An instructive ductal microenvironment is key for efficient *in vivo* modeling of estrogen receptor positive breast cancer

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Despite comprising 72% of all invasive breast cancers (1), and leading to a higher overall number of deaths than all other breast cancers combined, study of estrogen receptor positive (ER+) breast cancer in animal models has been hampered by numerous technical and biological problems which has made it difficult to effectively study to range of clinical subtypes that fall in this group. While each of the existing experimental systems has some favorable features, in many respects each is modeling a very particular aspect of breast cancer biology which may not be very generalizable to the diverse range of ER+ tumors arising in breast cancer patients.

Chemical carcinogenesis using DMBA reliably results in the formation of ER+ tumors, however the predominance of squamous histology (2), and the strong prevalence of Ras mutation in these tumors (3) in contrast to the rarity of this lesion in human ER+ breast cancer (4) raises concerns about its broader relevance. Hormonal carcinogenesis using medroxyprogesterone acetate also yields ER+ tumors with longer latency however, in contrast with the human disease, the growth of these tumors is inhibited by exogenous estrogen (5).

Genetically engineered mice that recapitulate many aspects of breast cancer biology have been generated, however the vast majority of these models yield estrogen receptor negative tumors (6). The most prominent exceptions are mice with alterations in p53. Although p53-null mice develop ER+ tumors, a wide spectrum of other malignancies also arises in these animals, often with shorter latency than the mammary tumors. Transplanting p53-null mammary epithelium into the cleared mammary fat pads of syngeneic p53 proficient hosts has been performed to circumvent this problem (7), but these experiments are time consuming and involve technically challenging surgical manipulations. Restricting p53 mutation to the mammary epithelium through the use of a Cre-regulated Lox-STOP-Lox approach is another way around the complication of lymphoma incidence in p53-null mice, and yields 67% ER+ tumors (8). *PIK3CA* is altered in 29–45% of human ER+ luminal tumors (4). Mice with mammary-specific *PIK3CA* mutation yield tumors with a number of histologies. Of these, adenomyoepitheliomas were enriched in ER+ cancer cells (9). Mice lacking the STAT1 transcription factor also develop ER+ tumors (10) but their STAT1 deficiency necessitates housing in completely pathogen-free conditions and they are likely less suitable to study the tumor-immune system interactions than other genetically engineered models.

In recent years, *ex vivo* manipulation of primary cells has also been used to develop mouse models of breast cancer. In one instance, mouse mammary epithelial subpopulations were FACS isolated and lentivirally infected with the polyoma virus middle T (PyMT) antigen leading to a lineage-dependent enrichment in ER+ tumors. Although ER+, these tumors were not estrogen dependent (11) perhaps reflecting the aggressive nature of PyMT driven tumors. Nonetheless, this strategy does offer the possibility of transforming this key subpopulation of mammary epithelial cells with weaker viral antigens or mutant genes more relevant to human ER+ breast cancer, in which case

this approach may prove very useful.

In parallel with these models in which tumors are induced in mouse mammary epithelial cells, a variety of human-in-mouse models have been developed using immunocompromised animals. These range from xenografts of long-established breast cancer cell lines to more recent innovations in patient-derived xenografts (12). In all cases, the efficiency of establishing ER+ xenografts is substantially lower than the success rate obtained with ER-negative tumors. Thus, while models do exist, much of the very extensive literature in this area is based on models that represent only a few original transforming events in a small number of original human patients—a troubling lack of diversity given the inter-individual complexity of this disease (4).

In the March 14th issue of Cancer Cell, George Sflomos, Cathrin Brisken and their colleagues report important new developments in the modeling of ER+ human breast cancers *in vivo* (13). Building on an earlier intraductal injection model developed to study DCIS (14), they injected single cell suspensions of a series of ER+ cell lines and patient-derived tumors directly into the mammary ducts of SCID mice. Injected cell lines developed as ductal carcinomas *in situ* for a period of several months before acquiring the ability to invade into the local tissue parenchyma, and metastasize to distant organs, with secondary sites that are frequently seen in the patient population. Importantly, the take rate was significantly greater than prior reports for ER+ breast tumors, indicating that it should now be possible to generate a diverse and more broadly representative set of transplantable models with which to better study the human disease and response to therapy.

By performing some of their experiments in parallel using the more typical orthotopic mammary fat pad injections, Sflomos and colleagues revealed some important differences. MCF7 and other cell lines injected into the mammary fat pad adopt a more basal cell fate, likely instructed by local TGF-beta signaling, while growing intraductally allowed these cells to maintain a more luminal phenotype. In addition, intraductal tumor growth proceeded without requiring the estrogen supplementation typically required for engraftment of ER+ cell lines in the mammary fat pad. Intriguingly, the intraductal tumors also developed microcalcifications, one of the key diagnostic criteria for mammographic identification of breast cancer (15), which are generally not observed in tumors growing in the mammary fat pad.

Tumor growth was significantly slower in the ducts than in mammary fat pads, almost certainly reflecting the tight space constraints for growth within the lumen of the ductal tree, but raising the additional question of the extent to which the physical architecture, cell-cell contacts or signaling microenvironment of the ductal tree may be actively preventing tumor cell invasion. It was noteworthy that so many cell populations that had already progressed to invasive disease in the human apparently lacked the intrinsic ability to immediately grow beyond the ductal network in the mouse. Thus, while providing an exciting new tool with which to study a wider range of ER+ tumors, the authors also highlight the strong constraints imposed by the local ductal microenvironment on progression to invasive disease. The relative contributions of physical barriers, such as the basement membrane and the layer of myoepithelial cells, and of soluble signaling molecules to this progression delay can now start to be understood using *in vivo* models with the appropriate architecture.

This new model offers the possibility of more diverse patient-relevant models for evaluating new drug candidates, which is to be welcomed in view of the well-publicized failure rate of new agents in clinical trials, as we will now be able to test a single drug on a great many tumors in the pre-clinical phase. At the opposite end of the spectrum, this advance offers the possibility of testing many drugs on tumors of a single origin, which may prove to be a fruitful personalized medicine approach for drug selection in individual patients. Finally, we anticipate that there will also be opportunities to develop much-needed clinically relevant models of resistance to endocrine and other therapies.

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Footnote

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