Published in final edited form as:

J Mammary Gland Biol Neoplasia. 2018 December ; 23(4): 207–221. doi:10.1007/s10911-018-9409-z.

Breaking Through to the Other Side: Microenvironment Contributions to DCIS Initiation and Progression

Andrew C. Nelson^{1,2}, Heather L. Machado³, and Kathryn L. Schwertfeger^{1,2,*}

¹Department of Lab Medicine and Pathology, University of Minnesota, MN

²Masonic Cancer Center, University of Minnesota, MN

³Department of Biochemistry and Molecular Biology, Tulane Cancer Center, Tulane University School of Medicine, LA

Abstract

Refinements in early detection, surgical and radiation therapy, and hormone receptor-targeted treatments have improved the survival rates for breast cancer patients. However, the ability to reliably identify which non-invasive lesions and localized tumors have the ability to progress and/or metastasize remains a major unmet need in the field. The current diagnostic and therapeutic strategies focus on intrinsic alterations within carcinoma cells that are closely associated with proliferation. However, substantial accumulating evidence has indicated that permissive changes in the stromal tissues surrounding the carcinoma play an integral role in breast cancer tumor initiation and progression. Numerous studies have suggested that the stromal environment surrounding ductal carcinoma in situ (DCIS) lesions actively contributes to enhancing tumor cell invasion and immune escape. This review will describe the current state of knowledge regarding the mechanisms through which the microenvironment interacts with DCIS lesions focusing on recent studies that describe the contributions of myoepithelial cells, fibroblasts and immune cells to invasion and subsequent progression. These mechanisms will be considered in the context of developing biomarkers for identifying lesions that will progress to invasive carcinoma and/or developing approaches for therapeutic intervention.

Keywords

mammary gland; ductal carcinoma in situ; DCIS; tumor microenvironment; myoepithelial; fibroblast; immune

INTRODUCTION

Ductal carcinoma in situ (DCIS) is a neoplastic proliferation of clonally related epithelial cells that remains confined to the luminal space of the mammary ductal-lobular system. The morphology, genetics, cellular biology, and clinical behavior of DCIS are heterogeneous. The incidence of DCIS in the United States increased dramatically after the introduction and

* Corresponding author: Kathryn L. Schwertfeger, 2231 6th St SE, University of Minnesota, Minneapolis, MN 55455, 612-626-9419 (phone), schwe251@umn.edu.

dissemination of mammographic screening programs through the 1990s, but subsequently plateaued with approximately 60,000 new cases diagnosed per year [1]. Given this burden of disease, understanding the biologic complexity of DCIS and delineating biomarkers associated with progression to invasive cancer are clear needs to tailor treatment strategies for each individual patient.

Microscopically, the neoplastic cells of DCIS fill and distend the ductal-lobular structures without penetrating through the myoepithelial cell layer and surrounding basement membrane (Figure 1). The morphology of DCIS is classified using six distinct growth patterns (such as solid, cribriform, comedo, papillary, micropapillary and Paget disease) and three grades of atypical nuclear features (low, intermediate, and high) [2]. The morphology of the tumor microenvironment surrounding *in situ* lesions demonstrates wide variability in both the amount of collagen deposition (termed desmoplasia) and in the distribution of stromal cells including: fibroblasts, macrophages, lymphocytes, and other cell types (Figure 1). Panels 1A and 1B demonstrate variability in the accumulation of acellular collagen and number of fibroblasts within the stroma. Further, the number and distribution of mononuclear immune cells can be highly variable (compare Figure 1C vs. 1D). Later sections of this review describe how the presence and function of specific immune cell subsets have been associated with a higher risk for progression/ recurrence.

There are several key components to the clinical diagnostic assessment of DCIS. Based on features of the cell nuclei, DCIS is classified as low, intermediate, or high grade [2, 3]. The critical diagnostic consideration is exclusion of micro-invasive carcinoma (defined as invasive foci no larger than 1 mm; Figure 1E, 1F) admixed within the *in situ* tumor. Several immunohistochemical stains for myoepithelial cells can be used to assist in the clinical diagnosis of DCIS vs. invasive carcinoma, although diagnostic pitfalls exist with any one specific marker and therefore utilization of several stains is advised to most accurately assess for the presence of a myoepithelial cell layer [4]. Further, as described later in this review, decreased or variable expression of particular myoepithelial markers may in fact contribute to altered myoepithelial cell function leading to invasive progression. Diagnostic assessment of estrogen receptor (ER) and progesterone receptor (PR) expression is standard of care, but analysis for overexpression or amplification of HER2 is not routinely performed in the clinical diagnosis and management of DCIS [2].

Surgery is the primary treatment modality of DCIS, with or without adjuvant radiation therapy based on the clinical and pathologic features of each individual patient's disease [5]. Adjuvant endocrine therapy is considered if the DCIS is hormone receptor positive. Most prognostic algorithms consider a combination of: lesion size, surgical margin status, nuclear grade and presence of necrosis with or without additional personal and family medical history components [6]. In addition, a limited 12-gene expression profile has been validated as a prognostic tool to predict recurrence risk of DCIS [7], but this profile is heavily weighted on the measurement of proliferation-related genes. Currently, assessment of stromal features or inflammatory infiltrates is not used clinically for patient management or estimating the prognosis for recurrence.

The somatic genomic alterations of DCIS overlap significantly with invasive breast carcinoma. Similar to invasive cancer, recurrent mutations in *PIK3CA*, *TP53*, and *GATA3* as well as commonly occurring copy number alterations have similarly been identified in DCIS [8, 9]. A number of studies have utilized genomic analyses of paired DCIS and invasive carcinoma samples from the same patient to investigate whether recurrent mutations are restricted to or over-represented in the invasive disease. Subclonal genomic heterogeneity is reproducibly identified in both the DCIS and invasive components, and often the prevalence of individual subclones is different between the DCIS and invasive disease from the same patient [10]. Although individual studies have suggested certain genomic events are preferentially gained in invasive breast cancer (IBC) vs. DCIS components of paired samples [11, 12] no single or group of genetic hits has been recurrently shown to drive the progression to invasion in the majority of cases. These observations support a model of stochastic clonal evolution [13] occurring during the proliferation of *in situ* carcinoma prior to invasion. In review of these findings, Cowell and colleagues [10] described two hypothetical models of invasive progression: 1) a convergent phenotype model wherein multiple genomic and epigenomic events across diverse subclones of disease converge onto similar pathways that drive progression of the disease as a whole, and 2) an evolutionary bottleneck model wherein only one subclone develops the necessary combination of genomic alterations for progression.

To further address these hypotheses, Casasent et al. [14] recently used a sophisticated technique for spatial mapping of single cell copy number analysis (termed topographic single cell sequencing, TSCS) combined with high depth exome DNAseq analysis of paired DCIS and invasive carcinoma samples. The TSCS technique enabled investigators to develop single cell copy number variation (CNV) profiles of cells discretely mapped from 2-dimensional tissue sections of mixed *in situ* and invasive human breast carcinoma. They found a range of 1–5 abnormal CNV clones in each of the 10 analyzed patients, and in all cases all distinct clones could be identified in both DCIS and IBC components. The prevalence of individual subclones did vary between components, but the level of clonal diversity was not significantly different. Further, all CNV subclones within each patient shared a common (stem) abnormality, consistent with a single intraductal cell of origin and subsequent stochastic evolution within the ducts. In parallel, the authors performed high depth DNAseq analysis of micro-dissected DCIS and invasive cancer areas, finding that >87% of nonsynonymous mutations were concordant between the components for each patient. The conservation of clonal diversity (inferred from mutation profiles) between DCIS and IBC appeared nearly complete but the relative proportion of individual clones was variable, consistent with their single-cell CNV analysis. From these findings, the authors proposed a model of multi-clonal progression in which genetic heterogeneity develops within the duct system prior to invading into the surrounding tissue in a co-mingled fashion encompassing all of the genetic subclones. While the proportional enrichment of specific subclones within invasive cancer is compatible with the hypothesis that distinct genotypes are more supportive of invasive behavior, the investigators did not identify a specific genomic alteration that was enriched within the IBC of more than one patient. Although this study is limited by the number of individual patients studied (n=10) and a bias toward high grade cancers (8/10 cases), these results provide additional strong evidence that genomic

heterogeneity is largely maintained during progression from DCIS into IBC. Therefore epigenetic or other non-genomic biologic factors are likely important for invasive progression.

Transcriptomic studies comparing human DCIS and IBC have shown that regulation of cell-cell signaling, cellular movement, and cellular growth pathways are consistently altered between these two disease states [15]. Many differentially expressed genes are implicated in modulation of the extracellular matrix (ECM), immune regulation, or other biologic functions of the tumor microenvironment, including upregulation of *MFAP2*, *MMP11*, *MMP2*, *COL1A2*, *VIM*, and *PLAU* and downregulation of *CDH1* and *F2RL1* in invasive disease compared to isolated DCIS. Further, gene expression analysis of high grade DCIS (from basal and HER2-enriched molecular subtypes) show signatures consistent with a tumor-associated immunosuppressive environment [8].

Genomic analyses of human DCIS to date have not identified recurrent alterations that are consistently enriched during the transition to invasive disease. In parallel, transcriptomic comparisons of human DCIS and IBC implicate biologic modulation of the tumor microenvironment as a likely mechanism of phenotypic convergence for a genetically heterogeneous disease process. Herein, we review the expanding evidence describing functional changes in myoepithelial, fibroblast, and immune cell populations of the tumor microenvironment that contribute to either suppressing or promoting DCIS progression (summarized in Figure 2).

MYOEPIHELIAL CELLS

Myoepithelial cells in the mammary gland

In the normal mammary gland, the myoepithelial cell layer separates the luminal epithelial layer from the surrounding basement membrane. Myoepithelial cells express basal markers such as cytokeratin (CK)5 and CK14 as well as integrins such as $\beta 4$ -integrin and $\alpha 1$ -integrin, which contribute to binding the basement membrane [16, 17]. Numerous studies have demonstrated that myoepithelial cells contribute to differentiation, proliferation and polarity of the luminal epithelial cells [18, 19]. The use of three dimensional (3D) culture models has been particularly instrumental for demonstrating the importance of myoepithelial cells for maintaining polarity of luminal epithelial structures. For example, plating luminal epithelial cells in collagen I results in the formation of acini with reversed polarity. However, the addition of myoepithelial cells to the cultures was found to reverse this polarity, allowing luminal epithelial cells to generate acini with proper polarity along with lumen formation [18]. One possible explanation for this result is that myoepithelial cells provide a source of laminin-1, which is required for correct polarization of luminal epithelial cells [20]. In addition to contributing to luminal epithelial polarity, myoepithelial cells contribute to ductal elongation and branching in part through production of ECM molecules as well as proteases that help to remodel the basement membrane [21]. Furthermore, loss of P-cadherin, a cell adhesion molecule found in myoepithelial cells in the normal mammary gland, results in precocious alveolar development and formation of hyperplasias, suggesting that myoepithelial cells may also negatively regulate aspects of mammary gland development [22]. Myoepithelial cells are critical for lactation, during which they contract in response to

oxytocin, resulting in expulsion of milk into the ductal lumen for release. In the lactating mammary gland, alveolar myoepithelial cells form a contractile network of cells that surround the secretory epithelium. In addition to the intermediate filament proteins CK5 and CK14, myoepithelial cells express smooth muscle-specific proteins including α -smooth muscle actin (SMA), smooth muscle myosin heavy chain (SMMHC), calponin, tropomyosin and smooth muscle alpha-actin (ACTA2), which contribute to their contractile function during milk ejection [23–25]. Results from studies using ACTA2 knock-out mice represent an example of the requirement for contractile proteins in myoepithelial cell function during milk ejection. Analysis of these mice revealed that loss of ACTA2 is associated with a reduction in the ability of the myoepithelial cells to contract, resulting in reduced milk ejection [24]. Thus, these cells play critical roles in normal mammary gland development and function.

Because loss of myoepithelial cells is a key factor that distinguishes DCIS from IBC, there is extensive interest in understanding the mechanisms through which myoepithelial cells are lost and whether they modulate tumor cell invasion. There are several established markers that can be used to identify myoepithelial cells including SMA, SMMHC, h-caldesmon, S100, p63, maspin, CD10, and cytokeratin 14/17. The development of intraductal DCIS xenograft models has allowed for the ability to study the mechanisms of DCIS progression [26]. A widely used model utilizes MCF10DCIS.com cells, which when injected into the mammary ductal system lead to the formation of intraductal lesions that invade into the surrounding tissue [26]. Using this model, Russell et al. analyzed myoepithelial cells and found that prior to invasion, several markers were lost in myoepithelial cells, including p63, calponin and α SMA [27]. Loss of these markers was confirmed in human DCIS samples, suggesting that myoepithelial differentiation is disrupted prior to invasion and that loss of such markers may serve as a marker of DCIS progression. Further studies on human biopsy samples have shown that reduced expression levels of both SMMHC and CD10 in the myoepithelial cells of DCIS lesions of core needle biopsies is associated with higher risk for concurrent stromal invasion on the subsequent surgical excision [28]. Studies comparing these cells with derivatives or other cell lines with less invasive phenotypes in the intraductal DCIS xenograft model could potentially identify paracrine factors released from the tumor cells that reduce expression of myoepithelial differentiation markers. Following validation in human cohorts, identification of these factors would lead to additional potential markers of DCIS invasion.

Evidence for myoepithelial cells as suppressors of invasion

Myoepithelial cells have been shown to exhibit anti-angiogenic, anti-proliferative and anti-invasive properties, suggesting that they may provide a natural barrier to invasive breast cancer growth. A recurring theme of myoepithelial function in DCIS progression has focused on their ability to modulate protease activity within the microenvironment. Both *in vitro* and *in vivo* studies have demonstrated that myoepithelial cells are capable of suppressing tumor cell migration and invasion. For example, early studies found that myoepithelial cells isolated from the breast were capable of suppressing invasion of MDA-MB-231. Interestingly, these cells were found to produce high levels of protease inhibitors that were sequestered within a myoepithelial-produced matrix, suggesting a potential

mechanism through which myoepithelial cells may effectively inhibit proteases produced by invading breast cancer cells [29]. Specific protease inhibitors have been studied in detail. Tissue inhibitor of metalloproteinase (TIMP)-1, which is a potent inhibitor of matrix metalloproteinase (MMP) activity, was also found to be produced by myoepithelial cells [30]. Because breast cancer cells produce high levels of MMPs, this provides another potential mechanism through which myoepithelial cells inhibit protease activity. Plasminogen activator inhibitor 1 (PAI-1), which is a serine protease inhibitor that inhibits urokinase plasminogen activator (uPA), has also been implicated in promoting DCIS invasion. Using an MCF10DCIS-based 3D co-culture model, Sameni et al. demonstrated that myoepithelial cells produce PAI-1 and that blocking uPA activity reduced the sizes of DCIS structures and collagen degradation products [31]. More recent studies have further implicated inhibitors of cysteine cathepsins, specifically stefin A, in the repression of invasion by myoepithelial cells [32]. Specifically, results from these studies demonstrated that high levels of cathepsin inhibitors, including stefin A and stefin B, in the N1ME immortalized myoepithelial cell line were capable of inhibiting invasion of MDA-MB-231 cells. Furthermore, siRNA-mediated knock-down of stefin A led to the inability of myoepithelial cells to restrain tumor cell invasion. Finally, analysis of human DCIS samples revealed high levels of stefin A in hyperplastic and low-grade DCIS, with loss of expression observed in intermediate and high-grade DCIS, suggesting that loss of stefin A may predict invasion. Taken together, these studies support the conclusion that myoepithelial cells are capable of producing protease inhibitors that may inhibit DCIS invasion. Further identification of the specific mechanisms that modulate the levels of expression of protease inhibitors from myoepithelial cells may lead to novel therapeutic approaches for inhibiting tumor cell invasion and for identifying high risk DCIS.

In addition to producing protease inhibitors, myoepithelial cells are also capable of reducing protease production by tumor cells. Co-culture of primary breast myoepithelial cells with a panel of different breast cancer cell lines led to reduced invasion as well as a decrease in expression of MMP-2, MMP-9 and MT1-MMP in MBA-MD-231 and MBA-MD-468 cells [33]. Interestingly, co-culture of myoepithelial cells with fibroblasts also led to reduced MMP gene expression, suggesting a general impact of myoepithelial cells on protease production in a paracrine manner. In addition, myoepithelial cell expression of specific MMPs may also maintain extracellular matrix homeostasis and suppress invasion. For example, MMP-8 expression in myoepithelial cells was found to increase adhesion to ECM molecules and reduce myoepithelial cell migration. Furthermore, MMP-8 expression also suppressed TGF β signaling and gelatinolytic enzyme activity, which corresponded with a reduced ability of the myoepithelial cells to promote tumor cell invasion. Confirmatory of these experimental studies, MMP-8 expression was found to be significantly reduced in samples of DCIS with invasion compared with pure DCIS [34]. The mechanisms by which myoepithelial cells inhibit MMP production in DCIS cells remain to be determined; however these findings, along with the studies demonstrating that myoepithelial cells produce protease inhibitors, suggest that there are likely to be multiple potential mechanisms through which myoepithelial cells are capable of reducing protease activity within the tumor microenvironment.

Myoepithelial cells have also been shown to produce anti-angiogenic factors that are capable of inhibiting endothelial cell chemotaxis and proliferation [35, 36]. For example, myoepithelial cell lines have been shown to produce factors such as maspin, a non-inhibitory serpin [37] that is highly expressed in normal myoepithelial cells but is reduced in the context of DCIS [38, 29]. Maspin is a potent inhibitor of angiogenesis [39] along with tumor cell migration and invasion [40, 41]. These cells also produce thrombospondin-1 (TSP-1), which inhibit angiogenesis by directly impacting endothelial cell proliferation, migration and survival, and through antagonizing vascular endothelial growth factor (VEGF) [42]. Furthermore, myoepithelial cells have been found to produce soluble basic fibroblast growth factor (bFGF) receptors that have the ability to inhibit activity of FGFs, which are potent angiogenic factors [36]. The ability of myoepithelial cells to inhibit angiogenesis by these and other mechanisms highlights the complexities through which myoepithelial cells may contribute to suppression of DCIS progression.

Finally, a number of other myoepithelial-associated molecules have been linked to tumor suppression through promoting tumor suppressive activities, including Caveolin-1, activin, relaxin, MEPI and Neogenin, which are summarized in [30]. Taken together, these studies indicate that myoepithelial cells suppress invasive progression through paracrine effects on tumor cells and through maintenance of homeostasis in the microenvironment. Further characterization of the paracrine factors from myoepithelial cells that limit growth, motility and protease expression in tumor cells, particularly in human samples, would be needed to develop therapeutic strategies that limit invasion. Furthermore, additional experiments with using *in vivo* model systems are required to better define the relative importance of the anti-angiogenic and anti-proteolytic activities of myoepithelial cells in preventing progression.

Evidence for myoepithelial cells as promoters of invasion

In contrast to the studies described above that suggest a suppressive role for myoepithelial cells, a number of studies have identified potential mechanisms through which myoepithelial cells may be capable of promoting invasion. Molecular profiling studies using human DCIS samples have been performed to identify potential changes in paracrine interactions between myoepithelial cells and tumor cells [43]. Using magnetic beads, myoepithelial cells and tumor cells were isolated from normal, DCIS and invasive breast tissues and assessed for gene expression profiles using SAGE analysis. The authors demonstrated an increase in chemokines including CXCL12 and CXCL14 in the myoepithelial and myofibroblast compartments. CXCR4, which is the receptor for CXCL12, was found on breast epithelial cells with increasing expression levels in invasive cancers compared with DCIS. CXCL12 has been shown in many studies to promote tumor cell migration and invasion through its interactions with CXCR4 [44]. Interestingly, the receptor for CXCL14 is not known, but CXCL14 was capable of promoting migration and invasion of MDA-MB-231 cells, suggesting that tumor cells express a functional CXCL14 receptor and implicating this chemokine in promoting tumor cell invasion.

Recent studies have identified additional mechanisms through which myoepithelial cells promote tumor cell invasion in the context of DCIS. For example, while normal myoepithelial cells do not normally express high levels of $\alpha v \beta 6$ integrin, increased

expression was found on myoepithelial cells associated with DCIS in human tumor samples [45]. Further analysis of expression levels demonstrated that 96% of DCIS associated with invasion were found to express $\alpha v\beta 6$ in their myoepithelial cells. $\alpha v\beta 6$ activation is associated with induction of MMPs and consistent with this, overexpression of $\alpha v\beta 6$ in myoepithelial cells led to a TGF β -dependent upregulation of MMP-9. Experimental studies demonstrated that coinjection of MDA-MB-231 cells with myoepithelial cells that are expressing $\alpha v\beta 6$ led to enhanced tumor growth. Together, these studies suggest that $\alpha v\beta 6$ may be able to identify patients at high risk for progression.

To identify additional mechanisms through which myoepithelial cells interact with DCIS cells *in vitro*, Lo et al. described the use of a co-culture model containing Hs578bst myoepithelial cells and MCF10DCIS cells [46]. These studies demonstrated that the MCF10DCIS cells induced expression of TGF β by the myoepithelial cells, which is a well-established inducer of EMT and invasion. Consistent with these findings, changes in EMT-associated genes were found in the MCF10DCIS cells, including increased FOXC2, SNAIL, Vimentin, ZEB1 and ZEB2, along with a decrease in E-cadherin, which was reversed upon knock-down of TGF β 1 in the myoepithelial cells. Finally, *in vivo* studies demonstrated that co-injection of myoepithelial cells with the MCF10DCIS cells into the mammary fat pad led to increased tumor volume and increased TGF β signaling. A study of 209 patients showed that increased SNAIL1 expression in isolated DCIS on biopsy was associated with a higher risk for upstaging to invasive carcinoma at surgical excision [47], suggesting this pathway may be important in human disease. Further biomarker studies are needed in patient cohorts to establish the clinical relevance of TGF β secretion from myoepithelial cells as a driver of invasion.

These contrasting results of invasion-suppressing and invasion-promoting functions of myoepithelial cells suggest that there are complex and dynamic interactions between MECs and luminal epithelial cells with neoplastic potential. There appear to be two potential mechanisms: 1) loss of “normal” myoepithelial cells that maintain tissue homeostasis and 2) a shift of MECs toward abnormal gain-of-functions that help support neoplastic growth and invasion. Further studies are needed to address the balance of these two potential mechanisms in the human condition in order to define the most viable clinical approaches to predict and prevent invasive tumor behavior.

FIBROBLASTS IN DCIS

Fibroblasts are located within the stroma and are typically found in close proximity to mammary epithelial structures. Early studies of fibroblast-epithelial interactions demonstrated that fibroblasts can enhance 17 β -estradiol-induced proliferation of epithelial cells *in vitro* [48]. Stromal fibroblasts also produce factors that act in a paracrine manner to enhance mammary epithelial stem and progenitor function [49, 50]. During mammary gland development, fibroblasts are located in proximity to the epithelium and are important for producing ECM molecules including collagen and fibronectin, which provide structural and mechanical support to the epithelial tissue and promote epithelial growth and differentiation [51, 52]. Fibroblasts also produce proteases, such as MMPs, which degrade the ECM, resulting in stromal reorganization and release of growth factors [51, 53, 54]. Together, these

fibroblast-derived factors contribute to branching morphogenesis in the developing gland. For example, MMP-3 is expressed in the stromal compartment and deletion of MMP-3 in mice results in reduced lateral branching in the mammary gland [55]. Because many of these factors, including hormone responsiveness, stem cell function, ECM remodeling and MMP production, are also involved in tumor progression, fibroblasts have been extensively studied for their potential contributions in the context of cancer.

In breast cancer, carcinoma associated fibroblasts (CAFs) have been linked to breast cancer progression via a variety of mechanisms, including the production of proteases, growth factors and angiogenic factors that are capable of contributing to breast cancer cell invasion (reviewed in [56–60]). In support of this, a number of studies have demonstrated that fibroblasts are capable of contributing to promoting DCIS invasion. Several *in vitro* models have demonstrated the capacity of fibroblasts to promote invasion of non-invasive cells. For example, using a 3D co-culture model in which MCFDCIS cells were co-cultured with fibroblasts in recombinant basement membrane, the presence of fibroblasts led to increased invasive branching in the matrix, which was accompanied by increased levels of MMP-14 and MMP-9 in the co-cultures [61]. Using a microfluidics approach, Sung et al. demonstrated that co-culture of MCF10DCIS cells with human mammary fibroblasts led to increased invasion of the MCF10DCIS cells, which was associated with changes in collagen organization surrounding the structures [62]. The microfluidics nature of this model will allow for high throughput identification of critical factors involved in fibroblast-mediated DCIS progression in future studies. Using a different 3D model approach, called the mammary architecture and microenvironment engineering (MAME) model, Osuala et al. co-cultured MCF10DCIS cells with human breast CAFs and found enhanced structure size, increased proliferation and the formation of branch-like connections between the structures [63]. Mechanistic studies demonstrated that CAF-derived IL-6 contributed to tumor cell invasion, which also required the cysteine protease cathepsin B. Taking advantage of the ability to utilize the MAME model for live-cell imaging, the authors found CAFs migrating with tumor cells at the leading edge, suggesting direct interactions between these cell types. More recent studies using the MAME model that incorporates myoepithelial cells along with fibroblasts demonstrated that myoepithelial cells effectively reduce the tumor promoting activity of the CAFs. This process involves inhibition of CAF-mediated proteolysis of the extracellular matrix in part through inhibiting the production of CAF-derived IL-6 [31]. Another recently identified CAF-derived factor that promotes invasion of *in situ* mammary lesions is the chemokine CXCL1 [64]. Using the MMTV-PyMT model on the C57BL/6 background, Bernard et al. [64] demonstrated that CXCL1 produced by CAFs isolated from tumors in MMTV-PyMT mice promoted invasion of epithelial cells using a co-transplant model. This was dependent upon fibroblast-derived CXCL1, which promoted invasion through CXCR2 and activation of downstream signaling pathways, including extracellular regulated kinase (ERK), nuclear factor kappa B (NFκB), Akt and signal transducer and activator of transcription 3 (STAT3) [64].

In vivo studies using xenograft models have also demonstrated the capacity for fibroblasts to promote invasion of DCIS. For example, using the MCF10DCIS model, Hu et al. demonstrated that subcutaneous co-injection of tumor cells and fibroblasts led to increased formation of invasive tumors compared with either tumor cells alone or tumor cells co-

injected with myoepithelial cells [61]. Increased invasion was found using fibroblasts isolated from normal breast tissue, invasive carcinomas or from patients with rheumatoid arthritis, suggesting a general ability of fibroblasts to promote DCIS invasion. Mechanistic studies demonstrated that fibroblast-mediated upregulation of cyclooxygenase-2 (Cox-2) in the tumor cells was found to be important in promoting invasion and that celecoxib treatment inhibited the progression to invasion. Additional studies used both orthotopic mammary fat pad and renal capsule transplant models demonstrated that CAFs significantly enhanced tumor size of DCIS xenografts and that these tumors were associated with increased collagen deposition [31]. Supportive of these experimental studies, analysis of human DCIS samples with microinvasion has demonstrated that myofibroblasts associated with DCIS lesions express uPA, uPAR and MMP-13 [65], which are all linked with promotion of invasion.

Recent studies have also implicated fibroblasts in modulating hormone responsiveness of tumor cells. For example, using a novel microfluidics-based organotypic model, Morgan et al. demonstrated that mammary fibroblasts enhanced ER activity and promoted proliferation of ER+ MCF-7 breast cancer cells [66]. Another study identified a cell surface marker, CD146, which allowed for the isolation of two distinct fibroblast populations human breast tumors [67]. CD146^{pos} fibroblasts and CD146^{neg} fibroblasts were found to have different effects on estrogen responsiveness of breast cancer cells. Interestingly, while exposure of tumor cells to CD146^{pos} fibroblasts led to maintained estrogen responsiveness and sensitivity to tamoxifen, CD146^{neg} fibroblasts suppressed ER expression and enhanced resistance of tumor cells to tamoxifen. While these studies were performed using fibroblasts from invasive breast cancer patients, it would be important to determine whether these subpopulations also exist in the context of DCIS, and whether they impact ER expression and/or responsiveness within these lesions.

As noted above, a key function for fibroblasts involves ECM deposition and reorganization. One of the consequences of altered ECM composition is the change in biophysical properties of the ECM. In particular, changes in the collagen matrix associated with orientation of collagen fibers have been linked with invasive breast cancer. Specifically, using multiphoton imaging and second harmonic generation, Provenzano et al. demonstrated distinct patterns of collagen signatures at different stages of tumor progression [68]. They found increased collagen density increased surrounding early stage lesions. As these lesions progressed, collagen fibers were found to stretch tangentially around the tumor. Finally, in invasive tumors, radially aligned collagen fibers were found and in some areas were found to be perpendicular to the tumor. Furthermore, localized cell invasion was associated with radially aligned collagen fibers, suggesting that this represents a permissive collagen alignment for tumor cell invasion.

Recent studies have also assessed collagen alignment in the context of DCIS [69]. These studies used second harmonic generation imaging to assess collagen alignment in patient samples of DCIS, which included a cohort of patients with recurrence. Perpendicular alignment of collagen fibers was found to be associated with DCIS lesions, compared to normal epithelial structures that were associated with parallel collagen fibers. While collagen alignment was not associated with recurrence, DCIS lesions associated with

comedo necrosis and were ER-, PR- and HER2+, which are features associated with DCIS with poor prognosis, were associated with more perpendicular fiber alignment. While the functional implications of collagen alignment with DCIS lesions remains unknown, the development of novel 3D models with tunable biophysical properties, many of which are described in this review, have the potential to provide insights into the links between ECM alterations and DCIS invasion. Furthermore, expanded access to and analysis of DCIS samples associated with long-term patient outcome will be important for determining whether ECM alterations can be used as biomarkers of invasion.

IMMUNE CELLS

Immune cells in mouse models of early stage progression

Studies using mouse models have demonstrated the importance of immune cell populations in both mammary gland development and in early stage tumorigenesis. Myeloid populations, including macrophages and eosinophils, are found in close proximity to developing epithelial structures in mammary glands from virgin mice [70]. Assessment of mammary gland development in mice lacking macrophages demonstrated the importance of this cell type in ductal elongation and alveolar development [70]. Macrophages were found to contribute to collagen fibrillogenesis surrounding the terminal end bud, providing a link between macrophages and ECM reorganization in the developing mammary gland [71]. More recent studies have demonstrated the presence of macrophages associated with epithelial structures during the estrous cycle, and have shown altered phenotypes depending upon the stage of the cycle [72]. Finally, macrophages are present within the involuting mammary gland and macrophage depletion prolongs the involution process, demonstrating their importance for normal mammary gland function [73]. In addition to macrophages, lymphocyte populations, such as T cells, have also been linked to negative regulation of ductal development in the mammary gland as mice lacking CD4+ and CD8+ T cells exhibited increased ductal outgrowth [74]. Together, these findings demonstrate the importance of both myeloid and lymphocyte populations in mammary gland development. Further characterizing the mechanisms by which immune cells regulate normal growth and homeostasis may identify immune pathways and biomarkers to assess in DCIS vs. invasive breast cancer.

As described in Behbod et al. (this issue), there are a number of mouse models of breast cancer that show evidence of hyperplasia and some that appear to progress through a process that mimics human DCIS progression. Some studies have demonstrated recruitment and function of immune cells at the early stages of tumorigenesis in these models. For example, the progression of MIN to carcinoma in the MMTV-PyMT model represents the transition to invasive cancer [75]. Analysis of immune cell localization by immunofluorescence revealed a progressive increase in both CD4+ T cells and macrophages as detected by F4/80, a commonly used macrophage markers, from early lesions to latestage carcinoma [76]. However, depletion of either CD4+ or CD8+ T cells in this model did not impact tumor latency or burden. In a separate study using the MMTV-PyMT model, macrophage depletion delayed histological progression of tumors and reduced metastasis, although did not impact primary tumor growth rates [77]. In another study, genetic deletion of the chemokine

receptor CCR6 in the PyMT-MMTV model led to delayed early stage tumorigenesis, which was correlated with reduced recruitment of pro-tumorigenic macrophages into the microenvironment [78]. Using a recently developed model of inducible macrophage depletion, in which CD68+ cells, which represent primarily macrophages although may impact some stromal populations as well [79], are deleted in a doxycyclinedependent manner, macrophage depletion reduced proliferation in tumors at the MIN/adenoma stage [80]. Taken together, these studies suggest that developing lesions in the MMTV-PyMT model effectively recruit both innate and adaptive immune cells. Furthermore, macrophages may have a functional contribution in early stage tumorigenesis, although the functional impact of adaptive immune cells in early stage progression in this model has been difficult to assess, possibly due to the aggressive nature of this model.

Using a less aggressive and inducible model of FGFR1-induced tumorigenesis, we have previously shown that early stage hyperplastic lesions and locally invasive lesions are associated with macrophage infiltration [81]. Furthermore, macrophage depletion in this model resulted in reduced epithelial proliferation and angiogenesis, demonstrating functional contributions of macrophages to early stage tumor formation. In further studies, we have identified several macrophage-derived factors that are capable of contributing to epithelial and tumor cell migration and invasion in 2D and 3D cultures models, including CXCR2-binding chemokines [82], suggesting potential mechanisms through which macrophages may contribute to local invasion of *in situ* tumors.

A recent study was published using a pre-malignant model of p53-/- outgrowth, which shows disruption of the basement membrane during the transition to invasive cancer, mimicking the *in situ* to invasive transition in human breast cancer [83]. Macrophage recruitment was assessed in PN1a-derived lesions, which progress to invasive cancer, and PN1b-derived lesions, which remain as low-grade hyperplastic lesions. Increased numbers of macrophages were recruited to the PN1a lesions (Figure 3A,B), and these macrophages exhibited a pro-tumorigenic phenotype along with the capacity to induce a malignant phenotype of PN1a cells in 3D culture. Furthermore, depletion of macrophages in this model using clodronate liposome-mediated depletion led to reduced progression to invasive tumors.

Finally, a recent study has also implicated macrophages in early stage lesions using the MMTV-HER2 model [84]. Macrophages were found to infiltrate early stage lesions in a CCL2-dependent manner and were found in association with areas of myoepithelial disruption and loss of E-cadherin junctions. Inhibition of macrophages using an antibody to colony-stimulating factor 1 receptor (CSF1R), which is a receptor present on macrophages, led to a reduction in hyperplastic ducts and restoration of E-cadherin expression. These results led to further assessment of macrophages in a small cohort of human DCIS samples, which confirmed the presence of intra-epithelial macrophages and loss of E-cadherin junctions. Taken together, these studies collectively suggest that macrophages contribute to early onset of tumorigenesis and localized invasion during the transition from DCIS to invasive cancer.

Many additional models are known to be associated with immune cell infiltration in late stage tumors, such as the MMTV-ErbB2, MMTV-Wnt and C3(1)Tag models [85–87].

Further studies are required to determine the presence of immune cells within these lesions and the functional contributions of these cells to early stage tumorigenesis in these models. For example, early lesions in the C3(1)Tag model are associated with F4/80+ macrophage infiltration (Figure 3C), although their functional contributions to tumorigenesis in this model have not been established. Assessing both immune infiltration and the functional contributions of these immune populations to early stage tumorigenesis would provide important information regarding the impact of innate immune cell populations in the context of DCIS.

Immune cell populations in human DCIS samples

The success of immune-based therapies in other solid tumors has led to significant interest in understanding immune responses in breast cancer. Recent studies have focused on profiling immune cell populations within tumors to better understand the immune environment within the distinct breast cancer subtypes. Analysis of DCIS by flow cytometry has demonstrated an increase in the general leukocyte population, including both innate and adaptive immune cells, in both DCIS and IBC compared with normal breast [88]. In invasive breast cancer, increased levels of tumor infiltrating lymphocytes (TILs) have been linked to therapeutic responsiveness and better outcome in HER2+ and triple negative breast cancers [89]. As a result, current studies are focusing on developing immunotherapies for patients with TIL infiltration and for developing approaches for enhancing anti-tumor immune responses in breast cancer associated with minimal immune infiltrates. This raises the possibility that similar immunotherapeutic approaches may be relevant in DCIS as well.

Several studies have been published that have examined TILs in human DCIS samples. Using a cohort of 138 cases of pure DCIS, Hendry et al. quantified TILs in H&E stained sections [90]. Higher levels of TILs were found in higher-grade, ER-negative and HER2-positive DCIS. Within this cohort, 27 cases were associated with subsequent recurrence. While the TIL score was not found to be associated with recurrence, there was a trend for recurrent lesions to have less TILs than the primary tumors. The loss of TILs in recurrent lesions may reflect suppression of anti-tumor adaptive immune responses during recurrence. Because the TIL population represents a variety of lymphocyte subsets, recent studies have focused on examining specific T cell subsets associated with DCIS lesions. There are a number of T cell subsets that vary in function and are found within the tumor microenvironment. CD8+ T cells are cytotoxic and are typically associated with anti-tumor immunity, although their activity can be suppressed in the tumor microenvironment. There are a number of subsets of CD4+ T cells that have different functions within the tumor. T helper 1 (Th1) cells represent a subset of the CD4+ T cell population that typically express high levels of interferon (IFN) γ , which is associated with anti-tumor functions [91]. The Th2 subset produces cytokines such as IL-4 and promotes an environment associated with a pro-tumorigenic environment. For example, Th2-derived IL-4 has been shown to promote polarization of macrophages towards a pro-tumorigenic state, resulting in enhanced tumor progression [76]. Regulatory T cells (Tregs) are defined by expression of the transcription factor Foxp3 and are typically involved in suppressing the function of cytotoxic T cells [92]. A recent study of 27 cases of DCIS, 24 of which were pure DCIS, higher numbers of TILs were found in ER-DCIS, and a higher CD8/Treg ratio was found in ER+ DCIS [93]. This

ratio demonstrates that more cytotoxic T cells are present, suggesting a more effective anti-tumor immune response within the microenvironment of ER+ DCIS, although this remains to be confirmed functionally. While analysis of recurrence was limited in this study, the two cases associated with recurrence were found to have high levels of Foxp3+ Tregs. Because Tregs represent a highly immunosuppressive subset of T cells, this suggests that recurrence may be associated with suppression of anti-tumor immune responses. Another analysis of a cohort of age-matched high-grade (52 cases) and non-high-grade (65 cases) DCIS found increased Foxp3+ T cells in high-grade lesions, suggesting that high-grade lesions are associated with an immunosuppressive environment [94]. Further analysis of this cohort was performed to assess the presence of cytotoxic CD8+ T cells that express human leukocyte antigen-antigen D related (HLADR). HLADR is a receptor for major histocompatibility (MHC) class II and serves as a marker for activated T cells. Thus, the presence of high levels of CD8+HLADR+ T cells likely represents an anti-tumorigenic immune environment. Within this cohort, low numbers of CD8+HLADR+ cells were associated with increased risk of recurrence, suggesting that a low level of anti-tumor immune responses corresponds with higher risk of recurrence. Conversely, lesions with high CD8+HLADR+ and low CD8+HLADR-cells were associated with reduced risk of recurrence, suggesting that the presence of an anti-tumor immune response within the tumor microenvironment is likely to be beneficial for the patient. While these studies demonstrate the importance of defining specific subsets of T cells for understanding the link to recurrence, the specific mechanisms that drive changes in T cell populations during DCIS recurrence are not known. Analysis of gene expression within the lesions prior to and following recurrence would provide guidance in terms of identifying immunosuppressive mechanisms that might contribute to recurrence.

Recent studies assessed immune infiltrates using FACS, multi-color immunofluorescence and gene expression profiling in a cohort that included primarily HER2+ and triple negative DCIS [88]. These studies found higher numbers of T cells associated with HER2+ DCIS compared with HER2-DCIS as well as higher numbers of T cells associated with high-grade compared with low-grade DCIS. These findings suggest that HER2+ DCIS and high-grade DCIS may be associated with a more immunogenic environment, thus these patients may exhibit better responsiveness to immune-based therapy. Furthermore, more T cells were found to be associated with DCIS that is adjacent to invasive cancer than pure DCIS. In terms of localization, T cells were found to be localized within the stroma and at sites of focal myoepithelial disruptions. While the functional implications of T cell localization in these areas remains unclear, the further identification of the specific T cell subtypes present within these regions would provide insights into the potential functional implications of T cells in DCIS.

In addition to infiltrating lymphocytes, studies have suggested the presence of myeloid cells associated with DCIS. For example, increased neutrophils have been found to be associated with DCIS compared with normal breast [88]. Given that neutrophils have been ascribed both pro-tumorigenic and antitumorigenic functions in breast cancer [95, 96], the functional implications of neutrophil accumulation in DCIS lesions are not known. Furthermore, high-grade DCIS was found to have higher percentages of macrophages as identified by the macrophage marker CD68, compared with non-high-grade DCIS [94]. Within this cohort, higher levels of CSF1R, which is a receptor present on macrophages, were associated with

increased risk of recurrence. Numerous mouse models of early stage progression suggest functional roles for myeloid cells, including macrophages, in DCIS progression. Furthermore, macrophages can be found surrounding DCIS lesions (Figure 3D). Therefore, the presence and localization of macrophages within human DCIS suggest that macrophages may functionally contribute to DCIS invasion and/or recurrence. However, tumor associated macrophage function is complex and anti-tumor functions for macrophages within the tumor microenvironment have also been implicated. For example, immunohistochemical analysis of macrophage localization in human breast cancer samples revealed that the presence of macrophages in gaps of ductal tumor structures correlated with reduced lymph node metastasis, suggesting that depending on their localization within the tumor, some macrophages may exhibit protective functions [97]. Therefore, further studies on the specific localization and activation states of macrophages within the tumor microenvironment are needed in patient cohorts with isolated DCIS and long-term follow-up for clinical recurrence to provide more definitive data on the functional roles of macrophages in progression.

Immune-based therapeutic targeting in DCIS

The recent success of checkpoint blockade therapy in some cancers, such as melanoma and lung cancer, has led to interest in determining the efficacy of checkpoint blockade in breast cancer. While this therapy is being actively pursued in invasive breast cancer, potential efficacy in DCIS is not known. To determine whether checkpoint blockade would be a feasible approach in the context of DCIS, some studies have examined expression of immune checkpoint molecules in DCIS. Inhibitory immune checkpoint molecules are cell surface proteins that inhibit T cell activity upon interaction with ligand. For example, programmed death 1 (PD-1) is a receptor expressed on T cells that suppresses T cell activation following binding to programmed death ligand 1 (PD-L1), which can be expressed on numerous cell types, including tumor cells and immune cells within the tumor microenvironment. PD-1 and PD-L1 are clinically relevant therapeutic targets and are being examined extensively in the context of invasive breast cancer. Another checkpoint molecule of interest is T immunoreceptor with Ig and ITIM domains (TIGIT), which is an inhibitory receptor expressed on T cells that binds CD155 and CD112 on dendritic cells, macrophages and tumor cells [98]. In a study examining TIGIT+ T cells and PD-1+ T cells in DCIS, initial analysis of T cell activation markers demonstrated that T cells in DCIS are typically more activated than in invasive cancer [88]. Furthermore, there were more TIGIT+CD3+ T cells associated with DCIS than invasive cancer although there were no differences in the presence of PD-1+CD3+ T cells, suggesting that there may be differences in mechanisms of T cell suppression in DCIS and invasive cancer. Interestingly, some differences in PD-L1 expression were observed. For example, myoepithelial cells in a luminal DCIS area demonstrated high PD-L1 expression although the tumor cells were negative, whereas in invasive regions PD-L1 was highly expressed on tumor cells. In another study of 27 cases of DCIS, PD-L1 expression was not detected on tumor cells, but was found on infiltrating lymphocytes in 81% of samples [93]. Finally, the study by Hendry et al. also found low levels of PD-L1 expression in DCIS [90]. These studies collectively support the observation that PD-L1 is not overexpressed on tumor cells in DCIS, but the possibility that PD-L1 is expressed in the microenvironment warrants further examination.

In addition to checkpoint blockade, vaccination-based strategies have also been examined as a potential immunotherapy approach for DCIS patients. Based on the observation that progression of HER2+ breast cancers is associated with a loss in anti-HER2 Th1 responses, Lowenfeld et al. developed a dendritic cell-based vaccination strategy [99]. Monocytic dendritic cell precursors were isolated from patients with HER2-positive DCIS and pulsed with HER2 peptides with the goal to inject these cells into patients and elicit a T cell response to the peptides presented by the dendritic cells. Six weekly injections of pulsed dendritic cells were administered to patients in either the breast or groin lymph nodes. Minimal side effects were observed in the 54 patients treated, suggesting that this vaccine is well-tolerated. Furthermore, the majority of patients (81.1%) exhibited enhanced immune responses as shown by an increase in either CD4 or CD8 T cells following vaccination, and the increase in immune response was independent of route of administration. Pathologic complete response was achieved in 13 patients, and this correlated with an increase in immune response within the sentinel lymph node. While further studies are required to determine the efficacy of vaccination-based approaches in HER2+ and other subtypes, this study suggests that this may represent a viable strategy for some DCIS patients.

CONCLUSION

DCIS is one of the few non-invasive neoplasms that is treated with multi-modality therapy, including surgery, radiation, and pharmacologic therapies. These current clinical approaches for DCIS have resulted in an extremely low breast cancer-specific mortality rate of 3.3% overall at 20 years [100]. However, there is growing consensus that DCIS is over-treated and that many women diagnosed with DCIS might not ever experience disease progression if left untreated [101]. There are significant morbidities associated with the multi-modal treatment approaches currently used in DCIS management; therefore, there is a critical clinical need to distinguish DCIS lesions with a high potential for invasive progression/recurrence from those which are essentially benign. Furthermore, the development of specific therapies that directly target mechanisms of progression could potentially have more tolerable side-effect profiles than current approaches. There is considerable complexity and heterogeneity in the biology of invasive progression, and these studies have further outlined how multiple components of the tumor microenvironment may be pushing that evolution. Future work on the role of the tumor microenvironment in DCIS progression has the potential to help individualize our treatment approaches and improve patient's quality of life.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Molly Klein for providing images. This work was supported by Minnesota Masonic Charities Eastern Star Scholarship (A.C.N.), Susan G. Komen CCR16377665 (H.L.M.), NIH R01 CA212518 (H.L.M.) and NIH R01 CA215052 (K.L.S.).

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin.* 2015;65(1):5–29. doi: 10.3322/caac.21254. [PubMed: 25559415]
2. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL et al. Protocol for the examination of specimens from patients with ductal carcinoma in situ of the breast. *Arch Pathol Lab Med.* 2009;133(1):15–25. doi:10.1043/1543-2165-133.1.15. [PubMed: 19123730]

3. Silverstein MJ, Poller DN, Waisman JR, Colburn WJ, Barth A, Gierson ED et al. Prognostic classification of breast ductal carcinoma-in-situ. *Lancet*. 1995;345(8958):1154–7. [PubMed: 7723550]
4. Rakha EA, Miligy IM, Gorringer KL, Toss MS, Green AR, Fox SB et al. Invasion in breast lesions: the role of the epithelial-stroma barrier. *Histopathology*. 2017. doi:10.1111/his.13446.
5. Network NCC. Breast Cancer (Version 1.2018). In: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf 2018 <http://mdanderson.libanswers.com/faq/26180>. Accessed May 5, 2018.
6. Gorringer KL, Fox SB. Ductal Carcinoma In Situ Biology, Biomarkers, and Diagnosis. *Front Oncol*. 2017;7:248. doi:10.3389/fonc.2017.00248. [PubMed: 29109942]
7. Solin LJ, Gray R, Baehner FL, Butler SM, Hughes LL, Yoshizawa C et al. A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast. *J Natl Cancer Inst*. 2013;105(10):701–10. doi:10.1093/jnci/djt067. [PubMed: 23641039]
8. Abba MC, Gong T, Lu Y, Lee J, Zhong Y, Lacunza E et al. A Molecular Portrait of High-Grade Ductal Carcinoma In Situ. *Cancer Res*. 2015;75(18):3980–90. doi: 10.1158/0008-5472.CAN-15-0506. [PubMed: 26249178]
9. Pang JB, Savas P, Fellowes AP, Mir Arnau G, Kader T, Vedururu R et al. Breast ductal carcinoma in situ carry mutational driver events representative of invasive breast cancer. *Mod Pathol*. 2017;30(7): 95263. doi:10.1038/modpathol.2017.21.
10. Cowell CF, Weigelt B, Sakr RA, Ng CK, Hicks J, King TA et al. Progression from ductal carcinoma in situ to invasive breast cancer: revisited. *Mol Oncol*. 2013;7(5):859–69. doi:10.1016/j.molonc.2013.07.005. [PubMed: 23890733]
11. Corzo C, Corominas JM, Tusquets I, Salido M, Bellet M, Fabregat X et al. The MYC oncogene in breast cancer progression: from benign epithelium to invasive carcinoma. *Cancer Genet Cytogenet*. 2006;165(2):151–6. doi:10.1016/j.cancergencyto.2005.08.013. [PubMed: 16527609]
12. Heselmeyer-Haddad K, Berroa Garcia LY, Bradley A, Ortiz-Melendez C, Lee WJ, Christensen R et al. Single-cell genetic analysis of ductal carcinoma in situ and invasive breast cancer reveals enormous tumor heterogeneity yet conserved genomic imbalances and gain of MYC during progression. *Am J Pathol*. 2012;181(5):1807–22. doi:10.1016/j.ajpath.2012.07.012. [PubMed: 23062488]
13. Nowell PC. The clonal evolution of tumor cell populations. *Science*. 1976;194(4260):23–8. [PubMed: 959840]
14. Casasent AK, Schalck A, Gao R, Sei E, Long A, Pangburn W et al. Multiclonal Invasion in Breast Tumors Identified by Topographic Single Cell Sequencing. *Cell*. 2018;172(1–2):205–17 e12. doi: 10.1016/j.cell.2017.12.007. [PubMed: 29307488]
15. Carraro DM, Elias EV, Andrade VP. Ductal carcinoma in situ of the breast: morphological and molecular features implicated in progression. *Biosci Rep*. 2014;34(1). doi:10.1042/BSR20130077.
16. Koukoulis GK, Howedy AA, Korhonen M, Virtanen I, Gould VE. Distribution of tenascin, cellular fibronectins and integrins in the normal, hyperplastic and neoplastic breast. *J Submicrosc Cytol Pathol*. 1993;25(2):285–95. [PubMed: 7686813]
17. Lazard D, Sastre X, Frid MG, Glukhova MA, Thierry JP, Koteliansky VE. Expression of smooth muscle-specific proteins in myoepithelium and stromal myofibroblasts of normal and malignant human breast tissue. *Proc Natl Acad Sci U S A*. 1993;90(3):999–1003. [PubMed: 8430113]
18. Gudjonsson T, Adriance MC, Sternlicht MD, Petersen OW, Bissell MJ. Myoepithelial cells: their origin and function in breast morphogenesis and neoplasia. *J Mammary Gland Biol Neoplasia*. 2005;10(3):261–72. doi:10.1007/s10911-005-9586-4. [PubMed: 16807805]
19. Polyak K, Kalluri R. The role of the microenvironment in mammary gland development and cancer. *Cold Spring Harb Perspect Biol*. 2010;2(11):a003244. doi:10.1101/cshperspect.a003244. [PubMed: 20591988]
20. Gudjonsson T, Ronnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumorderived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. *J Cell Sci*. 2002;115(Pt 1):39–50. [PubMed: 11801722]

21. Adriance MC, Inman JL, Petersen OW, Bissell MJ. Myoepithelial cells: good fences make good neighbors. *Breast Cancer Res.* 2005;7(5):190–7. doi:10.1186/bcr1286. [PubMed: 16168137]
22. Radice GL, Ferreira-Cornwell MC, Robinson SD, Rayburn H, Chodosh LA, Takeichi M et al. Precocious mammary gland development in P-cadherin-deficient mice. *J Cell Biol.* 1997;139(4):102532.
23. Deugnier MA, Teuliere J, Faraldo MM, Thiery JP, Glukhova MA. The importance of being a myoepithelial cell. *Breast Cancer Res.* 2002;4(6):224–30. [PubMed: 12473168]
24. Haaksma CJ, Schwartz RJ, Tomasek JJ. Myoepithelial cell contraction and milk ejection are impaired in mammary glands of mice lacking smooth muscle alpha-actin. *Biol Reprod.* 2011;85(1):1321. doi:10.1095/biolreprod.110.090639.
25. Sopel M The myoepithelial cell: its role in normal mammary glands and breast cancer. *Folia Morphol (Warsz).* 2010;69(1):1–14. [PubMed: 20235044]
26. Valdez KE, Fan F, Smith W, Allred DC, Medina D, Behbod F. Human primary ductal carcinoma in situ (DCIS) subtype-specific pathology is preserved in a mouse intraductal (MIND) xenograft model. *J Pathol.* 2011;225(4):565–73. doi:10.1002/path.2969. [PubMed: 22025213]
27. Russell TD, Jindal S, Agunbiade S, Gao D, Troxell M, Borges VF et al. Myoepithelial cell differentiation markers in ductal carcinoma in situ progression. *Am J Pathol.* 2015;185(11):3076–89. doi:10.1016/j.ajpath.2015.07.004. [PubMed: 26343330]
28. Aguiar FN, Cirqueira CS, Bacchi CE, Carvalho FM. Morphologic, molecular and microenvironment factors associated with stromal invasion in breast ductal carcinoma in situ: Role of myoepithelial cells. *Breast Dis.* 2015;35(4):249–52. doi:10.3233/BD-150416. [PubMed: 26397772]
29. Sternlicht MD, Kedeshian P, Shao ZM, Safarians S, Barsky SH. The human myoepithelial cell is a natural tumor suppressor. *Clin Cancer Res.* 1997;3(11):1949–58. [PubMed: 9815584]
30. Pandey PR, Saidou J, Watabe K. Role of myoepithelial cells in breast tumor progression. *Front Biosci (Landmark Ed).* 2010;15:226–36. [PubMed: 20036817]
31. Sameni M, Cavallo-Medved D, Franco OE, Chalasani A, Ji K, Aggarwal N et al. Pathomimetic avatars reveal divergent roles of microenvironment in invasive transition of ductal carcinoma in situ. *Breast Cancer Res.* 2017;19(1):56. doi:10.1186/s13058-017-0847-0. [PubMed: 28506312]
32. Duivenvoorden HM, Rautela J, Edgington-Mitchell LE, Spurling A, Greening DW, Nowell CJ et al. Myoepithelial cell-specific expression of stefin A as a suppressor of early breast cancer invasion. *J Pathol.* 2017;243(4):496–509. doi:10.1002/path.4990. [PubMed: 29086922]
33. Jones JL, Shaw JA, Pringle JH, Walker RA. Primary breast myoepithelial cells exert an invasion-suppressor effect on breast cancer cells via paracrine down-regulation of MMP expression in fibroblasts and tumour cells. *J Pathol.* 2003;201(4):562–72. doi:10.1002/path.1483. [PubMed: 14648659]
34. Sarper M, Allen MD, Gomm J, Haywood L, Decock J, Thirkettle S et al. Loss of MMP-8 in ductal carcinoma in situ (DCIS)-associated myoepithelial cells contributes to tumour promotion through altered adhesive and proteolytic function. *Breast Cancer Res.* 2017;19(1):33. doi:10.1186/s13058-017-0822-9. [PubMed: 28330493]
35. Barsky SH, Karlin NJ. Myoepithelial cells: autocrine and paracrine suppressors of breast cancer progression. *J Mammary Gland Biol Neoplasia.* 2005;10(3):249–60. doi:10.1007/s10911-005-9585-5. [PubMed: 16807804]
36. Nguyen M, Lee MC, Wang JL, Tomlinson JS, Shao ZM, Alpaugh ML et al. The human myoepithelial cell displays a multifaceted anti-angiogenic phenotype. *Oncogene.* 2000;19(31):3449–59. doi:10.1038/sj.onc.1203677. [PubMed: 10918603]
37. Law RH, Irving JA, Buckle AM, Ruzyla K, Buzza M, Bashtannyk-Puhlovich TA et al. The high resolution crystal structure of the human tumor suppressor maspin reveals a novel conformational switch in the G-helix. *J Biol Chem.* 2005;280(23):22356–64. doi:10.1074/jbc.M412043200. [PubMed: 15760906]
38. Rohilla M, Bal A, Singh G, Joshi K. Phenotypic and Functional Characterization of Ductal Carcinoma In Situ-Associated Myoepithelial Cells. *Clin Breast Cancer.* 2015;15(5):335–42. doi:10.1016/j.clbc.2015.01.004. [PubMed: 25700939]

39. Zhang M, Volpert O, Shi YH, Bouck N. Maspin is an angiogenesis inhibitor. *Nat Med.* 2000;6(2):196–9. doi:10.1038/72303. [PubMed: 10655109]
40. Streuli CH. Maspin is a tumour suppressor that inhibits breast cancer tumour metastasis in vivo. *Breast Cancer Res.* 2002;4(4):137–40. [PubMed: 12100737]
41. Shao ZM, Nguyen M, Alpaugh ML, O'Connell JT, Barsky SH. The human myoepithelial cell exerts antiproliferative effects on breast carcinoma cells characterized by p21WAF1/CIP1 induction, G2/M arrest, and apoptosis. *Exp Cell Res.* 1998;241(2):394–403. doi:10.1006/excr.1998.4066. [PubMed: 9637781]
42. Lawler PR, Lawler J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. *Cold Spring Harb Perspect Med.* 2012;2(5):a006627. doi:10.1101/cshperspect.a006627. [PubMed: 22553494]
43. Allinen M, Beroukhi R, Cai L, Brennan C, Lahti-Domenici J, Huang H et al. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell.* 2004;6(1):17–32. doi:10.1016/j.ccr.2004.06.010. [PubMed: 15261139]
44. Guo F, Wang Y, Liu J, Mok SC, Xue F, Zhang W. CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene.* 2016;35(7):816–26. doi:10.1038/onc.2015.139. [PubMed: 25961926]
45. Allen MD, Thomas GJ, Clark S, Dawoud MM, Vallath S, Payne SJ et al. Altered microenvironment promotes progression of preinvasive breast cancer: myoepithelial expression of alphavbeta6 integrin in DCIS identifies high-risk patients and predicts recurrence. *Clin Cancer Res.* 2014;20(2):344–57. doi:10.1158/1078-0432.CCR-13-1504. [PubMed: 24150233]
46. Lo PK, Zhang Y, Yao Y, Wolfson B, Yu J, Han SY et al. Tumor-associated myoepithelial cells promote the invasive progression of ductal carcinoma in situ through activation of TGFbeta signaling. *J Biol Chem.* 2017;292(27):11466–84. doi:10.1074/jbc.M117.775080. [PubMed: 28512126]
47. Szynglarewicz B, Kasprzak P, Donizy P, Bieчек P, Halon A, Matkowski R. Epithelial-mesenchymal transition inducer Snail1 and invasive potential of intraductal breast cancer. *J Surg Oncol.* 2017;116(6):696–705. doi:10.1002/jso.24708. [PubMed: 28570750]
48. Haslam SZ. Mammary fibroblast influence on normal mouse mammary epithelial cell responses to estrogen in vitro. *Cancer Res.* 1986;46(1):310–6. [PubMed: 3940197]
49. Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L et al. Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc Natl Acad Sci U S A.* 2004;101(14):4966–71. doi:10.1073/pnas.0401064101. [PubMed: 15051869]
50. Makarem M, Kannan N, Nguyen LV, Knapp DJ, Balani S, Prater MD et al. Developmental changes in the in vitro activated regenerative activity of primitive mammary epithelial cells. *PLoS Biol.* 2013;11(8):e1001630. doi:10.1371/journal.pbio.1001630. [PubMed: 23966837]
51. Inman JL, Robertson C, Mott JD, Bissell MJ. Mammary gland development: cell fate specification, stem cells and the microenvironment. *Development.* 2015;142(6):1028–42. doi:10.1242/dev.087643. [PubMed: 25758218]
52. Luhr I, Friedl A, Overath T, Tholey A, Kunze T, Hilpert F et al. Mammary fibroblasts regulate morphogenesis of normal and tumorigenic breast epithelial cells by mechanical and paracrine signals. *Cancer Lett.* 2012;325(2):175–88. doi:10.1016/j.canlet.2012.06.014. [PubMed: 22776560]
53. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol.* 2007;8(3):221–33. doi:10.1038/nrm2125. [PubMed: 17318226]
54. Simian M, Hirai Y, Navre M, Werb Z, Lochter A, Bissell MJ. The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development.* 2001;128(16):3117–31. [PubMed: 11688561]
55. Wiseman BS, Sternlicht MD, Lund LR, Alexander CM, Mott J, Bissell MJ et al. Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. *J Cell Biol.* 2003;162(6):1123–33. doi:10.1083/jcb.200302090. [PubMed: 12975354]
56. Kalluri R The biology and function of fibroblasts in cancer. *Nat Rev Cancer.* 2016;16(9):582–98. doi:10.1038/nrc.2016.73. [PubMed: 27550820]

57. Erdogan B, Webb DJ. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. *Biochem Soc Trans.* 2017;45(1): 229–36. doi:10.1042/BST20160387. [PubMed: 28202677]
58. Gascard P, Tlsty TD. Carcinoma-associated fibroblasts: orchestrating the composition of malignancy. *Genes Dev.* 2016;30(9):1002–19. doi:10.1101/gad.279737.116. [PubMed: 27151975]
59. Liao Z, Tan ZW, Zhu P, Tan NS. Cancer-associated fibroblasts in tumor microenvironment - Accomplices in tumor malignancy. *Cell Immunol.* 2018. doi:10.1016/j.cellimm.2017.12.003.
60. Santi A, Kugeratski FG, Zanivan S. Cancer Associated Fibroblasts: The Architects of Stroma Remodeling. *Proteomics.* 2018;18(5–6):e1700167. doi:10.1002/pmic.201700167. [PubMed: 29280568]
61. Hu M, Peluffo G, Chen H, Gelman R, Schnitt S, Polyak K. Role of COX-2 in epithelial-stromal cell interactions and progression of ductal carcinoma in situ of the breast. *Proc Natl Acad Sci U S A.* 2009;106(9):3372–7. doi:10.1073/pnas.0813306106. [PubMed: 19218449]
62. Sung KE, Yang N, Pehlke C, Keely PJ, Eliceiri KW, Friedl A et al. Transition to invasion in breast cancer: a microfluidic in vitro model enables examination of spatial and temporal effects. *Integr Biol (Camb).* 2011;3(4):439–50. doi:10.1039/c0ib00063a.
63. Osuala KO, Sameni M, Shah S, Aggarwal N, Simonait ML, Franco OE et al. Il-6 signaling between ductal carcinoma in situ cells and carcinoma-associated fibroblasts mediates tumor cell growth and migration. *BMC Cancer.* 2015;15:584. doi:10.1186/s12885-015-1576-3. [PubMed: 26268945]
64. Bernard S, Myers M, Fang WB, Zinda B, Smart C, Lambert D et al. CXCL1 Derived from Mammary Fibroblasts Promotes Progression of Mammary Lesions to Invasive Carcinoma through CXCR2 Dependent Mechanisms. *J Mammary Gland Biol Neoplasia.* 2018. doi:10.1007/s10911-0189407-1.
65. Nielsen BS, Rank F, Illemann M, Lund LR, Dano K. Stromal cells associated with early invasive foci in human mammary ductal carcinoma in situ coexpress urokinase and urokinase receptor. *Int J Cancer.* 2007;120(10):2086–95. doi:10.1002/ijc.22340. [PubMed: 17290405]
66. Morgan MM, Livingston MK, Warrick JW, Stanek EM, Alarid ET, Beebe DJ et al. Mammary fibroblasts reduce apoptosis and speed estrogen-induced hyperplasia in an organotypic MCF7-derived duct model. *Sci Rep.* 2018;8(1):7139. doi:10.1038/s41598-018-25461-1. [PubMed: 29740030]
67. Brechbuhl HM, Finlay-Schultz J, Yamamoto TM, Gillen AE, Cittelty DM, Tan AC et al. Fibroblast Subtypes Regulate Responsiveness of Luminal Breast Cancer to Estrogen. *Clin Cancer Res.* 2017;23(7):1710–21. doi:10.1158/1078-0432.CCR-15-2851. [PubMed: 27702820]
68. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med.* 2006;4(1):38. doi:10.1186/1741-7015-4-38. [PubMed: 17190588]
69. Conklin MW, Gangnon RE, Sprague BL, Van Gemert L, Hampton JM, Eliceiri KW et al. Collagen Alignment as a Predictor of Recurrence after Ductal Carcinoma In Situ. *Cancer Epidemiol Biomarkers Prev.* 2018;27(2):138–45. doi:10.1158/1055-9965.EPI-17-0720. [PubMed: 29141852]
70. Gouon-Evans V, Rothenberg ME, Pollard JW. Postnatal mammary gland development requires macrophages and eosinophils. *Development.* 2000;127(11):2269–82. [PubMed: 10804170]
71. Ingman WV, Wyckoff J, Gouon-Evans V, Condeelis J, Pollard JW. Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Dev Dyn.* 2006;235(12):3222–9. doi:10.1002/dvdy.20972. [PubMed: 17029292]
72. Hodson LJ, Chua AC, Evdokiou A, Robertson SA, Ingman WV. Macrophage phenotype in the mammary gland fluctuates over the course of the estrous cycle and is regulated by ovarian steroid hormones. *Biol Reprod.* 2013;89(3):65. doi:10.1095/biolreprod.113.109561. [PubMed: 23926283]
73. O'Brien J, Martinson H, Durand-Rougely C, Schedin P. Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. *Development.* 2012;139(2): 269–75. doi:10.1242/dev.071696. [PubMed: 22129827]
74. Plaks V, Boldajipour B, Linnemann JR, Nguyen NH, Kersten K, Wolf Y et al. Adaptive Immune Regulation of Mammary Postnatal Organogenesis. *Dev Cell.* 2015;34(5):493–504. doi:10.1016/j.devcel.2015.07.015. [PubMed: 26321127]

75. Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am J Pathol.* 2003;163(5):2113–26. doi:10.1016/S0002-9440(10)63568-7. [PubMed: 14578209]
76. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell.* 2009;16(2):91–102. doi:10.1016/j.ccr.2009.06.018. [PubMed: 19647220]
77. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med.* 2001;193(6):727–40. [PubMed: 11257139]
78. Boyle ST, Faulkner JW, McColl SR, Kochetkova M. The chemokine receptor CCR6 facilitates the onset of mammary neoplasia in the MMTV-PyMT mouse model via recruitment of tumor-promoting macrophages. *Mol Cancer.* 2015;14:115. doi:10.1186/s12943-015-0394-1. [PubMed: 26047945]
79. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. *Proc Natl Acad Sci U S A.* 2012;109(8):2796–801. doi:10.1073/pnas.1104303108. [PubMed: 21825174]
80. Rumney RMH, Coffelt SB, Neale TA, Dhayade S, Tozer GM, Miller G. PyMT-MacLow: A novel, inducible, murine model for determining the role of CD68 positive cells in breast tumor development. *PLoS One.* 2017;12(12):e0188591. doi:10.1371/journal.pone.0188591. [PubMed: 29220404]
81. Schwertfeger KL, Xian W, Kaplan AM, Burnett SH, Cohen DA, Rosen JM. A critical role for the inflammatory response in a mouse model of preneoplastic progression. *Cancer Res.* 2006;66(11):567685. doi:10.1158/0008-5472.CAN-05-3781.
82. Bohrer LR, Schwertfeger KL. Macrophages promote fibroblast growth factor receptor-driven tumor cell migration and invasion in a CXCR2-dependent manner. *Mol Cancer Res.* 2012;10(10):1294–305. doi:10.1158/1541-7786.MCR-12-0275. [PubMed: 22893608]
83. Carron EC, Homra S, Rosenberg J, Coffelt SB, Kittrell F, Zhang Y et al. Macrophages promote the progression of premalignant mammary lesions to invasive cancer. *Oncotarget.* 2017;8(31):50731–46. doi:10.18632/oncotarget.14913. [PubMed: 28881599]
84. Linde N, Casanova-Acebes M, Sosa MS, Mortha A, Rahman A, Farias E et al. Macrophages orchestrate breast cancer early dissemination and metastasis. *Nat Commun.* 2018;9(1):21. doi:10.1038/s41467-017-02481-5. [PubMed: 29295986]
85. Moraes LA, Kar S, Foo SL, Gu T, Toh YQ, Ampomah PB et al. Annexin-A1 enhances breast cancer growth and migration by promoting alternative macrophage polarization in the tumour microenvironment. *Sci Rep.* 2017;7(1):17925. doi:10.1038/s41598-017-17622-5. [PubMed: 29263330]
86. Ruffell B, Chang-Strachan D, Chan V, Rosenbusch A, Ho CM, Pryer N et al. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell.* 2014;26(5):623–37. doi:10.1016/j.ccell.2014.09.006. [PubMed: 25446896]
87. Vallerand D, Massonnet G, Kebir F, Gentien D, Maciorowski Z, De la Grange P et al. Characterization of Breast Cancer Preclinical Models Reveals a Specific Pattern of Macrophage Polarization. *PLoS One.* 2016;11(7):e0157670. doi:10.1371/journal.pone.0157670. [PubMed: 27388901]
88. Gil Del Alcazar CR, Huh SJ, Ekram MB, Trinh A, Liu LL, Beca F et al. Immune Escape in Breast Cancer During In Situ to Invasive Carcinoma Transition. *Cancer Discov.* 2017;7(10):1098–115. doi:10.1158/2159-8290.CD-17-0222. [PubMed: 28652380]
89. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol.* 2018;19(1):40–50. doi:10.1016/S14702045(17)30904-X. [PubMed: 29233559]
90. Hendry S, Pang JB, Byrne DJ, Lakhani SR, Cummings MC, Campbell IG et al. Relationship of the Breast Ductal Carcinoma In Situ Immune Microenvironment with Clinicopathological and Genetic

Features. Clin Cancer Res. 2017;23(17):5210–7. doi:10.1158/1078-0432.CCR-17-0743. [PubMed: 28611201]

91. Disis ML, Park KH. Immunomodulation of breast cancer via tumor antigen specific Th1. Cancer Res Treat. 2009;41(3):117–21. doi:10.4143/crt.2009.41.3.117. [PubMed: 19809560]
92. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14(10):1014–22. doi:10.1038/ni.2703. [PubMed: 24048123]
93. Thompson E, Taube JM, Elwood H, Sharma R, Meeker A, Warzecha HN et al. The immune microenvironment of breast ductal carcinoma in situ. Mod Pathol. 2016;29(3):249–58. doi: 10.1038/modpathol.2015.158. [PubMed: 26769139]
94. Campbell MJ, Baehner F, O'Meara T, Ojukwu E, Han B, Mukhtar R et al. Characterizing the immune microenvironment in high-risk ductal carcinoma in situ of the breast. Breast Cancer Res Treat. 2017;161(1):17–28. doi:10.1007/s10549-016-4036-0. [PubMed: 27785654]
95. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell. 2011;20(3):300–14. doi:10.1016/j.ccr.2011.08.012. [PubMed: 21907922]
96. Uribe-Querol E, Rosales C. Neutrophils in Cancer: Two Sides of the Same Coin. J Immunol Res. 2015;2015:983698. doi:10.1155/2015/983698. [PubMed: 26819959]
97. Buldakov M, Zavyalova M, Krakhmal N, Telegina N, Vtorushin S, Mitrofanova I et al. CD68+, but not stabilin-1+ tumor associated macrophages in gaps of ductal tumor structures negatively correlate with the lymphatic metastasis in human breast cancer. Immunobiology. 2017;222(1):31–8. doi:10.1016/j.imbio.2015.09.011. [PubMed: 26391151]
98. Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M, Yang Y et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell. 2014;26(6): 92337. doi:10.1016/j.ccell.2014.10.018.
99. Lowenfeld L, Mick R, Datta J, Xu S, Fitzpatrick E, Fisher CS et al. Dendritic Cell Vaccination Enhances Immune Responses and Induces Regression of HER2(pos) DCIS Independent of Route: Results of Randomized Selection Design Trial. Clin Cancer Res. 2017;23(12):2961–71. doi: 10.1158/1078-0432.CCR-16-1924. [PubMed: 27965306]
100. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast Cancer Mortality After a Diagnosis of Ductal Carcinoma In Situ. JAMA Oncol. 2015;1(7):888–96. doi:10.1001/jamaoncol.2015.2510. [PubMed: 26291673]
101. Martinez-Perez C, Turnbull AK, Ekatah GE, Arthur LM, Sims AH, Thomas JS et al. Current treatment trends and the need for better predictive tools in the management of ductal carcinoma in situ of the breast. Cancer Treat Rev. 2017;55:163–72. doi:10.1016/j.ctrv.2017.03.009. [PubMed: 28402908]

Figure 1: Morphologic variation of human DCIS and the tumor-associated stroma.

A) Example of low grade, solid type DCIS. Black arrows point to examples of the retained basement membrane and myoepithelial cells of the involved ducts. The stroma has minimal (*) to moderate (+) amounts of pinkstaining, acellular collagen. The overall stromal cellularity is low (scale bar=200 uM). B) Low grade, cribriform type DCIS with marked accumulation of dark pink-staining collagen throughout the stroma. The black arrow points to an example of fibroblasts demonstrating slightly elongated (spindle) nuclei. (scale bar=200 uM). C) High grade, cribriform type DCIS; accumulation of apoptotic debris is beginning within lumen (black arrow). The stroma (*) has a moderate accumulation of collagen throughout, with moderate stromal cellularity. A small focus of lymphocytes is outlined at the top of the image (scale bar=200 uM). D) High grade, solid type DCIS; there is a heavy, extensive lymphocytic infiltrate (outlined in black) (scale bar=200 uM). E,F) Low power image of a high-grade, comedo type DCIS with microinvasion (scale bar=500 uM). The boxed area is shown in panel F at high power; DCIS with comedo necrosis (*) is outlined in red, while the black arrows point to foci of microinvasive carcinoma. There is a marked lymphocyte infiltrate throughout the stroma (scale bar=200 uM).

Figure 2: Summary of microenvironment contributions to DCIS progression.

Multiple cell types, including myoepithelial cells, fibroblasts, myeloid cells and lymphocytes have been examined in the context of DCIS progression. Specific molecules that contribute to both tumor suppression and promotion are listed as detailed in the respective sections within this review.

Figure 3: Macrophage infiltration in premalignant lesions.

F4/80 staining shows that macrophages are recruited to pre-invasive lesions in (A) $p53^{-/-}$ PN1a, (B) $p53^{-/-}$ PN1b, and (C) C3(1)TA_g mouse models. D) CD68 staining shows immune cell infiltration in human DCIS.