. The UPR then switches on the pro-survival function of autophagy, which helps maintain the viability of milk producing ductal cells. When pups start drinking again, lactation resumes and UPR/autophagy is turned down to its baseline level.

However, a considerable buildup of milk proteins in ducts—which occurs when mouse pups are weaned from the breast—creates profound cellular stress that leads to autophagy switching into pro-death signaling, accompanied by increase in apoptosis, together leading to irreversible involution.

It is the reversible stage pro-survival signals that may be sustaining pre-malignant cells, Wärri says.

"It is understandable that abnormal cells may develop in breast tissue, because the mammary gland undergoes many changes during a lifespan. The breast ductal system resembles a tree. From puberty on, each menstrual cycle prompts the tree to grow a bit, but it always looks like a leafless tree in winter," she says.

"But the tree grows leaves, as if it is summer, when a woman becomes pregnant and then starts to nurse. The cells in ducts differentiate in order to produce milk. During brief breaks in lactation, the 'leaves' shrink a little, but then bloom again when feedings resume. After weaning, the tree returns to its dormant, winter state," Wärri says. "This constant state of flux may contribute to accumulation of some abnormal cells."

Cancer may come in to play when autophagy helps abnormal cells survive, she says.

To understand the mechanisms at play, the researchers used both an

autophagy gene deficient mouse model and drug intervention studies on wild type mice to both inhibit (with chloroquine) and stimulate (with tunicamycin) autophagy. Chloroquine is a drug currently being studied in two clinical trials aimed at preventing ductal carcinoma in situ (DCIS) from spreading. DCIS is a collection of precancerous cells in the duct and most DCIS does not become invasive.

They found that chloroquine, a drug commonly used to prevent and treat malaria, inhibits autophagy during involution. That action allows apoptosis to proceed, pushing the breast to revert to its normal, non-lactating state. This finding provides support for the clinical trials testing chloroquine use to keep DCIS in check in women diagnosed with DCIS, Clarke says. Results with autophagy gene deficient mouse model were similar—involution was enhanced and advanced. In contrast, stimulation of autophagy gave opposite results: milk producing cells were sustained and involution was delayed.

Researchers say their study could also have an important public health implication. The findings help explain why some women in sub-Saharan African countries who take chloroquine for malaria may have trouble breastfeeding, Clarke explains. "If, as we believe, chloroquine could bring lactation to an early end, we may be able to provide alternative short-term therapies that would allow breastfeeding when needed. Also, the opposite strategy, a short term use of autophagy-stimulating drug, could help women with difficulties in milk production or irregularities in nursing."

"The link between breast remodeling and breast cancer is a huge puzzle, and we have an important new piece to add to the emerging picture," Wärri says.

Provided by Georgetown University Medical Center