Chapter 4

Early-Stage Progression of Breast Cancer

William Kietzman, Anna T. Riegel and Virginie Ory

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Abstract

Breast cancer can be defined as a group of diseases with heterogeneous origins, molecular profiles and behaviors characterized by uncontrolled proliferation of cells within the mammary tissue. Around one in eight women in the US will develop breast cancer in their lifetime, making it the second most frequently diagnosed cancer behind skin cancer [1]. In 2015, an estimated 231,840 cases of invasive carcinoma were diagnosed, and over 40,000 deaths were caused by breast cancer which accounts for almost 7% of all cancer mortality each year [1, 2]. In 2015, 60,290 cases of in situ breast cancer were diagnosed, representing over 14% of all new cancer cases among women and men [1]. The steep increase in diagnosis of early-stage breast cancer over the past 10 years is believed to be a result of more frequent mammography. However, since over half of these in situ lesions will not progress to invasive breast cancer, controversies have arisen about approaches to treatment and prevention of progression of early-stage in situ breast cancer. Understanding the mechanisms of transition of normal breast to in situ pre-neoplastic lesions and invasive breast cancer is currently a major focus of breast cancer research with implications for preventive and clinical management of breast cancer. In this review, we give an overview of current knowledge on the molecular and pathological changes that occur during early-stage progression of breast cancer and describe some of the current models that are used to study this process.

Keywords: ductal carcinoma in situ, molecular and cellular drivers of invasive progression, early-stage breast cancer models

1. Pathophysiology of breast cancer

1.1. Anatomy and histology of the normal mammary gland

Within each mammary gland, there are 15–20 lobes containing 20–40 smaller compartments called lobules. Each lobule is composed of 10–100 grapelike clusters of milk-secreting

glands termed acini, which are connected to lactiferous ducts [3]. The epithelium throughout the acini and ducts consists of two layers: an inner layer of polarized and cuboidal luminal cells that encapsulate a central lumen, and a basal outer layer of myoepithelial cells with contractile properties conferring these cells an active role in the milk excretion during lactation [3, 4]. Myoepithelial cells also ensure the maintenance of the adjacent luminal epithelial cell polarity and the synthesis of a laminin-rich basement membrane (BM) that forms a structural barrier separating the glandular epithelium from the stroma [5]. In the normal mammary gland, luminal epithelial cells are characterized by the expression of the luminal cytokeratins CK7, CK8 and CK18, sialomucin, epithelial-specific antigen, occludin and integrin β4 [5, 6]. On the other hand, myoepithelial cells express the basal cytokeratins CK5, CK14 and CK17 along with CD10/CALLA, alpha-smooth actin and P63 [6, 7]. The stroma surrounding the mammary gland consists of an insoluble extracellular matrix (laminin, fibronectin, collagen, proteoglycans), mesenchymal cells (fibroblasts, adipocytes, endothelial cells and resident immune cells), and various growth factors and cytokines [8]. Aberrant interactions between mammary epithelial cells and the stroma may lead to structural and functional alterations of the mammary gland biology and ultimately promote breast malignancy [8].

1.2. Hyperplasia/atypical hyperplasia and "in situ" carcinoma histopathology

Many suspicious mammograms or palpable findings turn out to be benign lesions following breast biopsy [9]. However, based on the histopathological report and family history, about 3–10% of these benign lesions are considered to be at high risk of later breast cancer and are referred to as atypical hyperplasia [10, 11]. Atypical hyperplasia is a premalignant lesion diagnosed based on the architectural pattern, cytology and the disease extent and is traditionally classified into two subtypes: atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH) [12]. The absolute risk of developing breast cancer has been estimated at about 30% for women diagnosed with atypical hyperplasia after 25 years of follow-up [13].

"In Situ" carcinoma, also known as stage 0 breast cancer, is defined by the clonal proliferation of neoplastic epithelial cells within the ducts (e.g., ductal carcinoma in situ) or the lobules (e.g., lobular carcinoma In Situ) of the mammary gland. "In Situ" means that cancer epithelial cells remain confined inside the mammary ducts or lobules with no evidence of cancer cell invasion into the surrounding stroma [1]. Ductal carcinoma in situ (DCIS) incidence has risen, and it accounts for 15–20% of the breast cancer currently diagnosed as a result of increased screening mammography in the past 30 years [14, 15].

Even if DCIS is not immediately life-threatening, 14–53% of untreated DCIS lesions will progress to invasive ductal carcinoma (IDC) with considerable inconsistency in the timing and nature of this transition [16–20]. The most widely accepted model of breast carcinogenesis is the model of "linear" progression that hypothesizes that DCIS is an obligate precursor of IDC evolving through sequential stages, dependent upon early genetic and/or epigenetic changes [21–26].

1.3. DCIS: treatment perspectives

Generally, surgery alone will reduce the risk of mortality following DCIS to less than 5% for lumpectomy and 1% for mastectomy [27]. Surgery followed by radiation and/or hormonal therapy may not alter overall survival dramatically but tends to reduce recurrence, and in the case of hormonal therapy, contralateral breast cancers [28–30]. Decisions regarding therapy are made on a case-by-case basis in function of the clinical presentation and patient choice. Though controversy remains surrounding treatment strategies, generally an argument with concerns of overtreatment voiced against fears of under-treatment; it seems unlikely that current paradigms will change without further research and understanding into the natural history of DCIS progression and identification of clinically actionable markers of risk.

1.4. Profiling of DCIS

Using molecular profiling, Perou et al. first described the subtypes of human breast cancer generating four intrinsic subtypes: luminal A, luminal B (that are frequently ER (estrogen receptor) and PR (progesterone receptor) positive); HER2+ (human epidermal growth factor receptor); and basal-like (frequently HER2-,ER-, PR- also known as triple negative) [31]. These subtypes are utilized currently to subcategorize breast cancer and have demonstrated clinical applicability as individual subtypes display differences in biology and behavior that inform prognosis and course of treatment [31–33].

In 2008, Tamimi et al. performed tissue microarrays for demonstrably reliable [34–37] surrogates for the intrinsic subtypes identified by Perou et al. in 2000 in order to evaluate the prevalence of these phenotypes among DCIS cases. A large cohort of both DCIS and IDC samples were analyzed by immunohistochemistry for ER, PR, HER2, cytokeratin 5/6 and epidermal growth factor receptor **(**EGFR) and were classified based on expression of these markers. It was discovered that though all of the subtypes are represented in DCIS, the relative frequency differs significantly between DCIS and IDC. HER2 and luminal B subtypes were significantly enriched in the DCIS samples (13.2 and 13.6%, respectively) relative to invasive lesions (5.2 and 5.7%, respectively). In contrast, it was observed that the luminal A subtype was significantly more frequent in invasive carcinoma (73.4%) than in DCIS (62.5%). The triple negative/basal-like category was somewhat more prevalent in invasive carcinoma (10.9%) than in DCIS (7.7%). These discrepancies have been recapitulated by several other studies utilizing genetic means that corroborate this and other histological studies [38–42].

2. Drivers of malignant progression of early-stage breast cancer

Two types of mechanisms drive the invasive progression of DCIS: genetic and/or epigenetic modifications occurring in tumor epithelial cells, or nongenetic aberrations as a result of the bidirectional interactions between cancer epithelial cells and their microenvironment. No molecular markers have been convincingly validated to predict which subsets of DCIS are expected to progress to IDC. Nevertheless, a plethora of studies have demonstrated that the most significant changes in gene expression profiles are observed during the transition from

normal tissue to DCIS [43] and revealed that the genetic patterns observed in IDC are already present in DCIS, suggesting a probable common origin [24, 44].

2.1. Genomic changes during progression

In addition to HER2, ER and PR status, similarities between DCIS and IDC begin broadly with chromosomal aberrations that are present in both invasive and in situ stages of disease [45–48]. Several studies have demonstrated that the majority of DCIS cases harbor large copy number alterations that appear to have origins in the transition from normal mammary epithelium to ADH, indicative of a role in early tumorigenesis [40, 48, 49]. The general profile and distribution of DCIS copy number variation have proven to very nearly match that which was previously established for invasive breast cancer with 63% of the peak regions overlapping and 21% within 10 Mb of peak regions established in IDC [46, 48, 50, 51]. Many of the most frequently observed and well-characterized alterations in invasive cancer, including gains on 1q, 5p, 8q, 12q, 16p, 20q and Xq along with losses on 8p, 9p, 11q, 13q, 14q, 16q, 17p and Xp, were similar among ADH, DCIS and IDC [45, 52]. It has been demonstrated that, on average, 83% (range 59–100%) of matched DCIS and IDC genome sample displays the exact same copy number status [48, 53]. A large body of work has failed to find significant, nonrandom discrepancies in the quantity or quality of DCIS copy number aberrations relative to invasive carcinoma [24, 25, 44, 52, 54–60]. These data support the notion of clonal disease origins and add evidence to the case that DCIS is a precursor lesion to invasive stages. Some studies have shown these genetic alterations to increase in frequency during progression from ADH through DCIS to IDC [24, 45, 49]. This suggests that at least some of the genomic alterations may be required for progression though they could be indicative of global genomic instability resulting in the accumulation of chromosomal gains and losses over time. Some studies utilizing matched synchronous ipsilateral DCIS and IDC have identified potential copy number variations that may be related to progression including amplification of genes mediating proliferative, invasive and migratory function such as the growth factor receptor, fibroblast growth factor receptor 1 [61], V-myc avian myelocytomatosis viral oncogene homolog (MYC) [62] and cyclin D1 [63] in invasive disease relative to in situ lesions. Despite these findings, it appears that these changes alone are not necessary or sufficient to drive the acquisition of an invasive phenotype and tend to be inconsistent across individual studies [45–47].

2.2. Transcriptomic and proteomic changes during progressions

In a 2015 study by Abba et al., the full exome, transcriptome and methylome of 30 pure high-grade DCIS cases were examined. 100% of DCIS cases displayed numerous somatic mutations, 62% harboring mutations in known and potential cancer driver genes; though moderately lower than invasive disease, overall the mutational profile of DCIS is remarkably similar to later stages [40]. Not all DCIS cases with chromosomal copy number variation also exhibit mutations in driver genes, which could suggest that chromosomal alteration proceeds some mutation and is a very early event in the natural history of breast cancer [25, 40]. In fact, only 10% of cases in this study displayed unaltered chromosomal copy number and cancer driver genes. They report evidence of P53 pathway inactivation in every lesion analyzed; regardless of its mutation status or intrinsic subtype, a provocative finding as P53 inactivation

is not commonplace in all varieties of invasive disease [64, 65]. Other studies have found P53 mutation in anywhere from 15 to 22% of cases [66–69]. A higher frequency was observed in high-grade lesions, a trend that is reflected in invasive breast cancer [66, 67]. Overall, this study concluded that on a whole, the molecular profiles they identified in DCIS were indistinguishable from invasive cancer suggesting that the known major genomic anomalies present in later stages of disease are present in their in situ origins. The results of this study corroborate earlier studies comparing the molecular profiles of ADH, DCIS and IDC, all of which find that the stages of breast cancer progression are extremely similar to one another, providing evidence in support of the now widely held notion that the gene and protein expression changes present in IDC proceed the transition from in situ [39, 40, 43, 70–75].

A recent study by Lesurf et al. compared DCIS and IDC after stratifying by intrinsic subtype and was able to identify differential and highly specific gene sets that distinguish IDC from DCIS [38]. The gene sets are remarkably distinct with no single gene present in every subtype's "invasion signature." It is thus possible that previous studies lack of stratification by subtype could have generated systematic errors in attempts to identify genetic predictors of progression. Strengthening this case, when genetic analysis is performed without intrinsic subtype stratification, it generates relatively inconclusive differentially expressed gene lists with significant overlap relative to previous lists generated without stratification [39, 43, 70, 74, 76, 77]. These previously generated gene lists have not be overwhelmingly useful as predictive tools for progression of DCIS, making Lesurf's findings of invasive signatures valuable. Further work needs to be done to validate these signatures before anyone can comfortably rely on them for consideration in therapy.

DCIS has also been analyzed at the proteomic level, and compared to normal ductal and lobular units, this has revealed that alterations in protein expression occur during carcinogenesis [72]. Proteins that have been identified to be significantly differentially expressed between normal structures and in situ carcinoma have functions ranging from control of cytoskeletal architecture, intracellular trafficking, apoptosis, chaperone functions to regulation of genomic stability [72]. Differential expression of actin-binding proteins was considered to be an unusual finding as remodeling of the actin cytoskeleton tends to be related to lamellar protrusions utilized in invasion and motility, and DCIS is defined as a pre-invasive lesion [72]. It seems that, in a manner similar to the genomic and transcriptomic variation observed in breast cancer progression, proteomic alterations are an early event in the natural history of tumor progression and do not display extensive changes in the transition from in situ.

2.3. Epigenomic changes during progression

Based on the overall lack of differences observed at both the global genomic and transcriptomic levels, some have postulated that epigenetic alterations, inheritable changes that do not modify DNA sequences, may be involved in the transition from in situ to IDC. DNA methylation is a common mechanism of gene promoter silencing [78–80] and has been demonstrated to increase in breast tumorigenesis [81–83]. In comparing normal breast epithelium, ADH, DCIS and IDC, Park et al. noted an increase in methylation status of interrogated breast cancer-specific CpG islands in the transition from normal to ADH and again when comparing

DCIS to ADH though DCIS and IDC did not differ in methylation levels or frequencies. Even the earliest morphologically identifiable stages of breast disease, columnar cell lesions, display an increase in the number of methylated genes, with a similar profile to DCIS and IDC [84]. This suggests that changes in methylation frequency and patterning are an early event in the natural history of breast cancer and may not significantly contribute to the transition between disease stages.

Outside of methylation status, chromatin remodeling via modification of histone residues results in differential gene transcription and has been linked to carcinogenesis [85–87]. Hints at the importance of chromatin remodeling in DCIS formation and progression have been demonstrated with over expression of chromatin remodeling proteins associated with transformation of premalignant lesions and poor prognosis in invasive disease [88, 89]. One study has even demonstrated that overexpression of the chromatin remodeling protein EZH2 is able to drive the acquisition of malignant phenotypes in immortalized mammary epithelial cells [88]. Chromatin remodeling also appears to be involved in the epithelial to mesenchymal transition, a process reported to be important in the DCIS transition to IDC [76, 90].

Another mechanism of epigenetic gene regulation is the expression of microRNAs (miRNAs) which are short noncoding sequences that are able to bind and repress translation of messenger RNAs [91, 92]. When compared to normal breast epithelium, DCIS miRNA profiles do display differences such as increased miR-21 and decreased miR-98 and let-7, though these changes are consistent between DCIS and IDC [93]. Some studies have identified potential miRNA invasive signatures highlighting differential expression of a subset of miRNAs in the transition from DCIS to IDC while others have found essentially no difference between the two [38, 40, 94]. Further studies will need to be done to validate the potential pro-invasive effect of miRNAs that could be involved in the transition to invasive carcinoma.

A more recently recognized mechanism of epigenetic regulation is the alternative splicing of mRNA transcripts, allowing for the generation of multiple unique proteins from the same message, which may have differential and even opposing functions [95, 96]. For example, our lab has studied the role of the nuclear co-activator and oncogene, amplified in breast cancer 1 (AIB1), and we have identified an alternatively processed transcript of the mRNA which generates a shorter form of the protein named AIB1-Δ4 [97]. We have demonstrated an upregulation of this variant in breast cancer and have associated the alternative processing with loss of a regulatory domain, potentiating the oncogenic function of AIB1 [97]. Our lab has also shown that AIB1 is upregulated in the transition from normal breast to DCIS and maintained in the transition to invasive carcinoma [98]. It is possible that given the enhanced oncogenic activity of Δ4 and correlation of this variant with metastatic capability that alternative splicing of this oncogene could play a role in progression from DCIS to invasive disease. Future investigation into alternative splicing in the acquisition of invasive capacity could yield fruitful results in understanding, predicting and potentially preventing progression.

Overall the epigenetic changes interrogated as a potential drivers of tumor progression have returned similar results to genetic and proteomic investigations—differences between invasive and in situ disease are minimal suggesting that epigenetic alterations seen in advanced disease are present from an early stage in the natural progression of breast cancer [81, 84, 99].

2.4. Tumor microenvironment components driving invasive progression

Breast cancer cells are integrated within a complex microenvironment that has been increasingly recognized to influence tumor initiation and invasiveness [100, 101]. The tumor microenvironment (TME) is composed of multiple stromal cells (e.g., myoepithelial cells, fibroblasts, immune cells and adipocytes), insoluble extracellular matrix (ECM), newly formed vasculature, as well as growth factors and cytokines [101].

2.4.1. Myoepithelial cells

The disruption of the myoepithelial cell layer that separates in situ lesions from the surrounding breast stroma is considered to be the initial step required for DCIS to progress to IDC [102–106]. Normal myoepithelial cells secrete proteinase inhibitors along with factors like thrombospondin, laminin and the oxytocin receptor that ensure the maintenance of BM integrity and suppress epithelial cell proliferation and invasion [104, 105]. By contrast, cancer-associated myoepithelial cells (CAMs) aid in BM destruction through proteinase production [5, 107]. Though they appear genomically normal, CAMs are significantly different from those associated with normal ductal structures in terms of gene expression and tumor-suppressive function [73, 102, 106, 108] and engage in paracrine signaling with adjacent cancer cells [109, 110]. One such signaling axis is the upregulation of the chemokine CXCL14 which has been demonstrated to positively influence proliferation, migration and invasion of cancer epithelial cells [73]. Loss of tumor-suppressive signaling and the loss of this physical cell barrier along with associated ECM signaling unleash progressive potential [5, 102, 106, 111, 112].

2.4.2. Immune cells

In response to impairment of the BM, tumor cells express chemokines (e.g., colony-stimulating factor 1 receptor) that attract macrophages within the TME [113, 114]. Tumor-associated macrophages (TAMs), mainly of M2 phenotype, can constitute up to 50% of the breast tumor mass [115], and increased TAMs density has been shown to relate poor prognosis in most human tumors [114, 116]. M2-type TAMs are critical modulators that potentiate the invasion of tumor cells through various mechanisms: secretion of chemotactic factors (e.g., EGF) [117], pro-angiogenic molecules (e.g., vascular endothelial growth factor) [118] and anti-inflammatory cytokines (such as interleukin-10) [119, 120], as well as remodeling of the ECM [121]. Tumor infiltrating lymphocytes (TILs) have also been identified as a prognostic factor in breast cancer, generally associated with improved survival, decreased distant recurrence and increased metastatic latency predicting a better response to therapeutic interventions and overall survival [122–126]. Though there have been many studies on the importance of immune presence and regulation in advanced breast cancers, the immune infiltrate in DCIS specifically is less well characterized and has only recently started to be evaluated. A novel 2016 study by Thompson et al. investigated the immune microenvironment of 27 DCIS cases of known intrinsic subtype [127, 128]. CD3+ T cells were the predominate lymphocyte subtype across all DCIS cases with CD4+ T-helper cells making up a slightly larger proportion compared to CD8+ effector T cells. Also present, though at a lower frequency were CD20+ B cells and FoxP3+ T regulatory cells. Interestingly, it was noted that the DCIS cases included that

had concurrent invasive disease tended to have more CD20+ B cell and CD8+ T cell infiltrate. Additionally, the DCIS cases known to recur later had greater CD8+ T cells than other subsets of DCIS cases and also displayed an increased relative presence of regulatory T cells than those that did not [127]. These findings suggest that an active adaptive immune response is mounted early in the natural history of breast cancer and that suppression of the host immune system constitutes another crucial step in the malignant progression through the inhibition of immune effector cells (e.g., myeloid-derived suppressor cells) and the stimulation of immunosuppressive cells (e.g., regulatory T cells) [129].

2.4.3. Fibroblasts

Cancer-associated fibroblasts (CAFs) are predominant components of the TME that enhance tumor growth and invasiveness by conferring a mesenchymal-like phenotype in premalignant mammary epithelial cells [130]. CAFs create a pro-tumorigenic environment through high deposition, cross-linking and remodeling of the ECM [131], and by regulating the immune polarization [52]. In breast cancer carcinoma, transforming growth factor beta (TGF-β), stromal cell-derived factor-1, platelet-derived growth factor α/β and interleukin 6 are the major tumor-derived factors that have been described to induce CAFs activation [132–134]. Reciprocally, CAFs secrete tumor-promoting factors, such as hepatocyte growth factor, that stimulate the invasive behavior of DCIS cells [135]. Co-implantation of CAFs with DCIS cells has been shown to increase the invasive capacity of the in situ lesions [136, 137]. For instance, the presence of CAFs resulted in activation of cyclooxygenase-2 (COX-2) in the epithelial component, driving cancer progression [136]. It should be noted that COX-2 expression was demonstrated to be one of three markers, along with P16 and Ki-67 that were found to be associated with significantly increased risk of invasive recurrence within 8 years of initial diagnosis and treatment of DCIS [138].

2.4.4. Extracellular matrix

Factors that mediate ECM remodeling and degradation have been of interest in studying the transition of DCIS to invasive disease as destruction of the BM is a hallmark of progression. Several studies have shown matrix metalloproteinases (MMPs) such as MMP1, 2 11, 12 and 13 as well as other proteases and protease inhibitors such as cathepsins, PLAU, SERPINS and metallopeptidase inhibitors to be regulated, up and down respectively, in both DCIS and invasive cancer-associated stromal cells [73, 74, 139]. These expression changes are further linked to poor prognosis and likely related to the acquisition of invasive capacity [73, 74, 139]. Lyons et al. suggested that mammary gland involution, which is a natural driving force of ECM remodeling following pregnancy [140–142], may recapitulate alterations that occur in the initiation of tumor progression [143]. They demonstrated in a mouse model of involution that xenografted MCF10DCIS.com cells grown in this environment formed larger more invasive lesions marked by increased fibrillar collagen deposition and COX-2 expression and that anti-inflammatory treatments with NSAIDs were able to at least partially prevent this progression [143].

2.4.5. Neovasculature

In order to sustain their expanding neoplastic growth and eventually disseminate to distant sites, tumors are capable of stimulating the formation of new blood vessels, a process referred to as angiogenesis [144]. Strikingly, the tumor-associated neovasculature is observed early during carcinogenesis in both murine and human premalignant, noninvasive lesions [145, 146]. The transition from dormant nonvascularized hyperplasia to vascularized proliferative tumor requires the cooperation of various TME cell types (e.g., endothelial and pericytes) and is regulated by counteracting molecules, of which the main pro- and anti-angiogenic factors are vascular endothelial growth factor-A and thrombospondin-1, respectively [147].

2.4.6. Adipocytes

Lastly, at earlier stages, the level of invasiveness of breast tumor ductal epithelial cells is increased as a result of the secretion and processing of ECM molecules by the mammary adipocytes, especially type VI collagen [148, 149].

3. Experimental models of early-stage breast cancer

Our understanding of the natural history of early-stage breast cancer remains challenging due to the tumor heterogeneity and requires the implementation of experimental models that are capable of mimicking all aspects of the disease. Cell lines and mouse models are valuable tools routinely used to investigate the mechanisms underlying the initiation and progression of breast cancer and will be reviewed in this section.

3.1. In vitro models

3.1.1. Normal breast epithelial cell lines

Most in vitro studies aiming to model early-stage breast cancer are based on the utilization of immortalized mammary epithelial cells

3.1.1.1. MCF10A cell line

MCF10A cell line is the most commonly used breast epithelial cell line to model normal breast epithelium. This immortal cell line was generated from the fibrocystic breast tissue of a 36-year-old patient and emerged spontaneously as a result of continuous trypsin-versene passages [150]. These cells are considered as "normal" breast epithelial cells based on various characteristics commonly found in the normal glandular epithelium, including lack of tumorigenicity, anchorage-dependent growth, as well as hormonal and growth factor-dependent proliferation in vitro. MCF10A cells are ER negative and express wild-type P53 [151] along with markers of basal-like cells, such as P63 [152, 153]. Although MCF10A cells are non-transformed and exhibit near diploidy, cytogenetic analyses revealed that these cells are karyotypically abnormal following immortalization. Their genetic abnormalities include amplification

of the oncogene MYC and the deletion of the chromosomal locus containing genes regulating the cellular senescence, especially P14ARF and P16 [150]. These latter molecular characteristics render MCF10A cell line particularly adapted for oncogenic transformations. Cui et colleagues recently reported that MCF10A cells do not fully recapitulate in vitro the architectural features of normal human breast tissue most likely due to epigenetic derivations driven by the immortalization process and a continuous culture [154].

3.1.1.2. Primary mammary epithelial cell lines

In vitro systems using primary human mammary epithelial cells (HMEC) are believed to be a more reliable model of normal breast epithelial cells. Usually easily isolated from reduction mammoplasty tissues, the life span and propagation of HMEC in vitro remain challenging as they stop doubling and undergo cellular senescence after several passages [155]. In addition, these cells tend to lose their lineage commitment as well as their capacity to grow and normally differentiate when cultured ex vivo. To overcome the senescence block, primary human epithelial cells have been immortalized using exogenous expression of viral oncogenes [156] and the telomerase reverse transcriptase [157]. None of these strategies is capable of maintaining these cells in culture without permanently altering their normal phenotype and genetic background. Schlegel and colleagues in collaboration with our laboratory recently established a novel method that can be used to indefinitely propagate a wide range of normal primary epithelial cells, including breast cells [158, 159]. This technique is based on the coculture of primary epithelial cells in presence of irradiated fibroblasts and requires the utilization of a specialized medium containing a Rho-kinase inhibitor. The resultant cells, also referred to as conditionally reprogrammed cells (CRCs), although highly proliferative, remain karyotypically normal, non-tumorigenic [158] and exhibit hallmarks of adult stem cells [159, 160]. Because human breast CRCs can be genetically modified in culture and implanted into mouse models as discussed below, the CRC system appears as an in vitro method of choice to study the phenotypic and molecular alterations underlying the benign to malignant transition in breast cancer.

3.1.2. Early-stage breast cancer cell lines

To explore the mechanisms promoting the invasive progression of DCIS, the scientific community has at its disposal few cellular models, although no single cell line is capable of fully recapitulating the different subtypes of DCIS tumors.

3.1.2.1. MCF10DCIS.com cell line

The majority of research studies focused on early-stage breast cancer utilized the premalignant MCF10A series established by Miller and Colleagues [161, 162]. One of these variants, termed MCF10DCIS.com, was isolated upon successive passages in culture of lesions obtained from xenografted MCF10AT cells [162]. At the molecular level, MCF10DCIS.com is a ER-negative basal-like cell line that expresses high levels of signaling proteins well-known to play a crucial role in malignant progression, including CD44v, HER2, COX-2, Smad4, Stat3, Pak4 and the phosphorylated forms of ERK and AKT [163]. Similarly, a gain of function mutation

conferring an increased oncogenic potential to the phosphatidylinositol 3-kinase has also been found in MCF10DCIS.com cells [164]. Of note, although MCF10DCIS.com cells are considered as a model of early-stage disease, these cells secrete a significant amount of the metastatic galectin-3-binding protein [165], which suggests that they also contain precursors with metastatic capacities. The essential advantage of using MCF10DCIS.com cells relies on their ability to give rise to fast-growing and comedo-like DCIS tumors when injected into xenograft mouse model [162, 166]. The particular features of the tumors derived from MCF10DCIS.com xenograft will be described in the next section.

3.1.2.2. SUM cell lines

Eleven breast cancer cell lines, referred to as SUM, have been generated by Forozan et al. from different subtypes of primary breast tumors [167]. Two of them, called SUM-102 and SUM-225 cells, were immortalized from human DCIS tumors containing microinvasive lesions or from recurrent lesions formed in the chest wall of a patient with DCIS history that did not receive chemotherapy treatment, respectively. SUM-102 cells express CK19 and are considered as basal B-type breast cancer cells [167, 168]. These cells also overexpress Cyclin D1 while they possess mutations in PIK3CA, P16 [169] and checkpoint kinase 2 genes [170]. On the other hand, SUM-225 cells are ER and PR negative, whereas they are amplified for HER2 and are thus classified as luminal epithelial cells [167, 171]. Of note, a P53 missense mutation frequently observed in breast cancer within the sequence encoding the DNA-binding region has also been found in SUM-225 cells [171]. Like MCF10DCIS.com cells, SUM-225 cells generate tumors resembling human DCIS lesions when injected into immuno-compromised mice [166].

3.1.2.3. 21T cell lines

Band and colleagues developed another series, named 21T, including four cell lines established from the tumor tissues of a 36-year-old woman that was first diagnosed with stage 3 intraductal carcinoma, then developed lung metastases 1 year later [172]. 21PT and 21NT cells were both isolated and immortalized from the primary breast tumor and were found to resemble ADH and DCIS, respectively. Phenotypically, 21PT cells are normal spindle epithelial cells, whereas 21NT cells are polygonal-shaped tumor cells of different sizes. At the molecular level, these two cell lines are aneuploid, HER2-amplified and are believed to not express ER and PR, reflecting the original patient biopsy. The most striking difference between 21PT and 21NT cells relies on their ability to form tumors when grown into immunodeficient mice, and this tumorigenic property was shown to be restricted to 21NT cells [172, 173].

3.1.2.4. Other immortalized cell lines

To date, two additional early-stage breast cancer cell lines have been reported: h.DCIS.01 cells established from columnar cell hyperplastic lesions [77] and FSK-H7 cells isolated from human DCIS tumors positive for HER2 [166]. Similar to the previous cell lines, h.DCIS.01 and FSK-H7 cells are capable of producing xenograft tumors in vivo as reviewed previously [77, 166]. In addition to these established cell lines, various technologies have been developed to

generate primary breast cancer cell lines representing the full spectrum of human breast cancer subtypes, such as the mammary-optimized EpiCult-B technology and the CRC system (described above) [158, 159, 174]. The CRC system is particularly advantageous as it allows for isolation and rapid expansion of tumor cells from a core needle biopsy of human or murine breast cancer [158, 174]. Notably, primary murine CRCs are able to form tumors recapitulating the original carcinoma when implanted orthotopically into syngeneic mice [159].

As previously discussed in this chapter, signals from the breast microenvironment play a key role in the differentiation and maintenance of normal breast epithelial cells [8], as well as during breast cancer initiation and progression [101]. For these reasons, homotypic culture of breast cancer cell lines does not provide the optimal system for studying the multicellular complexity of breast carcinogenesis. This latter limitation emphasizes the importance of developing in vitro culture systems that allow investigations of the cross talk between breast cancer epithelial cells and the surrounding stroma.

3.2. In vitro models for tumor-stromal interactions

Breast cancer cells cultured ex vivo in three-dimensional and heterotypic systems represent advanced and effective tools for elucidating the morphological and molecular changes governing the epithelial-stromal interactions during breast cancer invasive progression. Besides recapitulating the breast cellular complexity, organotypic 3D cultures are also practicable systems for experimental research and manipulations of cell lines.

3.2.1. Three-dimensional culture systems

Morphogenesis and homeostasis of the normal breast epithelium depend on the balanced relationship between ductal epithelial cells and the ECM [175]. Conversely, the altered communication between epithelial cells and ECM results in the loss of polarity together with the invasion of epithelial cells through the ECM and ultimately contributes to cancer initiation and progression. Breast epithelial cells grown in three-dimensional (3D) systems can recapitulate the architectural and functional features of the glandular epithelium in vivo in response to molecular signals provided by the ECM substratum [176, 177]. The development of biologically relevant 3D models relies on matching specific matrices and culture media with specific cell types. Particularly, mammary epithelial cells are capable of forming differentiated and functional organoids that resemble normal mammary acini when grown within substrata rich in collagen I [178] or laminin [179], and under culture conditions allowing their survival and proliferation [180, 181]. The vast majority of the 3D cultures of breast epithelial cells imply the utilization of an ECM secreted by the Englebreth-Holm-Swarm mouse tumor cells and commercially available as Matrigel™ [179]. Matrigel™ is composed of laminin, collagen IV, entactin and proteoglycans and is supposed to mimic the ductal BM. Monotypic and Matrigel™-based 3D culture assays recapitulating the breast epithelial signaling have been originally developed by Brugge, Bissell and colleagues in order to unravel the morphogenetic processes supporting the normal mammary gland development and its tumorigenesis [180–182]. Notably, Petersen et al. reported that normal breast cells seeded singly within Matrigel™ are capable of forming spherical, polarized and growth-arrested acini-like structures with a central hollow lumen

and deposition of BM rich in laminin V and collagen IV [176, 180]. By contrast, breast tumor cells failed to adopt acini-like phenotypes and instead evolved into poorly differentiated, non-polarized and highly disordered aggregates when grown in Matrigel™ [176].

3D culture systems offer the opportunity to investigate a large spectrum of phenotypic effects mediated by oncogenes and tumor suppressors on the architecture of breast epithelia by using two main strategies. The first strategy intends to reconstruct the tumorigenic phenotypes as a result of targeted genetic modifications in normal epithelial cells. For example, Muthuswamy et al. demonstrated that MCF10A cells genetically engineered to overexpress the oncogene HER2 can give rise to hyperproliferative multi-acinar structures showing filled lumina and loss of the apicobasal polarization when cultured in homotypic 3D assays [183]. These disorganized structures are characterized by the absence of invasive properties and thus mirror the histopathological hallmarks of precancerous epithelial cells, especially human DCIS. The second strategy aims to genetically manipulate breast cancer cells to possibly restore the organized and polarized phenotype observed in the normal breast duct. Recently, our laboratory followed this strategy to delineate the functional role of the oncogene AIB1 during DCIS progression [98]. We demonstrated that small hairpin RNA (shRNA)-mediated knockdown of AIB1 in MCF10DCIS.com cells generates more normal acini-like spheroids with deposition of laminin V to the periphery when these knockdown cells are grown in Matrigel™, overall suggesting that AIB1 plays a key role in the maintenance of the DCIS-like structure in 3D culture.

Besides alterations of the normal breast acinar architecture, the invasion of malignant epithelial cells into the surrounding stroma relies on their migratory properties and implies the protease-mediated disruption of the BM barrier [73]. 3D culture techniques can be applied to assess the migratory behavior of breast cancer cells through specific 3D matrices in response to particular cytokines [184]. As an illustration, Zaman and colleagues revealed that the co-overexpression of the oncogenes HER2 and 14-3-3ζ in MCF10A cells significantly induces their motility within 3D type I collagen matrices in a stiffness-dependent manner [185].

Homotypic organoid models involving a single cell type grown within reconstituted BM matrices remain the most simplistic approach used to appreciate the epithelial-stromal interactions. Significant technological progresses had been made in the past decade to provide the cancer cell scientists with a variety of evolved 3D coculture systems reflecting more faithfully the breast cellular complexity in vivo.

3.2.2. Heterotypic 3D culture systems

Multiple organotypic coculture systems have been elaborated to selectively investigate the cross talk between preneoplastic breast epithelial cells and particular stromal cells, such as myoepithelial cells, fibroblasts and macrophages.

3.2.2.1. Myoepithelial cells

As previously discussed, myoepithelial cells play an essential role in preventing breast cancer dissemination by expressing genes specifically responsible of maintaining a polarized

bilayered acinar organization [186]. To decipher the molecular mechanisms underlying the tumor-suppressive role of myoepithelial cells, Petersen and colleagues recently developed 3D cocultures of human primary luminal and myoepithelial cells isolated from either normal breast reduction mammoplasty or breast tumor tissues [5]. They confirmed that luminal epithelial cells embedded in type I collagen matrices required the presence of normal myoepithelial cells to form polarized acini-like structures. By contrast, 3D collagen cocultures of luminal epithelial cells with tumor-derived myoepithelial cells fail to generate properly polarized organoids as a consequence of decreased synthesis of functional laminin I by tumor myoepithelial cells.

3.2.2.2. Fibroblasts

In addition to being the most abundant cancer-associated stromal cells present in the TME, CAFs have been largely shown to modulate the invasive transition of breast cancer [130]. To better comprehend the influence of the cross talk fibroblasts-epithelial cells during early-stage breast cancer, Sadlonova et al. grew premalignant breast MCF10AT cells on top of Matrigel™ in the presence of primary fibroblasts [187]. Only fibroblasts purified from normal breast reduction mammoplasty were able to notably suppress MCF10AT cell growth in 3D culture, whereas breast cancer-derived fibroblasts reduced the proliferation of these transformed cells to a lesser extent. Those results further indicate that upon their malignant conversion, breast cancer epithelial cells become insensitive to the tumor growth inhibitors synthesized by stromal fibroblasts, which underlines the importance of establishing new treatment paradigms for early-stage breast cancer based on recovering the tumor-suppressive function of fibroblasts.

3.2.2.3. Macrophages

Similar heterotypic 3D coculture strategies have been applied to elucidate the molecular mechanisms by which macrophages modulate the behavior of tumor cells during breast carcinogenesis [188, 189]. As an example, Balkwill and colleagues plated MCF-7 cells or human mammary epithelial cells genetically immortalized with hTERT, on top of Matrigel™ [188]. Then, they tested the invasive phenotype of these two breast cancer cells along with the level of expression of 22 inflammatory-related genes in the presence of macrophages, previously isolated from human bone marrow and seeded into a modified Boyden chamber to avoid direct cell-to-cell contact. This method permitted them to prove that the cancer cell ability to invade through Matrigel™ is induced when cocultured with macrophages. This invasive phenotype was further correlated to increased activation of JNK and NF-Kappa B pathways. Linde et al. investigated the macrophage-mediated invasive properties of tumor cells using an approach that integrates macrophages into cocultures of tumor cells with fibroblasts [189]. They plated tumor cells on top of collagen I-rich dermal equivalents containing macrophages derived from bone marrow alone or together with primary dermal fibroblasts. Using this tri-culture model, they concluded that activation of macrophages toward M2 phenotype promotes cancer cell invasion through the proteolytic degradation of the BM likely due to increased levels of MMP-2 and MMP-9 [189]. Although this model was developed using tumor cells derived from squamous cell carcinoma, it was successfully generated in both a

murine and human background, and its applicability can presumably be extended to study the interactions between early-stage breast tumor cells and TAMs.

The establishment of a wide collection of heterotypic 3D models and their interchangeable utilization have allowed the extensive study of the molecular roles of each cellular component within the breast TME during carcinogenesis. In addition, these models are easily reproducible and particularly handy for the development of targeted therapies. However, even if organotypic 3D cultures are preferred to in vitro models for the identification of new stromal targets for breast cancer progression, only in vivo models have yet been able to thoroughly recapitulate the complexity of the tumor-stromal interactions that govern breast tumor initiation and invasiveness.

3.3. Mouse models

3.3.1. Xenograft mouse models

Xenografts of human breast cancer cell lines or tissues implanted into immuno-compromised recipient mice represent powerful tools for understanding the multiple aspects of the human disease within an in vivo context. Athymic nude, severe combined immune deficient (SCID), NOD/SCID IL2Rgamma^{null} (NGS) and "humanized" NSG mice are the most commonly utilized immunodeficient animals [190]. Two types of xenograft mouse models of breast cancer are usually established: through subcutaneous injection [162], or after orthotopic transplantation of cancer cells or tissues into the mammary fat pad or the duct [166, 173, 191]. Breast cancer cell lines can be genetically manipulated prior to being grafted into mice, which allows to study the tumorigenic properties of specific factors on the tumor take, growth and dissemination in vivo [98]. On the other hand, the actual human cancer tissue can be used to rapidly generate xenograft tumors recapitulating the cellular and molecular heterogeneity inherent to a particular cancer [191]. As a result, xenograft mouse models are frequently employed to predict the tumor response to clinically relevant drugs as well as their possible adverse effects and thus have served as preclinical models for multiple clinical trials [190]. In order to identify the possible factors driving the invasive transition of DCIS, various xenograft models of human DCIS have also been developed based on the utilization of the DCIS cell lines reviewed above, especially MCF10ADCIS.com [162, 166, 192], 21NTci [173] and SUM-225 cells [166].

3.3.1.1. MCF10ADCIS.com xenograft models

As previously discussed, MCF10ADCIS.com cells are capable of engendering tumors containing comedo-like DCIS lesions that resemble human high-grade DCIS and that spontaneously evolve to IDC in a time-dependent manner when implanted into immunodeficient mice [162, 192]. Polyak and colleagues extensively studied this animal model and revealed that MCF10DCIS.com cells are unique bipotent progenitors capable of differentiating into both luminal and myoepithelial cells following subcutaneous injection into female nude mice [192]. MCF10ADCIS.com xenograft tumors displayed DCIS-like lesions filled with luminal epithelial cells expressing ESA, CD44, CK17, cadherin 1 and vimentin, surrounded by an outer

layer of myoepithelial cells positive for P63, CD10 and α-smooth muscle actin, and by a BM rich in laminin V. In addition, they demonstrated that luminal and myoepithelial cells, purified from MCF10ADCIS.com tumors, differentially expressed factors that belong to signaling pathways known to modulate their functional phenotype, especially TGF-β, hedgehog, cell adhesion and P63 signal transduction pathways. Interestingly, these gene expression patterns were similar to those observed in primary DCIS tissues. As mentioned above, the tumorigenic properties of factors possibly involved in breast cancer progression can be investigated using xenograft animal models upon implantation of genetically engineered breast cancer cells. Our laboratory recently used this approach to elucidate the pro-oncogenic function of AIB1 in early-stage breast cancer using the MCF10ADCIS.com xenograft model [98]. Because high levels of AIB1 were found in MCF10ADCIS.com cell line, we transduced these cells with shRNAs directed against AIB1 prior to injection into nude mice. Decreased expression of AIB1 in MCF10ADCIS.com cells resulted in reduced tumor growth and development in vivo. Additionally, MCF10ADCIS.com xenograft tumors deficient in AIB1 exhibited smaller DCIS lesions containing fewer tumor-initiating cells (TIC) and myoepithelial progenitor cells, and those cellular alterations were correlated with downregulation of NOTCH, HER2 and HER3 signaling pathways. Overall, our data indicated for the first time that AIB1 is required for the initiation and preservation of DCIS lesions in part through maintenance of the TICs subpopulation, thus suggesting that AIB1 may serve as a novel therapeutic target for early-stage breast cancer.

3.3.1.2. 21T xenograft model

As detailed previously, 21PT and NT cell lines are primary tumor-derived cells believed to mimic early-stage breast cancer when implanted into the mammary fat pad of female nude mice at 8–9 weeks of age [173]. Souter et al. reported that 21PT cells were not tumorigenic or metastatic in vivo and instead formed xenograft structures that shared features of ADH. Furthermore, atypical/neoplastic and normal/benign epithelial cells were shown to coexist within 21PT xenograft tissues. Conversely, 21NT cells were able to give rise to malignant lesions in about 20% of the mice, and the resultant tumors displayed intermediate to high-grade DCIS lesions, with no evidence of invasive progression. Interestingly, 21NT-derived lesions exhibited phenotypic traits identical to the ones obtained in MCF10ADCIS.com xenograft mouse model, although in contrast to 21NT tumors, MCF10ADCIS.com tumors ultimately progress to IDC as a result of RAS-induced transformation.

3.3.1.3. Mouse intraductal models

Most of the xenograft models are obtained upon subcutaneous injection of cancer cells and thus fail to replicate the early steps of breast carcinogenesis that occur inside the mammary duct. In order to better recreate the natural progression of DCIS within conditions mimicking the stromal microenvironment, Medina and colleagues developed a novel method based on the intraductal transplantation of human breast cancer cells into immunodeficient female mice [166]. To generate this unique mouse intraductal model, they injected the previously described DCIS cell lines, MCF10ADCIS.com, SUM-225 and FSK-H7 cells, through the

nipple directly into the mammary ducts of 6- to 10-week-old SCID-beige mice. Following intraductal transplantation, all three cells lines were able to form DCIS-like lesions that slowly evolved into IDC in 90% of the mice. Unlike subcutaneous injection, the intraductal transplantation of MCF10ADCIS.com cells into immuno-compromised mice gave rise to cribriform DCIS, whereas SUM-225 and FSK-H7 cells displayed comedo and apocrine-like DCIS lesions, respectively. Using immunostaining analyses, Behdoh et al. further reported that SUM-225 and FSK-H7 xenograft samples are characterized by the overexpression of HER2 along with moderate expression of CK8 and 19. MCF10ADCIS.com-derived tumors were also positively stained for CK8 although they contained lesions classified as basal-like as they express CK5. More recently, Behbod et colleagues extended the intraductal mouse model to primary human DCIS [191]. They successfully xenotransplanted DCIS cell lines, derived from eight different patient samples, within the mammary duct of 8- to 10-week-old virgin NSG mice. At 8 weeks, the tissues collected from the xenografted tumors exhibited noninvasive lesions that closely resemble human DCIS and that retain the histopathological and molecular hallmarks of the patient's original biopsy. Strikingly, the engraftment of primary DCIS cells obtained from human tumors was only permitted using mice depleted in both mature T and B cells, suggesting that T and B cells both regulate the tumor growth of primary cells into in vivo models**.** Altogether, the mammary intraductal mouse model thus appears as the most suitable xenograft model of DCIS to deciphering the cellular and molecular processes that underline the epithelial-stromal cell cross talk during the initiation and invasive transition of DCIS.

As the fundamental role exerted by the tumor microenvironment during early-stage breast cancer is increasingly recognized, the subcutaneous implantation of cancer cells into mouse models deficient in T and B lymphocytes remains problematic and may result in decreased tumor take and abnormal cancer progression. To overcome this critical limitation, "humanized" mouse models have been generated based on the engraftment of human $CD34^+$ hematopoietic stem cells onto irradiated NOD/SCID mice [193] and the orthotopic implantation into cleared mammary fat pads humanized using stromal cells of human origin [194]. Humanized mice represent very promising models for better defining the tumorigenic properties of tumor-associated stromal cells during breast cancer development. This strategy can further be used to create a wide range of analogous models reflecting the various subtypes of DCIS.

3.3.2. Genetically engineered mouse models

Genetically engineered mouse models (GEMM) constitute invaluable resources for experimentally examining the in vivo function of specific oncogenes or tumor suppressor genes within immunocompetent animals. The primary benefit of employing GEMM as model of human cancer is that this approach allows modulation of the expression of particular genes in a tissue and time-specific fashion. Many GEMM models of breast cancer are currently available and are of two kinds: transgenic models in which specific oncogenes are overexpressed, and knockout models created upon targeted deletion of tumor suppressors [195]. In the following section, we will focus on GEMM that were found to develop tumors containing early-stage breast cancer lesions, especially DCIS.

3.3.2.1. Transgenic mouse models

In transgenic mouse models, the transcription of particular oncogene is induced throughout the mammary luminal epithelium under the control of strong mammary-specific epithelial promoters, of which the mouse mammary tumor virus (MMTV) and the whey acidic protein (WAP) are the most frequently utilized [195]. Transgenic mammary carcinoma models usually give rise to highly penetrant tumors after short latency. To date, few transgenic mouse models of human breast cancer have been shown to develop premalignant and DCIS-like lesions. They are discussed below.

3.3.2.1.1. MMTV-neu mouse model

Given the undeniable association of *neu* (e.g., HER2) amplification with DCIS [196], two mouse models expressing high levels of *neu* driven by the MMTV promoter have been created. Jolicoeur and colleagues established a model by genetically modifying mice to strongly express the activated form of *neu* [197]. As expected, multifocal mammary adenocarcinomas were observed in 5- to 10-month-old transgenic females. The *c-neu* overexpressing tumors displayed undifferentiated lesions resembling human comedo-type DCIS mixed with cytologically normal epithelium. Interestingly, the majority of the primary tumors had the potential to metastasize to the lung. Muller and colleagues developed a transgenic model bearing the unactivated form of *neu* fused to MMTV promoter [198]. The latter transgenic animals gave rise to focal mammary tumors highly metastatic and histologically identical to those induced by the activated *neu*, although after a longer latency period. Using this model, our laboratory demonstrated that the development of early-stage lesions and invasive mammary cancer depends upon the oncogene AIB1 [199].

3.3.2.1.2. MMTV-PyV-mT mouse model

MMTV-PyV-mT mouse model is another frequently used mouse model of premalignant mammary cancer in which the MMTV promoter has been manipulated to target the expression of the polyomavirus middle T antigen (PyV-mT) [198]. The resultant transgenic mice rapidly developed multifocal tumors in all mammary glands associated with secondary lung metastases. Maglione et al. characterized this transgenic model and reported that 5-week-old MMTV-PyV-mT mice carry tumors which contain high-grade, poorly differentiated, ADH-like lesions [200]. Furthermore, those lesions were ER positive and exhibited aberrant vasculature and myoepithelium.

3.3.2.1.3. WAP-T mouse model

The WAP promoter sequence has also been employed to induce mammary intraepithelial neoplasia in mice. Tzen et al. initially applied this strategy to drive the murine mammary epithelial cell transformation through WAP-mediated increased transcription of the SV40 large T antigen (SV40 TAg) [201]. SV40 TAg possesses the unique properties to physically interact with both P53 [202] and the retinoblastoma protein [203] and abrogate their tumor-suppressive function, leading to cellular hyperproliferation. Deppert and colleagues

recently expanded the histological analysis of the WAP-T transgenic mouse model and reported that these animals carried multifocal DCIS-like carcinomas progressing to IDC after a short latency period [204]. Notably, the resultant in situ carcinoma was composed of differentiated lesions showing tubular and papillary architecture comparable to those diagnosed in human DCIS.

3.3.2.1.4. C3(1)-SV40 TAg mouse model

Because MMTV and WAP were shown to be regulated by hormones [205, 206], Dr. Green's laboratory engineered a novel transgenic model by transcriptionally overexpressing the SV40 TAg in the mammary and prostate glands under the hormone-independent control of the C3(1) component of the prostate steroid-binding protein (PSBP) 5′ flanking sequence [207]. All C3(1)-SV40 TAg female mice bore mammary tumors that were found to share similar histologic and molecular hallmarks of human infiltrating ductal carcinoma in a predictable time-dependent manner. Eight-week-old transgenic mice, in fact, did exhibit ADH-like lesions that evolve into DCIS at about 12 weeks, invasive carcinoma at 16 weeks of age and ultimately metastasized into the lung with a 15% incidence. In addition, well-known chromosomal and molecular aberrations driving mammary carcinogenesis were also observed in C3(1)-SV40 TAg mice, thus suggesting that this transgenic model provides the unique opportunity to assess the antitumor potential of targeted therapies.

3.3.2.1.5. MMTV-tTA/tetop-SV40 TAg/tetop ERα mouse model

Increased levels of ERα in mammary epithelial cells are believed to be correlated to initiation and progression of premalignant breast cancer [208]. In order to corroborate this hypothesis, our collaborator Dr. Furth established a unique mouse model with dominant gain of ERα by breeding together three types of transgenic animals expressing: (i) the tetracycline-responsive transactivator tTA under control of MMTV promoter (MMTV-tTA); (ii) SV40 TAg under control of the tetracycline-responsive promoter (tetop); (iii) FLAG-tagged ERα under control of tetop [209, 210]. Strikingly, 4-month-old triple transgenic mice (MMTV-tTA/tetop-SV40 TAg/tetop-ERα) gave rise to ERα positive and estrogen-sensitive mammary tumors that contained preneoplastic lesions identical to those found in human ADH and DCIS. Using the double transgenic mice MMTV-tTA/tetop ERα, Frech et al. further reported that the rate of mammary epithelial cell proliferation was higher in ADH and DCIS lesions and was accompanied by increased expression of cyclin D1 in the nuclear compartment of these cells [210]. Altogether, this work reported for the first time that high ERα expression can promote the benign to cancerous transformation of mammary epithelial cells in vivo and provided unique mouse models to unravel the neoplastic events regulated by ERα signalings.

3.3.2.1.6. GEMM-based syngeneic transplantation models

Due to the time-consuming nature of using GEMM and their costs, various methods have recently emerged based on the establishment of stable mammary intraepithelial neoplasia outgrowth (MIN-O) lines derived from tumors carried by GEMM, such as MMTV-PyV-mT [211], P53 knockout [212] and MMTV-*Neu* mice [159]. The orthotopic serial transplantation

of these MIN-O cell lines into immunocompetent syngeneic mice has been proven to faithfully mimic the human disease and offered the opportunity to well-characterize the molecular mechanisms underlying breast cancer progression. As an illustration, Gregg and colleagues obtained MIN-O lines from MMTV-PyV-mT premalignant lesions and serial transplanted these lines into the cleared mammary fat pads of FVB females [211]. Those PyV-mT lines formed DCIS-like lesions that were able to progress to IDC in a predictable manner and that were undistinguishable from the parental tumors with respect to their histology and molecular profiles. MIN-O lines purified from mice depleted in the suppressor gene P53 [213] and implanted into the cleared fat pads of P53 wild-type BALB/c mice developed DCIS-like and invasive lesions [212]. Similarly to the PyV-mT-derived MIN-O cells, the resultant ductal-like outgrowths were capable of recapitulating the different stages of DCIS progression from ADH to IDC. Our laboratory recently extended this approach to MIN-O lines isolated from MMTV-*Neu* tumors using the CRC technology described previously [159]. The main advantage of this strategy is to allow the indefinite propagation of those lines ex vivo without the need for serial transplantation. Remarkably, MMTV-*Neu* MIN-O cells orthotopically transplanted into syngeneic females gave rise to lesions that retained the histopathological features of the original mammary tumors, underscoring the unique potential of using this approach to generate a wide range of GEMM-based preclinical models.

4. Conclusion

In summary, the molecular and cellular profiling of early-stage breast cancer using in vitro and in vivo models that reflect the heterogeneity of the disease have drastically allowed for improved understanding of the mechanisms underlying invasive progression of DCIS. As no individual model is capable of recapitulating the complexity of the human tumors, efforts should be made in the future to develop integrated strategies for better defining the drivers of early-stage progression of breast cancer and thus open new avenues for targeted therapy.

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Author details

William Kietzman, Anna T. Riegel and Virginie Ory*

*Address all correspondence to: vo24@georgetown.edu

Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA

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