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The origin of breast tumor heterogeneity

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Abstract

How breast diversity is generated is a fascinating and fundamental question with important clinical implications. It is clear that the diversity of phenotypes displayed by breast cancer cells reflects the array of cell types present in the disease-free breast epithelium, including luminal, basal, and stem cells. Therefore, it is hypothesized that the molecular regulators governing normal development of the breast epithelium may double as engines of breast tumor diversity. In the past few years, a deepened understanding of the mammary epithelial hierarchy has prompted the search for the cellular precursors of breast tumors. At the same time, the use of novel experimental strategies as well as the new technology of massively parallel sequencing have provided insight into the origin and evolution of breast tumors. Here, we review the current understanding of the basis of the intrinsic subtypes and the sources of intertumor heterogeneity.

Keywords

Breast cancer; tumor heterogeneity; cell-of-origin; mammary gland

INTRODUCTION

Breast tumors exhibit striking genetic and phenotypic diversity. Features that vary widely among breast cancers include proliferation rate, invasiveness, metastatic potential, response to anticancer therapy, and the presence or absence of specific oncogenic driver mutations. Indeed, each of the ‘hallmarks of cancer’ originally identified by Weinberg and Hanahan (1,2) represents a potential axis of heterogeneity along which breast tumors may be distributed. Breast cancer diversity exists at several levels, encompassing differences both between tumors from different patients (inter-tumor heterogeneity) and among cancer cells within of a single tumor (intra-tumor heterogeneity).

For many years, breast cancers have been studied and classified based on their histologic appearance and the presence or absence of select biomarkers, including hormone receptors and cytokeratins. Since the early 2000s, the revelation that breast cancers can be robustly

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classified into discrete molecular subtypes based on their global gene expression profiles has fundamentally shaped the current understanding of inter-tumor heterogeneity (3–5). At least six distinct molecular subtypes of breast cancer have been identified on the basis of gene expression profiling, which include luminal A, luminal B, HER2-enriched, basal-like, and claudin-low tumors, as well as a normal breast-like group (for an excellent overview of molecular subtypes, see reference 6). Since these subtypes were originally derived from unsupervised hierarchical clustering of global gene expression data, they are not defined by arbitrary selection of specific histopathologic features, and are therefore referred to as the "intrinsic" subtypes. Though the intrinsic subtypes fall quite short of encompassing the full extent of cancer diversity, in general tumors that fall within the same subtype behave in similar ways and exhibit similar sensitivity to therapy (7–10). In addition, the fact that breast cancers show long-term consistency with regard to molecular subtype and exhibit a high concordance rate between primary tumors and recurrences/metastases (11) suggests that each subtype may represent a stable biological state. Dissecting the biology of these very distinct disease entities has become a major focus of contemporary breast cancer research.

THE MAMMARY EPITHELIAL HIERARCHY

The roots of breast tumor heterogeneity lie in the developmental hierarchy of the normal mammary gland (Figure 1). The mammary epithelium is a bilayered structure, containing an inner layer of luminal cells and an outer layer of basal or myoepithelial cells (basal/ME). Anatomically, the gland is a tree-like structure consisting of a network of ducts and lobules. Milk is produced by secretory luminal cells in the lobules, while smooth muscle contraction of the ductal and lobular myoepithelial cells is responsible for milk release (12,13). The epithelium undergoes cycles of regeneration and regression with successive pregnancies. In light of this observation, the existence of a long-lived mammary tissue stem cell (MaSC) has been postulated for almost 60 years. Using the cleared fat pad assay pioneered by DeOme, several pioneering groups demonstrated that transplanting fragments of mammary tissue or bulk suspensions of MECs could serially regenerate entire epithelial trees in the recipient mice, suggesting the existence of long-lived progenitor cells within the tissue (14–16).

In 2006, it was shown that these mammary stem cells, or MaSCs, were nearly exclusively contained within the $CD24^{+}/CD29^{high}/CD49f^{high}$ population of basal/myoepithelial cells. Singly transplanted cells from this fraction occasionally generated entire outgrowths in recipient mice, a *bona fide* demonstration of MaSC function and bipotency (16,17). However, a majority the basal/ME cells were unable to generate outgrowths. This may be an indication that the basal/ME epithelial compartment is heterogeneous, containing both stem and non-stem cells. On the other hand, it may reflect incomplete or sporadic activation of MaSCs in the transplant assay and/or technical limitations of the assay itself.

Historically, studying MaSCs in the human gland has been difficult because of a lack of a comparable assay to the cleared fat pad transplant. However, even before the definitive identification and isolation of mouse MECs, the existence of a human analog was suspected based on contiguous regions of identical X-chromosome inactivation in human mammary lobules (19). Pioneering work in this area involved *in vitro* colony-forming assays, where sorted subsets of human MECs are grown in tissue culture and their progeny are

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characterized. Using this approach, several groups reported the existence of “bipotent” human mammary stem cells that are able to generate both luminal and basal progeny (Stingl et al. 2001, another references). Recently, with the development of a model allowing the growth of human mammary epithelial cells (HMECs) in humanized mouse stroma (termed human-in-mouse or HIM), it has become possible to evaluate the behavior of these putative MaSCs *in vivo* as well (22). In particular, a CD49f+/EpCAM− population of human basal/ME cells, analagous to the CD24+/CD29^{high} population in mice, is also enriched for repopulating ability in HIM xenografts (23). Alternatively, one group identified an aldehyde dehydrogenase (ALDH)-positive subpopulation in the human epithelium with enhanced ability to generate epithelial structures in the HIM model (24). In general, engraftment potential is much lower in HIM transplants than in orthotopic mouse transplantation, likely because of inadequate supporting stromal milieu or residual immune activity in recipient mice. Hence, unlike in mice, the existence of a *bona fide* human MaSC capable of generating a fully functional tissue from a single cell has yet to be definitively demonstrated.

Evidence also exists for unipotent stem or progenitor cells that maintain the luminal or basal cell population. Currently, the dominant paradigm is a hierarchical model of mammary development, with bipotent MaSCs residing at the apex of the hierarchy, and dedicated luminal and myoepithelial progenitors giving rise to terminally differentiated progeny. Human luminal progenitor cells display a CD49f+/EpCAM+ immunophenotype, while their mouse counterparts are CD24^{high}/CD49f^{low}/CD61+/Sca1−/CD133$^{low/-}$ (20,25,26). No specific combination of markers has yet been identified to purify myoepithelial progenitors, but these cells can be derived from serial passage of the MaSC-enriched fraction, implying that they indeed lie downstream in the hierarchy (20).

Until recently the evidence for the bipotentiality of MaSCs was limited to the observation that these cells could clonally generate both lineages in fat pad transplantation assays, which may or may not be an accurate reflection of the behavior of these cells *in situ*. Recently, several groups have taken advantage of sophisticated *in vivo* lineage tracing approaches to study the behavior of MaSCs in their native tissues. These studies have made use of lineage-specific inducible *Cre* alleles to allow for labeling of specific sets of cells at different developmental stages. Using this approach, multiple groups have identified a population of K14-expressing basal cells that makes long-term contributions to the luminal lineage (25–27). A small subset of basal cells marked by *Procr* expression seem to be highly enriched for stem cells, since clonal lineage tracing analysis demonstrated that 93% of labeled *Procr* + clones contained both luminal and basal cells after a 6-week chase. However, using a very similar lineage-tracing approach that also employed an inducible *K14*−Cre allele, van Keymeulen et al. (28) recently reported the existence of self-renewing, unipotent luminal and basal stem cells within the mammary gland. These cells did not appear to contribute to the opposite lineage to any significant extent, suggesting that MaSCs were not essential for maintenance of the mammary gland *in situ* under normal physiologic conditions. Likewise, Prater et al. (29) recently described a set of cells labeled by smooth muscle actin (SMA) that behave similarly and remain restricted to the myoepithelial lineage. Therefore, the role of MaSCs *in vivo* and the relevance of the fat pad transplantation assay is currently a topic of some debate.

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Determinants of breast cancer heterogeneity

How does the mammary hierarchy relate to breast tumor heterogeneity? All breast tumors arise from the accumulation of oncogenic hits in a genetically normal precursor. Breast tumors may differ in either the nature of those genetic mutations, or in the identity of the initiating cell. This precursor, or "cell-of-origin" undergoes clonal expansion during the earliest stage of tumor progression, and therefore has a critical impact on the behavior and progression of the resulting tumor. Presumably, the characteristics of the normal precursor cell are epigenetically passed on to the tumor cells (and their progeny) following transformation (Figure 2A). As such, the identity of the cell-of-origin for various tumor types has been a topic of great interest.

Notably, the cell-of-origin concept is closely related to, but quite distinct from, the cancer stem cell concept (30). The cell of origin is the normal cell which receives the initial genetic insult that eventually results in a full-blown tumor, while the cancer stem cell is operationally defined as the cell that can maintain tumorigenicity and seed metastases. All tumors have a cell-of-origin, but they may not necessarily contain cancer stem cells. Also, since the CSC phenotype could emerge at some later stage of tumor progression, these two cells need not be identical or even similar. This has been a source of some confusion in the literature, since cancer stem cells are also frequently referred to as tumor-initiating cells.

As stated earlier, a second and equally important determinant of tumor phenotype is the particular collection of mutations that lead to oncogenesis, different from tumor to tumor. In recent years, massively parallel DNA sequencing technologies has enabled whole-exome analysis of a large number of human tumors. Comprehensive molecular profiles of breast and other cancer types have revealed associations between the panel of mutated genes and particular tumor subtypes (31–33). Recent data suggests that many frequently mutated genes may act as determinants of tumor differentiation in addition to exerting oncogenic effects. Therefore, through a combination of genetic and cell-of-origin effects, both epigenetic and genetic influences can serve as engines of tumor diversity.

MaSCs as precursors of breast tumors

From where in this complex cellular hierarchy do breast tumors originate? Is there a single tumor precursor for all breast tumors, or can multiple cell types initiate a tumor? If so, which cells in the mammary gland are likely tumor precursors for which types of breast cancer?

Historically, MaSCs were theorized to play a key role in breast tumor initiation. In theory, these cells should have a lower threshold for oncogenic transformation than progenitor cells (which lack self-renewal) or differentiated cells (which have lost both self-renewal and proliferative capacity). Additionally, MaSCs are long-lived within the epithelium and therefore are at increased risk for acquiring the stepwise genetic alterations required for transformation, whereas transit-amplifying progenitors and terminally differentiated cells presumably have a more limited lifespan.

There is convincing evidence that MaSCs may be the targets of transformation in certain mouse models of breast cancer (34–36). Several lines of evidence suggest that MaSCs are the precursors of the spontaneous mammary tumors which arise in transgenic *MMTV-Wnt1*

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mice. These tumors are notable for considerable intra-tumor heterogeneity, containing luminal-like and basal-like cells. Originally, the presence of a shared secondary deletion of *Pten* in both types of cells suggested a common bipotent precursor, though a recent deep-sequencing study demonstrated that only half of *MMTV-Wnt1* tumors follow this type of hierarchy (37). In any event, mammary glands from all *MMTV-Wnt1* mice have a massively expanded basal/MaSC subpopulation prior to tumorigenesis (36,38–40). In addition, these tumors have a gene expression profile that is similar to the MaSC-enriched basal epithelial subpopulation (41). Other mammary tumor models driven by deletion of *Apc* or mutant *B-catenin* also show preneoplastic changes consistent with stem cell overexpansion, suggesting that tumors in these mice likely arise from MaSC transformation (42,43). Indeed, conditional deletion of *Apc* produces tumors only when targeted to *Krt14*-expressing basal cells/MaSCs, although the tumors were molecularly distinct from *MMTV-Wnt1* tumors (44).

Collectively, these findings are reflective of the role of the Wnt signaling pathway in regulating the expansion and proliferation of MaSCs and of stem cells in general (25,45). To what extent, however, do *Wnt*-driven mouse mammary tumor models accurately recapitulate human tumor development? While there is substantial evidence that aberrant Wnt signaling may contribute to tumor progression and metastasis, recurrent Wnt-activating mutations in B-catenin or APC appear to be relatively rare in breast cancer. As such, most human tumors seem to follow a distinct oncogenic pathway compared with *MMTV-Wnt1* mouse tumors, and the generalizability of the *MMTV-Wnt1* model to human breast cancer is an open question. Certainly, aberrant Wnt signaling is a critical component of breast tumor biology, and it is possible that human tumors may indeed be initiated from the transformation of MaSCs, but this has not yet been definitively demonstrated.

Origin of BRCA1-associated breast cancer

The origin of BRCA1-associated breast cancer has been a topic of recent interest. Tumors arising in BRCA1 carriers are unusual in that they are usually of the basal subtype, which is relatively uncommon in sporadic cases (~15%). These tumors also tend to be quite heterogenous, and express markers associated with mammary stem cells including KRT5/6, KRT14 and TP63, while lacking expression of hormone receptors (46). In the past, perceived similarities between basal-like breast cancer cells and MaSCs fueled speculation that these tumors are initiated by the transformation of MaSCs (47–49). This notion was supported by direct evidence from mouse models that indicated that selective loss of *Brca1* in basal cells led to the generation of basal-like tumors (50). Recently, however, the initiating cell for these murine tumors has been investigated in more detail. Molyneux et al. (51) employed a model where deletion of the BRCA1 tumor suppressor was targeted to the basal/MaSC population with a *Krt14-Cre* allele (on a p53-heterozygous background) or to luminal ER- cells with a *BRG1-Cre* allele. Interestingly, while deletion BRCA1 in basal cells/MaSCs did indeed lead to basal-like tumor formation, the tumors were malignant adenomyoepitheliomas that did not resemble the invasive adenocarcinomas most commonly in human *BRCA1*-associated cancer. However, when deletion of *BRCA1* was specifically targeted to to β-lactoglobulin-expressing luminal cells using a *Blg-Cre* allele, they generated high-grade invasive ductal carcinomas (IDC) similar to those seen in BRCA1 mutation carriers. Moreover, the majority of these tumors showed a basal-like phenotype, expressing

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basal-specific markers such as *Krt14* and *Tp63*. This result suggested that luminal cells, rather than MaSCs, may be the more likely precursors for basal-like disease, at least in the setting of BRCA1 loss.

Interestingly, in humans, BRCA1 haploinsufficiency is associated with lineage commitment defects prior to tumorigenesis. *BRCA1* mutation carriers harbor altered luminal progenitor cell phenotypes (23,52). These luminal progenitors cells are characterized by decreased expression of mature luminal differentiation markers and abnormal expression of markers of basal epithelial cells (52). This observation has been interpreted as a defect in luminal lineage commitment and maturation, the cause of which appears to be aberrant accumulation of the Slug transcriptional repressor in luminal progenitors. In normal tissues, Slug represses luminal differentiation in basal cells and is rapidly turned over via protesomal degradation (52–54). In BRCA1-mutant tissues, however, Slug protein is aberrantly stabilized in luminal cells. Therefore, BRCA1 loss leads to abnormal maturation and differentiation of the luminal lineage, and subsequently the formation of basal-like tumors.

Origin of sporadic breast cancer

The revelation that BRCA1-associated tumors likely arise from luminal progenitors, rather than MaSCs, raises the question of whether sporadic breast tumors also arise from luminal precursors. Recent work suggests that this indeed may be the case. Apart from the *Brca1/p53* example, luminal cells have been implicated as targets of transformation in other mouse models as well. Tao et al. (26) used an innovative approach where oncogenes could be delivered to the mammary epithelium in a cell type-specific manner. By introducing the *Etv6-NTRK3* fusion oncogene into *Krt8*-expressing luminal cells, they observed a range of tumor phenotypes with variable expression of luminal and basal markers. A caveat is that this fusion protein is typically present at high frequency only in secretory breast cancer, a rare subtype.

Aberrations in the PI3K pathway occur in a large fraction of sporadic breast cancers, including luminal and basal tumors (32,33,55). Meyer et al. (56) generated a mouse model in which a constituitively active mutant form of PI3K was conditionally expressed in the luminal population driven either by *WAP* (which targets alveolar progenitors) or *MMTV* (which targets a heterogeneous group of progenitor cells). Interestingly, tumors initiated from either population were heterogeneous, and displayed evidence of both luminal and basal differentiation. A recent study by Melchor et al. (57) expanded on these findings by using a conditional knockout approach to examine the phenotype of tumors driven by either *Brca2* or *Pten* deletion (either with or without concommitant p53 loss). In this study, variation of the tumor-initiating mutation as well as the cell of origin allowed for untangling of the effects of these two factors on tumor phenotype. In line with the previous findings, the cell-of-origin determined tumor phenotype in *Brca2*-deleted mice. However, interestingly, deletion of *Pten* and/or *p53* in an identical subset of BLG-expressing, ER-negative luminal cells led to distinct phenotypes depending on the initiating lesion. While deletion of *Brca2* in this population always resulted in a basal-like tumor, deletion of *Pten* or *p53* resulted in tumors that were variably luminal A/B, basal-like, or the so-called “normal breast-like”

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subtype. Thus, much of the spectrum of heterogeneity seen in human tumors can be recapitulated in mouse tumors that are initiated in a luminal cell of origin.

Even so, mouse models are inherently limited in their ability to fully represent all incarnations of breast cancer in human patients. To investigate the cell of origin for human tumors Keller et al. (58) isolated luminal cells from human breast reduction tissues on the basis of cell surface marker expression, induced tumorigenesis by lentiviral transduction of the cells with multiple combinations of oncogenes, and studied the resulting tumors that formed in immunodeficient mice. Interestingly, the tumors that formed were variably luminal-like or basal-like, encompassing much of the heterogeneity seen in sporadic human tumors. On the other hand, paralleling the findings of Molyneux et al., transformation of CD10-expressing basal/ME cells generated tumors that did not resemble common forms of human cancer. Instead, these tumors were poorly differentiated carcinomas with metaplastic features, and were molecularly similar to claudin-low breast cancer. Similar tumor xenografts generated from the transformation of cultured mammary epithelial cells (HMECs), which are derived from the CD10+ fraction and display a predominately basal phenotype. When transduced with telomerase, oncogenic *KRAS* and SV40 large T antigen (termed HMLER), these cells form poorly differentiated or metaplastic carcinomas in mice that show heterogenous areas of squamous, papillary, giant cell, and glandular histologies (58,59). Claudin-low breast tumors often display similar features (7). Therefore, luminal progenitors likely serve as the origin of luminal and basal-like human breast cancers, while MaSCs or basal/ME progenitor cells likely initiate claudin-low tumors.

Genetic determinants of luminal and basal subtype

If transformation of luminal progenitors with the same oncogenes can variably lead to either a basal-like or luminal-like tumor, as in the examples cited previously, what ultimately determines tumor phenotype? One possibility is that the choice of promoters used in the previously discussed studies (i.e. *Wap, Krt18, Blg)* target a heterogeneous population of cells, and that cell-of-origin effects remain dominant in determining whether the tumor displays a luminal or a basal-like phenotype (Figure 2B). Indeed, recent evidence suggests that the luminal progenitor population is heterogeneous. In mice, most luminal progenitors appear to lack expression of estrogen receptor, but at least a portion of ER+ cells also show clonogenicity (60). In humans, several distinct subsets of luminal progenitors have been identified, including a cryptic *ERBB3*-expressing class that does not appear to be present in all individuals (61). However, whether these varying cell types contribute to different subtypes of breast cancer remains an open question.

An alternate possibility is that distinct initiating and/or secondary mutations occurring in each precursor cell are critical in determining the eventual tumor phenotype. In recent years, the development of massively-parallel sequencing technologies has made genome- or exome-wide sequencing of breast tumors a reality. This technology has been used to compare large numbers of individual tumors in attempt to identify recurrently mutated genes. Alternatively, deep or even single-cell sequencing has been used to reconstruct the life history of individual cancers. These studies have revealed significant differences in the mutational profiles of luminal-like and basal-like tumors. The most intuitive explanation for

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these associations is, of course, that particular oncogenic mutations may lead a developing tumor to adopt a particular differentiation state early in tumorigenesis. This notion is supported by the previously discussed findings where cells derived from BRCA1-mutation carriers show aberrant differentiation (52).

Luminal A/B cancers exhibit distinct mutational profiles, including mutations in *PI3K*, *MAP3K1*, *GATA3*, *FOXA1* and *TBX3*, key regulators of luminal differentiation in the normal mammary gland (33). A number of frequently mutated genes in luminal A/B tumors play a key role in luminal epithelial differentiation and/or hormone receptor signaling, including *GATA3*, *FOXA1*, and *TBX3*. In particular *GATA3* is known to play an essential role in the terminal differentiation of luminal progenitor cells (24,62). Meanwhile, *FOXA1* plays a critical role in ER-dependent transcription (62). Though it remains to be proven, it seems highly likely that aberrant functioning of these genes play some role in the determination of luminal tumor phenotype.

The mutational profile of basal-like tumors is notable in that there is a higher number of somatic mutations per tumor, but a lower number of frequently mutated genes and a higher degree of genomic instability (33,63). Clearly, this unique mutational profile indicates that basal-like breast cancer is a fundamentally different disease than luminal cancer, and is more reminiscent of non-mammary tumor types such as serous ovarian carcinoma, which has a similar mutational and phenotypic profile (33,64). A striking finding is the extremely high rate of p53 mutations in basal-like disease (over 80% in the TCGA dataset), which was the only gene mutated at high frequency in this subtype. Is, then, the loss of p53 a defining event in the genesis of basal-like breast cancer? Recently, there is direct evidence from mouse models that p53 loss can bias tumors toward a more basal-like or mesenchymal fate. Liu et al. () reported that double deletion of both *Pten* and *p53* led to the generation of triple-negative tumors that shared many features with the claudin-low subset, regardless of whether the initial deletion of *Pten* and *p53* was driven by the *MMTV* or *WAP* promoters.

In light of these findings, it is likely that early loss of p53 may lead to secondary mutations that alter MEC phenotype and lead to basal-like tumor formation. Based on their mutational profile, basal-like tumors are hypothesized to undergo rapid clonal evolution, and recent deep sequencing studies suggest that this may indeed be the case (32,66). The loss of the DNA repair mechanisms orchestrated by p53 may allow basal-like tumors to acquire secondary mutations which impart characteristics that would provide a competitive advantage over neighboring clones - such as rapid growth, invasiveness, and loss of cell-cell contacts – all of which are also features of basal-like tumors. There is some evidence that recurrent secondary mutations, amplifications or deletions also appear to contribute to the basal-like phenotype. Amplification of *TAZ*, a key component in the Hippo signaling pathway, is a frequent occurrence in basal-like tumors and has been shown to regulate the basal/MaSC phenotype during mammary gland development (67,68).

Role of cellular plasticity in basal-like breast tumor development

Does the luminal origin of basal-like breast cancer imply that de-differentiation of luminal cells is an essential component of basal tumor initiation?

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On the one hand, as has been pointed out previously, it may simply be the case that the tumor subtypes are inaccurately named. Lim et al. (23) directly compared the gene expression profiles of normal mammary epithelial subsets (i.e. basal/MaSC, luminal progenitor, and mature luminal cells) to those of breast tumors falling into each of the intrinsic subtypes, including basal, luminal A/B, and claudin-low. They found that the luminal A and B subtypes were most similar to the EpCAM+/CD49f− mature luminal cells, while unexpectedly, the luminal progenitor gene expression signature was most highly associated with the basal-like subtype. Meanwhile, the MaSC-signature was closely in line with the claudin-low subtype. This observation has since been noted by other groups as well (6). In fact, many of the immunohistochemical and molecular markers that define the basal-like subtype, such as cytokeratins 14 and cytokeratin 5/6, are also expressed by luminal cells in human tissues (69). Therefore, the so-called “basal-like” gene expression signature may actually be a luminal progenitor signature. Likewise, the claudin-low tumors show similarities with the basal/MaSC subpopulation of epithelial cells, a finding that is in line with the generation of metaplastic, poorly-differentiated tumors from basal/ME precursors.

On the other hand, it is possible that under the influence of certain oncogenic mutations, luminal cells become developmentally plastic and dedifferentiate to re-acquire a basal or stem-like phenotype, thereby giving rise to a basal-like tumor. Some have suggested that such a differentiation may involve a complete or partial epithelial-to-mesenchymal transition (EMT), where luminal cells lose apicobasal polarity, become motile and express markers characteristic of basal or mesenchymal cells. An EMT signature is enriched in basal-like tumors and to a greater degree in claudin-low tumors (7). Moreover, several groups have shown that EMT is intimately linked with an undifferentiated or embryonic stem-like state (70,71). However, many breast tumor specimens fail to show evidence of EMT, and the *in vivo* relevance of this process has been questioned (72,73). Nonetheless, amplification or overexpression of several EMT inducers have been identified in basal-like tumors, including alterations in *SNAI2*, *SNAI1*, *TWIST1*, and *YAP/TAZ* (74,67).

Cellular plasticity has also been shown to occur by stochastic mechanisms in cancerous cells as well as in normal breast epithelial cells. Recently, Chaffer et al. (75) reported that a subpopulation of mammary epithelial cells retained the capacity to spontaneously generate stem-like cells *in vitro*. When transformed, these cells were enriched for stem cell markers and exhibited enhanced tumorigenicity in xenotransplantation assays. Similar cell-state transitions have been observed in cultured breast cancer cell lines, where normal or malignant non-stem cells purified by FACS and cultured separately were observed to regenerate the stem cell population at a rate too rapid to be explained by cell sorting impurities (53,76). Since the *in vitro* tissue culture microenvironment is presumably more or less homogenous, these transitions were hypothesized likely to occur randomly rather than in a directed manner. The transitions were modelled by Gupta et al. as a Markov process, where the cells stochastically transition between luminal-like, basal-like and stem-like states at characteristic frequencies.

Recently, Phillips et al. (53) extended these findings to normal epithelial cells as well, and showed that expression of the transcription factor Slug was critical for interconversion of more differentiated luminal and basal cells to stem cells. Interestingly, mice lacking Slug

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were completely protected against tumor development when crossed with the *MMTV-myc* strain, suggesting that these cell-state transitions governed by Slug were critical for tumorigenesis. As a caveat, the *in vivo* prevalence of stochastic transitions between stem and non-stem cells in breast cancer has not yet been explored. Recently, however, several groups have reported *in vivo* evidence of stochastic interconversion between stem-like and non-stem cells in other cancer types, including Wnt-driven intestinal tumors (77).

Origin of HER2-enriched breast cancer

Regarding the pathogenesis, cell-of-origin and genetic determinants of HER2-enriched breast tumors, considerably less is known. This subtype is more heterogeneous with respect to hormone receptor expression than the other subtypes, with the majority being negative for hormone receptors but a sizeable minority showing expression of ER or PR. Furthermore, only 70% of these tumors are clinically HER2-positive (7). At a molecular level, however, all HER2-enriched tumors are defined by high expression of a common set of HER2-regulated genes. Therefore, it is tempting to speculate that HER2-enriched tumors driven by a common oncogenic signaling pathway (i.e. dysregulation of HER2/EGFR signaling) and may not necessarily share a common cell of origin. Efforts to model HER2+ disease have made extensive use of the *MMTV-neu* mouse (78).

To date, the most likely candidates for transformation in these mice are the so-called parity induced mammary epithelial cells (PI-MECs). These cells are identified as lacZ-expressing cells that arise in nulliparous *WAP-Cre/Rosa26-lacZ* mice, undergo significant expansion at pregnancy, and survive involution (79–81). PI-MECs were originally described as multipotent alveolar progenitors that contribute to the basal and luminal layers of alveoli in subsequent cycles of pregnancy and lactation. Recent work, however, suggests that PI-MECs reside in the luminal layer and exclusively give rise to ER- secretory alveolar luminal cells (82).

Several lines of evidence support the notion that PI-MECs are the targets of *MMTV-neu* tumorigenesis. First, mice deficient in cyclin D1 activity cannot maintain a functional PI-MEC population, and additionally are resistant to tumorigenesis when crossed with HER2-neu mice (83). Second, conditional ablation of PI-MECs was shown to reduce the onset of tumor development in *MMTV-neu* mice (84). Could HER2-enriched breast tumors develop from a similar population present in human tissues? To date, this has not been definitively demonstrated for several reasons. First, the existence of PI-MECs in human tissues has not been proven. Currently, no specific markers are available to prospectively isolate PI-MECs, so there is no way to identify or study these cells in human epithelia. Second, the extent to which the *MMTV-neu* model represents HER2-enriched breast cancer is unclear, as these tumors have a global gene expression profile that is more similar to the luminal A and B subtypes (41,85,86). Thus, the definitive origin of HER2-positive tumors remains to be elucidated.

SUMMARY AND CONCLUDING REMARKS

Recent years have witnessed impressive advances in the understanding of how breast tumors are initiated. The emerging consensus is that the most common subtypes, including luminal

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A/B and basal-like tumors likely arise as a result of transformation of a luminal progenitor cell of origin. In contrast, rare metaplastic and claudin-low breast tumors may have a different origin, either from a unipotent myoepithelial stem cell or a MaSC. While a few of the genetic determinants that drive basal or luminal tumor phenotype have been elucidated, such as BRCA1 loss, many more remain to be discovered. Importantly, a majority of basal-like tumors do not show loss of BRCA1 expression or BRCA1 mutation; in these tumors, a different genetic driver may allow the luminal progenitor cell of origin to transdifferentiate or adopt basal-like features during tumor development. Lastly, the origin of HER2-enriched breast cancer remains somewhat of a mystery. Better models of HER2-mediated tumorigenesis are needed to resolve the life history of this subtye.

In this review, we have focused our discussion mainly on inter-tumor heterogeneity, but also acknowledge that it is only one facet of the tremendous diversity exhibited by breast tumors. Individual clones and even single cells within each breast tumor may differ markedly in their oncogenic makeup and in the range of phenotypes manifest by the tumor cells, a feature that is usually not captured by ‘omics’ approaches such gene expression profiling. Nonetheless, the intrinsic subtypes represent fundamentally distinct disease processes with very different origins and patterns of evolution. In the future, a better understanding of the basic biology of each subtype should lead to an enhanced ability to diagnose and treat women with different forms of breast cancer.

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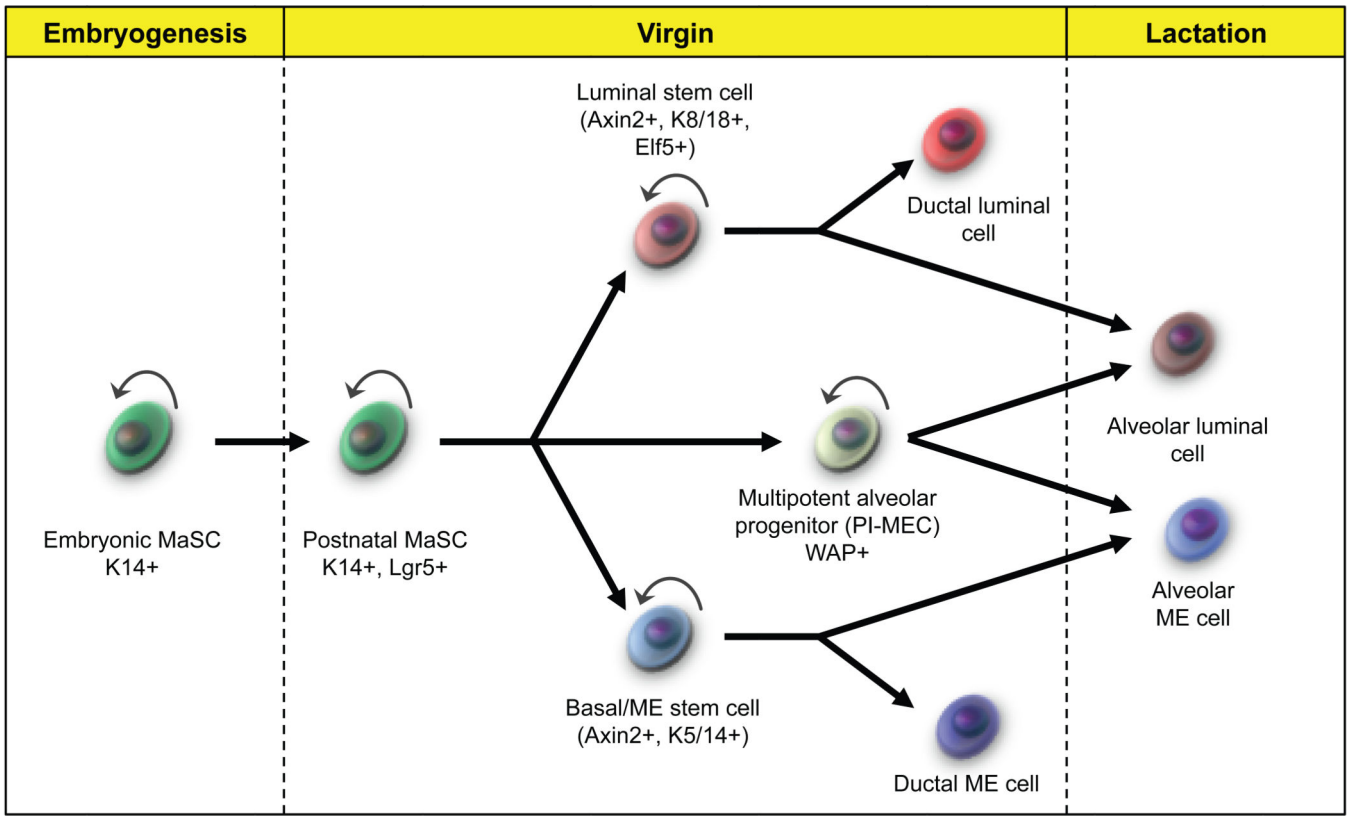

FIGURE 1. The mammary epithelial hierarchy

A simplified schematic depicting the known relationships between stem, progenitor, and mature cell populations with the mammary epithelium. Bipotent MaSCs have been identified in lineage tracing in both embryonic and adult glands. More recently, unipotent stem cells have also been identified in both the luminal and basal lineages. There is also evidence for a long-lived, multipotent alveolar progenitor cell population (PI-MECs) which expands during pregnancy and survives involution. However, recent evidence suggests this population may only contribute to the luminal alveolar lineage.

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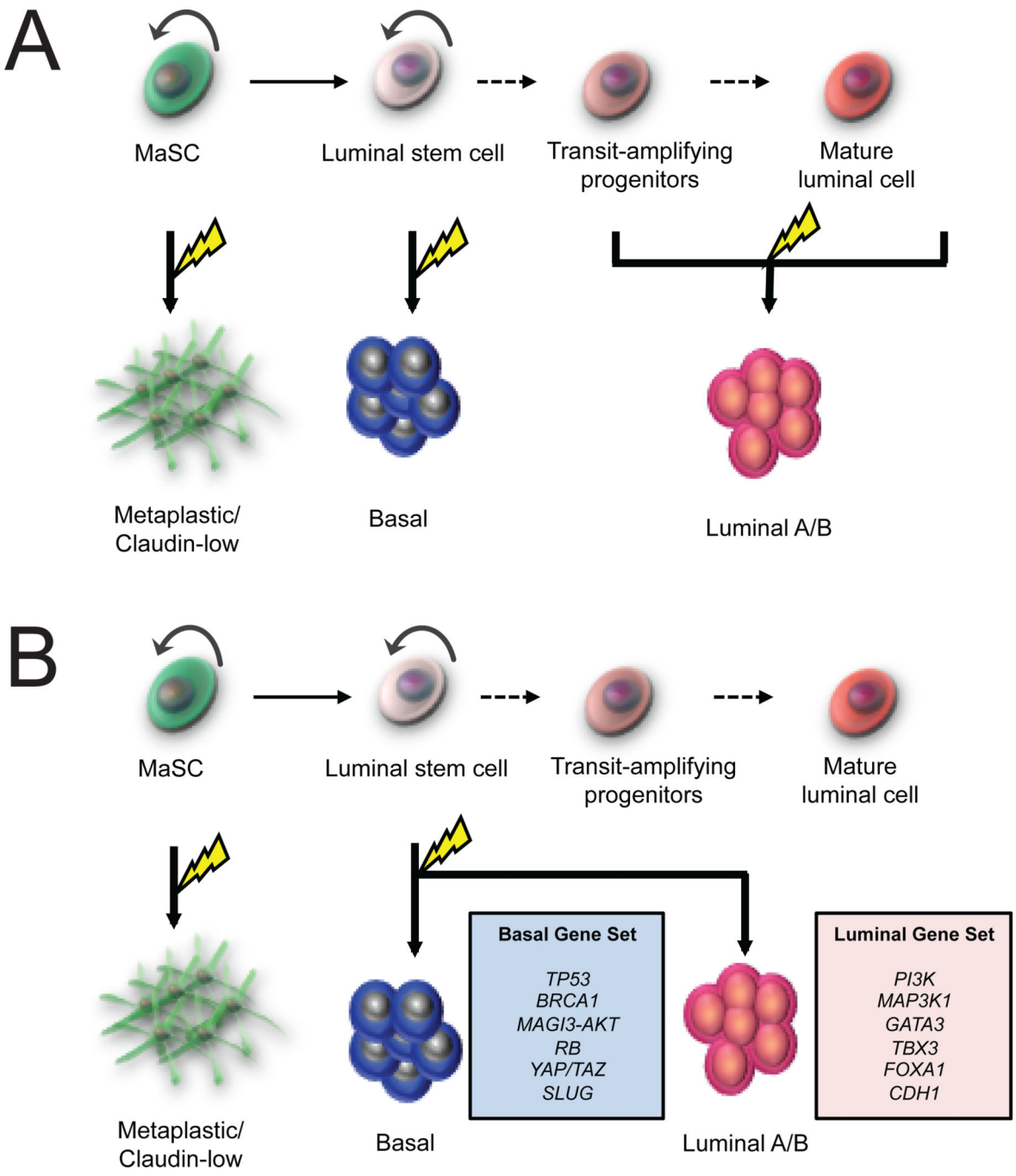

FIGURE 2. Cellular origins of luminal and basal tumors

A, Cell-of-origin model of breast tumor heterogeneity. In this model, luminal tumors arise from transformation of more committed progenitors, and maintain their differentiation during tumor progression. On the other hand, basal-like tumors arise from earlier progenitors or from unipotent luminal stem cells. **B**, Genetic mutation model. In this scenario, basal and luminal tumors can both arise in similar precursors but the nature of the oncogenic signal determines the eventual phenotype of the tumor.