Unlocking the Mysteries of Lobular Breast Cancer Biology Needs the Right Combination of Preclinical Models

Shaymaa Bahnassy[1], Matthew J. Sikora[2], and Rebecca B. Riggins[1]

ABSTRACT

Preclinical model systems are essential research tools that help us understand the biology of invasive lobular carcinoma of the breast (ILC). The number of well-established ILC models is increasing but remain limited. Lower incidence of ILC, underrepresentation of patients with ILC in clinical trials, and intrinsic ILC tumor characteristics all contribute to this challenge. Hence, there is significant need to continually develop better model systems to recapitulate the essential characteristics of ILC biology, genetics, and histology, and empower preclinical therapeutic studies to be translated back into the clinic. In this Perspective, we highlight recent advances in *in vivo* experimental models, which recapitulate key features of ILC biology and disease progression and potentially reshape the future of ILC translational research. We assert that all existing *in vitro* and *in vivo* ILC preclinical models have their strengths and weaknesses, and that it is necessary to bridge key deficiencies in each model context as we move forward with ILC research. Thus, unlocking the mysteries of ILC will be best achieved by choosing the right combination of preclinical model systems.

Introduction

Invasive lobular carcinoma of the breast (ILC) accounts for 15% of all breast cancer cases and is the most common special histologic subtype versus breast cancer of no special type i.e., invasive ductal carcinoma (IDC; refs. 1, 2). ILCs are histologically defined by single-file growth and a targetoid pattern of invasion (3). Ninety-five percent of ILCs are estrogen receptor alpha—positive (ER^+) and strongly estrogen-driven; however, ILC has important clinical features distinct from other ER^+ breast cancers. ILCs are typically more difficult to detect by standard imaging and have higher risk of late recurrence. Disease-free survival (DFS) and overall survival (OS) are typically favorable for patients with ILC compared with other breast cancers within 5 years of diagnosis; however, studies in the United States and Europe confirm that after ∼6 to 8 years post-diagnosis, both DFS and OS are worse for ILC regardless of ER status (4–6). Compared with IDC, patients with ILC showed 54% higher risk of recurrence 6 years after diagnosis, and 50% higher risk of death 10 years after diagnosis (4). In the NCI SEER database, IDC had a reduced risk of death after 5 years (IDC:ILC OS; HR = 0.775, $P < 0.0001$; ref. 6). Further, beyond typical ER^+ breast cancer metastatic spread sites, ILC also metastasizes to ovaries, gastrointestinal tract, peritoneum, leptomeninges, and orbital cavity (3, 7). Profiling primary tumors by three independent consortia cast light on defining features of ILC versus IDC (8–10) beyond the hallmark loss of *CDH1*/E-cadherin underlying ILC's discohesive morphology (8, 9, 11). *PIK3CA*, *PTEN*, *ERBB2*, *ERBB3*, and *FOXA1* mutations, and *ESR1* amplifications, are enriched in primary ILC versus IDC (8, 9). In addition, increased tumor mutational burden in overall ILC metastases and specifically enriched *RHOA* mutations in ovarian ILC metastases (12, 13) suggest that the unique metastatic pattern of ILC is accompanied by unique genetic changes.

Laboratory research on ILC as a distinct entity is in its infancy – fewer than ∼50 papers on ILC were published annually until 2011. As this field grows, we must leverage the new wealth of ILC tumor data and advancement in breast cancer modeling systems to identify ideal preclinical models for specific research questions and clinical contexts. Patients with ILC are under-represented in clinical trials due to challenges with imaging, tumor measurement, and inclusion and/or response criteria (14). It is therefore critical that preclinical ILC studies choose the most appropriate models at research outset to ultimately inform the design of more inclusive clinical trials.

Challenges to Modeling ILC

Many challenges in preclinical modeling, for example, the lack of tumor-immune microenvironment (TIME), are not unique to ILC. However, unique features of ILC biology, genetics, and histology with historically limited model development can confound the choice of model to study specific aspects of human ILC. A new "atlas" of ILC models comprehensively reviews existing *in vitro*, *ex vivo*, and *in vivo* preclinical ILC models (15). Thus, we propose that integrating several of these models have significant potential to bridge individual key deficiencies (**Fig. 1**) and advance ILC research.

ER positivity and E-cadherin loss each make model establishment, organoid formation, and xenograft implantation difficult. However, human ILC cell lines have been essential tools to define ER function and estrogen response (16–19), capture intrinsic resistance to endocrine therapies (17, 20, 21), and interrogate anchorage independence and active signaling pathways in ILC tumors (22–24). Although ILC cell lines are unique in capturing endocrine response, currently available ILC lines are derived from metastatic disease, and do not model TIME. Tumor organoids better capture disease heterogeneity and microenvironment, and show high phenotypic, genomic, and drug response fidelity to their originating tumors, making them great platforms for drug screening (25, 26). However, loss of E-cadherin expression, and defective adherens junctions, hinders development and expansion of ILC organoids (15). Hence, few ILC patient-derived

[1]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC. [2]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO.

Corresponding Authors: Matthew J. Sikora, University of Colorado Anschutz Medical Campus, 12801 E. 17th Avenue, Rm 5117 Research Complex 1 South, Aurora, CO 80045. E-mail: matthew.sikora@cuanschutz.edu; and Rebecca B. Riggins, Department of Oncology, Georgetown University Medical Center - Lombardi Comprehensive Cancer Center, 3970 Reservoir Road NW, E412 Research Bldg, Washington, DC 20057. E-mail: rbr7@georgetown.edu

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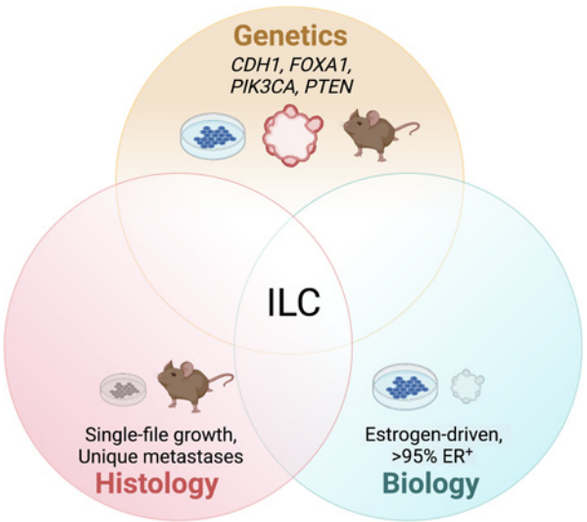

Figure 1.
Experimental models representing each of the three essential characteristics of human ILC. Icons for *in vitro* (cell line), *ex vivo* (organoid), and *in vivo* (GEMM). Smaller sized and grayed out ILC model icons are shown where they may partly, but not ideally, allow investigation of that characteristic.

organoids exist (15, 26). Genetically engineered mouse models (GEMM) of ILC are driven by mammary-specific *CDH1* loss (reviewed in refs. 15, 27), and capture ILC's discohesive single-file growth (**Fig. 1**). However, current GEMMs do not fully recapitulate other aspects of human ILC, as they metastasize to some but not all ILC-specific metastatic sites. The development of mammary tumors in multiple glands and at different rates can complicate the ability of ILC GEMMs to model drug efficacy (15, 28). Importantly, ILC GEMMs lack the ER expression seen in >95% of human ILC (28), and no ILC GEMM has shown either bona fide estrogen responsiveness or estrogen dependence.

Robust and reliable xenograft models that recapitulate the unique features of ILC will empower preclinical therapeutic studies to be translated to the clinic and inform better patient care. However, ER^+ ILC cell lines and tumor xenografts grow slowly and exhibit low engraftment rates when conventionally implanted in mammary fat pads of immunodeficient mice (29, 30). Of 351 breast cancer patient-derived xenografts (PDX) in pdxfinder.org, only two are ER^+ ILC PDX. In addition, there is a lack of ER^+ breast cancer immune-humanized preclinical models. Altogether these challenges heavily affect *in vivo* modeling of ILC, which is >95% ER^+. We highlight two recent studies that overcame these limitations to develop *in vivo* ER^+ ILC models.

Emerging *In Vivo* ILC Model Systems

Sflomos and colleagues used the mouse intraductal (MIND) xenograft model, which markedly improves engraftment of ER^+ breast cancer through intraductal injection of cells into murine mammary ducts (28, 31). Cells from two ILC cell lines, and patient tumors, engrafted into immunocompromised mice using MIND recapitulated histology, growth, endocrine response, progression, and metastatic patterns of ILC (28). These innovative studies highlight the essential contribution of local microenvironment to *in vivo* ILC models, and demonstrate that the MIND technique can advance our understanding of ILC biology throughout the disease course. However, MIND tumors are slow to develop and progress—primary tumors form within 4 to 6 months, with macro-metastases forming in 10 to 12 months (28)—and so the "complete" nature of this model comes with a heavy cost of time.

Conventional ILC PDX models closely resemble the original tumor at the genomic and molecular level (17, 25), but cannot model TIME crosstalk because they are grown in immune-deficient mice. ILC has distinct immune cell composition, tumor-infiltrating lymphocytes levels, and immune activity versus IDC that impacts clinical outcomes (3, 32, 33), highlighting the need to include human TIME in ILC models. Scherer and colleagues developed the first immune-competent PDX model of ER^+ ILC using the HCI-013 ILC PDX that naturally harbors the *ESR1* Y537S mutation in a mouse with a human-like immune system (34). After myeloablation, NSG-SGM3 mice were implanted with PDX fragments one day following engraftment with $CD34^+$ hematopoietic stem cells, to minimize allograft reaction and maximize T-cell tolerance. However, this immune-humanized model supports growth of an estrogen-independent HCI-013 variant (HCI-013EI) but not the original estrogen-dependent HCI-013 ILC PDX (exogenous estrogen induced severe anemia in NSG-SGM3 mice). The immune-humanized HCI-013EI ILC PDX recapitulates lymphoid-sparse and myeloid-rich immune milieu of human ER^+ breast tumors (34). Complementary studies in the Riggins lab wherein HCI-013EI is implanted after maturation of human-like immune system (14–16 weeks post-$CD34^+$ engraftment) are consistent with these studies, and these mice models develop spontaneous metastases (unpublished). While still in their infancy, humanized immune models of ILC should allow us to resolve the ILC-specific TIME more clearly and may provide promising platforms to test immunotherapies.

What's On the Horizon for ILC Preclinical Models?

Patient-derived explants (PDE) allow testing of short-term response to treatments in tumor tissue fragments *ex vivo* while preserving tissue architecture, TIME, and interperson heterogeneity (35). Stires and colleagues (36) showed response to a combination of fulvestrant and glutamate release inhibitor riluzole in PDEs from ILC and IDC primary tumors, demonstrating that PDEs are another valuable tool in the repertoire of ILC preclinical models.

A recent observational trial (NCT01322893) reported that circulating tumor cell (CTC) counts in patients with metastatic ILC are higher than IDC (37). However, CTC-derived preclinical models are scarce. The CTC line CTC-ITB-01 was derived from peripheral blood of an individual with bilateral breast cancer (ILC left, IDC right). CTC-ITB-01 is ER^+ and expresses E-cadherin protein but carries a *CDH1* mutation (D402N) that is distinct from that of primary ILC tumor (R598* premature stop; ref. 38), so it is unclear whether this model represents ILC. However, CTC-derived ILC xenograft models may be essential tools to better capture disease heterogeneity, especially for metastatic breast cancer.

Current and future model development efforts must be accompanied by publicly accessible and rigorous credentialing of how well each model embodies essential characteristics of ILC. Exciting efforts underway in collaboration with the Cancer Cell Line Encyclopedia and Cancer Dependency Map Project, and from the LOBSTERPOT-CA19138 action, provide a roadmap for how such efforts should proceed (https://reporter.nih.gov/search/q5YBPU02NEagyz5qAgj5SA/project-details/10219509 and https://www.cost.eu/actions/CA19138/, respectively).

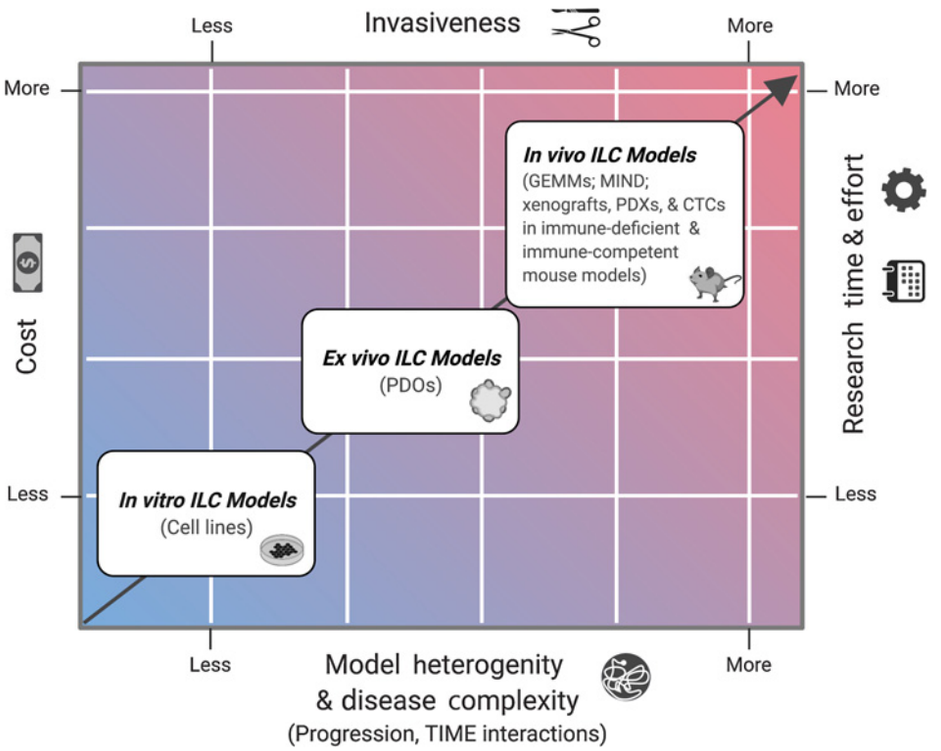

Figure 2.
Critical factors influencing the selection of experimental models used in ILC research projects. Abbreviation: PDO, patient-derived organoid.

Closing Thoughts

There is no one-size-fits-all model that captures essential histologic, genetic, and biological features of ILC. Accelerating the development and characterization of ILC MIND and TIME models has exciting potential to usher in a new era of ILC preclinical research. However, new and emerging *in vivo* ILC models do not support abandoning less complex *in vitro* ILC cell lines. The latter currently remain the most appropriate and accessible models for mechanistic studies of the ER-driven biology of ILC. Integration of multilevel model systems to complement and compensate for limitations associated with each is our best-practice recommendation to ensure rigor and reproducibility in ILC research (**Fig. 2**). When funding, time, and/or other external factors become limiting, choosing a smaller number of best-fit ILC model(s) that best address the specific research question(s) and most relevant feature(s) of ILC to that study should be prioritized.

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