A New Paradigm for Mammary Glands

What role do basal cells play in the genesis of breast cancer?

Efforts to understand the changes that the breast undergoes during pregnancy and lactation, and during the early development of cancer, have mostly focused on luminal cells, one of two types of epithelial cells in the mammary gland. Luminal cells carry out the important functions in lactation, lining the milk ducts and alveoli where they secrete milk. One type of luminal cell, known as a luminal progenitor, may also be the precursor to some of the most aggressive forms of breast cancer. The other epithelial cells, basal cells, are thought to function during lactation as mere contractile elements that circulate milk to the nipple, and have seemed to have a negligible role in cancer development.

Now, however, Leif Ellisen, MD, PhD, program director of Breast Medical Oncology at Massachusetts General Hospital Cancer Center, has discovered that it’s actually basal cells that initiate the changes that occur in luminal cells during pregnancy and lactation. This happens via a previously unknown basal-to-luminal cell signaling involving the gene p63, a master regulator of basal (continued on page 2)

Basal Cells and Luminal Cells

The maturation of luminal cells is spurred by a signal from basal cells, according to new research.

- **a** Puberty
- **b** Mature virgin
- **c** Pregnancy
- **d** Lactation

Basal cells provide structural support for milk-producing luminal cells in the mammary gland’s milk ducts and alveoli, and help circulate milk during lactation. New research at Mass General Cancer Center has identified a previously unknown role for basal cells in initiating the luminal cells’ milk production.

Above Illustration by Bryan Christie
The Role of p63

Basal cells express p63, which signals the luminal cells to initiate lactation. Failing to do this may contribute to the development of triple negative breast cancer.

EARLY PREGNANCY

p63-regulated paracrine signaling

PARTURITION

Progenitor maturation/ lactogenesis

LEFT: Basal cells (red cells) express p63, which directs the expression of the growth factor hormone NRG1 (neuregulin). NRG1 binds specifically to ERBB4 on luminal progenitor cells (blue cells).

UPPER RIGHT: Normally, NRG1-mediated ERBB4 activation phosphorylates STAT5A (pS5) and induces luminal progenitor cell proliferation and differentiation (tan cells), resulting in milk production.

LOWER RIGHT: Loss of p63 results in luminal progenitors that are blocked during development and fail to differentiate. Cooperating genetic events may ultimately result in malignant transformation of such progenitors, which are known to be the precursors of a particularly aggressive form of breast cancer, triple negative breast cancer.

THE PARADOX OF TRIPLE NEGATIVE BREAST CANCER RISK

Triple negative breast cancer (TNBC), a very aggressive subtype, occurs in some women with BRCA1 mutations. Once TNBC tumors are established, they do not respond to hormone deprivation therapies. However, blocking hormones early, before the cancer develops, reduces the risk of this hormone-independent breast cancer. “This paradoxical clinical observation tells us that we don’t understand enough about the early steps of breast cancer development,” says Dr. Ellisen.

Breast cancer arises in the context of the whole mammary gland, in which basal and luminal cells are in close physical contact, yet little is known about their cellular interactions in either normal breast development or in cancer. However, researchers did know that rare germline mutations in the p63 gene affect mammary gland development. Dr. Ellisen’s group decided to delete p63 in just the breast’s basal cells in adult mice.

BASAL-TO-LUMINAL CELL SIGNALING

Surprisingly, without that gene, the luminal progenitor cells failed to differentiate as normal during pregnancy into mature milk-producing luminal cells. Consequently, the mothers did not lactate.

“Although we manipulated only the basal cells, the effects were all in the luminal cells,” Dr. Ellisen says. “This told us that the basal cells normally signal the luminal cells to initiate lactation, and the loss of p63 silenced the signal.”

This result provided the first evidence of basal-to-luminal cell signaling. The researchers next identified the signal produced by p63 as the growth factor hormone NRG1, also called neuregulin. Again, this was the first evidence that basal epithelial cells produce NRG1, and that it is regulated by p63.

NRG1 was known to bind to epithelial growth factor receptors, ERBB4, in the luminal progenitor cell, and that ERBB4 activation phosphorylates another signaling molecule, STAT5, triggering its activation. STAT5, in turn, causes the luminal progenitor cells to differentiate into mature luminal cells capable of producing milk. Dr. Ellisen’s study now connects all of the dots in this process, showing that p63 in the basal cell initiates this cascade of activity.

Deleting p63 in the basal cells blocked the ERBB4/STAT5 activity and maturation of the luminal progenitor cells, causing lactation failure. Dr. Ellisen thinks that failure of luminal progenitors to differentiate could also potentially set the stage for cancer.

CANCER RISK IMPLICATIONS

Women with BRCA1 mutations have abnormal luminal progenitor cells, and one theory holds that such aberrant cells may be precursors to the most aggressive cancers—TNBC and the more common luminal B subtype—in these women. “Because BRCA1 carriers also have altered proportions of basal and luminal cells,” Dr. Ellisen
Ceritinib: A potent second-generation ALK inhibitor for non-small-cell lung cancer

Will ceritinib prove to be a breakthrough therapy?

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer death in the United States, yet until recently it had no effective treatments. Now, however, by gaining a better understanding of the molecular biology of NSCLC, researchers have identified genetic subtypes that can be targeted with selected inhibitors. The Massachusetts General Hospital Cancer Center has led many of the studies and trials of first- and second-generation targeted therapies for specific NSCLC subtypes. The latest results concern NSCLCs driven by chromosomal rearrangements in the anaplastic lymphoma kinase (ALK) gene that have become resistant to the first-generation ALK inhibitor, crizotinib.

In a phase I trial, ceritinib caused marked tumor shrinkage in patients with advanced cancer who had developed resistance to crizotinib as well as in those who had received other treatments, according to Alice Shaw, MD, PhD, a thoracic oncologist at the Mass General Cancer Center and lead investigator of that trial. Trial results appeared in the March 27 New England Journal of Medicine\(^1\), while the March 27 Cancer Discovery\(^2\) published a molecular analysis of the treatment. (continued on page 4)

Lung Tumor Shrinkage

PET scan showing response to ceritinib in ALK-rearranged non–small-cell lung cancer (NSCLC). Positron-emission tomographic scans taken at baseline (left) and after 3.5 weeks of ceritinib treatment (right) in a patient with crizotinib-resistant disease. Subsequent computed tomographic scans after six weeks of ceritinib treatment showed a 52 percent reduction in tumor burden.

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explains, “the resulting altered cell-to-cell signaling interactions might underlie the genesis of many if not most breast cancers.”

Researchers have long observed that pregnancy and lactation reduce breast cancer risk. Dr. Ellisen speculates that this risk reduction may result from the depletion of the luminal progenitor cells at the end of pregnancy, when all of the cells have differentiated and are no longer potential cancer precursors. Because basal cell signaling initiates the differentiation of these luminal cells, Dr. Ellisen’s finding supports an emerging view of the basal cell as a tumor suppressor cell.

Dr. Ellisen is now collecting tissue from women with germline BRCA1 mutations who undergo mastectomies to prevent future breast cancers. He will analyze the samples to learn whether dysregulated basal-to-luminal cell signaling is associated with higher breast cancer risks. Is there a marker that can predict cancer before it develops that could facilitate earlier intervention? Once researchers understand how the dysregulated cell signaling leads to cancer, could they eventually develop preventive strategies?

More broadly, the discovery suggests that to understand cancer biology, researchers must study the entire microenvironment as the tumor develops rather than examining only a single tumor cell type once the cancer has formed.\(^2\)\(^-\)\(^3\). Says Dr. Ellisen, “We need to look at cancer in the context of how heterogeneous cells interact early in the genesis of the tumor so we can understand how cells cooperate during the initial stages of cancer formation.”
Progression-Free Survival Estimates

The median progression-free survival (PFS) for all 144 patients with advanced, ALK-rearranged NSCLC who received ceritinib at doses of 400 to 750 mg daily was 7.0 months. In the subgroup of 80 patients who had received crizotinib previously (yellow), the median PFS was 6.9 months. In the 34 patients who had not received crizotinib previously (blue), the median progression-free survival was 10.4 months. The PFS data for crizotinib-naïve patients is immature, as many patients are still responding to treatment.

TRIAL RESULTS

The preclinical results provided a strong rationale for a multi-center phase 1 trial of ceritinib in patients with locally advanced or metastatic NSCLC, which was open to patients who had or had not previously been treated with crizotinib. The dose escalation phase of the trial enrolled 59 NSCLC patients and established a maximum tolerable dose of 750 mg/day of this oral drug. Side effects involved mainly gastrointestinal issues that resolved when treatment was reduced or stopped. Another 71 patients enrolled in an expansion phase to assess response rate and progression-free survival (PFS).

Among the 114 patients who received at least 400 mg/day, the overall response rate was 58 percent and the median PFS was seven months. Those results included patients with various resistance mechanisms and those with brain metastases. Response rates and PFS were better for patients who had not previously been treated with crizotinib. Because some patients are continuing to respond to ceritinib, data on overall survival are not available.

The positive responses to ceritinib among crizotinib-resistant NSCLC patients stand in contrast to the situation with EGFR-mutated cancers, in which fewer than 10 percent of patients with acquired resistance to first-line EGFR inhibitors respond to second-generation inhibitors.


Preliminary data from this trial led the FDA to give ceritinib a “breakthrough therapy” designation one year ago, and to grant accelerated approval to ceritinib in April 2014.

Dr. Shaw is also involved in two phase II clinical trials of ceritinib that have completed enrollment. There are also two ongoing phase III trials that are enrolling patients. Information about these “LDK378” trials is available on clinicaltrials.gov.

**MOLECULAR BASIS OF RESISTANCE**

Dr. Shaw’s team undertook molecular analysis of ALK resistance and ceritinib activity in cancer cell lines and mouse models, including some derived from biopsy samples of trial participants. They found that ceritinib overcomes several known mutations that cancers acquire in developing resistance to crizotinib, but its potency varies according to which resistance mechanism is involved. “Our results suggest that the majority of crizotinib-resistant tumors remain dependent on the ALK oncogene, so they are still sensitive to ALK inhibition,” says Dr. Shaw, who notes that crizotinib may over time provide sub-therapeutic inhibition that a modified structure could improve.

Dr. Shaw’s team also identified two new mutations that promote resistance to both crizotinib and ceritinib, and the researchers are now testing other ALK inhibitors and combinations of inhibitors. “We want to develop additional options for patients who relapse following an initially successful targeted therapy,” she says.

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In a recent study published in Nature Genetics, Dr. Brastianos reported that each of the two subtypes has a high rate of mutations in a particular cancer gene, but the mutated genes in each subtype are mutually exclusive and have quite different biological activities. An astonishing 96 percent of the childhood adamantinomatous form of the tumor had a mutation in a β-catenin gene (CTNNB1), which affects cell-to-cell adhesion and gene transcription through the Wnt pathway, while 95 percent of the adult papillary tumor samples had a mutation in the BRAF gene, which affects cell growth rates. Surprisingly, few other mutations occurred in either subtype.

Moreover, the driving mutation occurred in 100 percent of the cancer cells in each tumor, indicating that the tumor is clonal: the mutation was present in the tumor initiating cell (cell of origin or cancer stem cell) and was passed down to each descendant cell as the tumor evolved. Consequently, because each subtype of craniopharyngioma tumor is dependent on only one driving mutation (β-catenin or BRAF), both subtypes should be particularly sensitive to selective inhibitors.

Dr. Brastianos says that because the mutations are mutually exclusive, identifying them can be used to confirm a diagnosis. Moreover, papillary craniopharyngioma patients may benefit immediately from today’s FDA-approved BRAF inhibitors. β-catenin and Wnt-signaling inhibitors are in development.

BRAIN METASTASES ON THE RISE

Many prevalent malignancies—lung cancer, breast cancer, melanoma, renal cell carcinoma—metastasize to the brain, which Dr. Brastianos calls “a sanctuary for cancer” that may not respond to targeted therapies. That’s a problem, because as cancer therapies for primary tumors improve, more patients are living long enough to develop brain metastases. Some 200,000 people a year now present with brain metastases, and half of those patients will die because of the cancer in their brain, often within months.

Dr. Brastianos leads a multidisciplinary brain metastasis clinic at Mass General Cancer Center to provide individualized treatment and support for such patients and to conduct clinical and translational research. The goal is to establish clinical protocols specifically for patients with brain metastases, and to integrate lessons from the clinic into work in the lab—and then to take what researchers learn in the lab back to patients. This is the first program of its kind in brain metastases in the country.

Dr. Brastianos collaborated with researchers worldwide to collect 101 matched primary tumor, brain metastasis and normal tissue samples from patients, and then conducted the largest, most comprehensive genotyping study to date characterizing those brain metastases and showing how they differ from the primary tumors. Dr. Brastianos worked with Scott Carter, PhD, and Gad Getz, PhD, at the Broad Institute of Harvard and MIT using the latest analytic sequencing tools to understand how brain metastases evolve from the primary tumor. Dr. Brastianos found that as the brain metastasis evolves, it develops a heterogeneous set of mutations that differ from those in the primary tumor—which also evolves. This research suggests that therapies may need to change as brain metastases continue to evolve with new mutations.

Dr. Brastianos hopes to identify the genetic changes that specifically drive brain metastases.

For Dr. Brastianos, this research is both professionally and personally urgent. “My mother recently passed away from metastatic breast cancer,” she says. “Her illness shaped me as a physician and researcher, and I’m dedicating my life to solving this problem.”


2 Abstract. Genomic characterization of 101 brain metastases and paired primary tumors reveals patterns of clonal evolution and selection of driver mutations: http://www.abstractsonline.com/Plan/ViewAbstract.aspx?tid=3404&uKey=7f0fa126-59bf-48fc-aaf5-457245a12ebc&cKey=dee7c0d8-525b-4ca9-a5e1-27605260d53b&mKey=ff1e444a-a164-476a-92e7-c2644874d93.
Selected Open Clinical Trials

The Massachusetts General Hospital Cancer Center conducts nearly 400 clinical trials in collaboration with the Dana-Farber/Harvard Cancer Center. Selected Mass General Cancer Center clinical trials currently enrolling new cancer patients are listed here. For a complete list, go to massgeneral.org/cancer/trials. To receive a monthly email about select open clinical trials at the Cancer Center, please send your contact information to MGHAdvancesinCancer@partners.org.

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2012P001355

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Pilot | Yi-Bin Chen, MD | 617-726-1124

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13-411

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**MELANOMA**

13-587

A dose-escalation, open-label, three-part study of the MEK inhibitor, trametinib, combined with the CDK4/6 inhibitor, palbociclib, to investigate the safety, pharmacokinetics, pharmacodynamics and anti-cancer activity in subjects with solid tumors

Phase I/II | Keith Flaherty, MD | 617-643-5817

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**MULTIPLE MYELOMA**

12-014

A dose escalation study of PVX-410, a multi-peptide cancer vaccine, in patients with smoldering multiple myeloma

Phase I/IIa | Noopur Raje, MD | 617-726-4000

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**PEDIATRIC CANCERS**

09-361

A study of proton beam radiotherapy for medulloblastoma and pineoblastoma: an assessment of acute toxicity and long-term neurocognitive, neuroendocrine and ototoxicity outcomes

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**TARGETED THERAPIES**

13-424

A study of dabrafenib, trametinib and navitoclax in BRAF mutant melanoma and other solid tumors

Phase I/II | Ryan Sullivan, MD | 617-643-3614

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**THORACIC CANCERS**

12-098

An open-label, safety, pharmacokinetic and preliminary efficacy study of oral CO-1686 in patients with previously treated mutant EGFR non-small-cell lung cancer

Phase I/II | Lecia Sequist, MD, MPH | 617-724-4000

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**A New Way to Access Targeted Clinical Trials: TargetedCancerCare.org**

At the Mass General Cancer Center, we are closing the gap between groundbreaking gene-based cancer research and life-changing treatments. We developed and launched an industry-leading website, TargetedCancerCare.org, that connects users with the Cancer Center’s innovative clinical research in the field of targeted cancer therapies.

TargetedCancerCare.org features:

- The latest information related to tumor-specific genetic and molecular biomarkers
- An interactive tool that enables users to search for targeted cancer therapy clinical trials. Users can input as much information as they have available, searching by disease type, cancer gene or specific mutation
- Customized search results
- A user-friendly format designed to guide physicians through what can be an increasingly complex field with unique opportunities.
New Physician Appointments

Massachusetts General Hospital is pleased to announce new physician appointments in the areas of Hematology/Oncology, Imaging, Radiation Oncology, and Thoracic Surgery. For more information about the Mass General Cancer Center or our new physicians, please visit massgeneral.org/cancer.

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