Immune Escape during Breast Tumor Progression

Carlos R. Gil Del Alcazar[1,2,3], Maša Alečković[1,2,3], and Kornelia Polyak[1,2,3]

ABSTRACT

Immunotherapy using checkpoint inhibitors is one of the most promising current cancer treatment strategies. However, in breast cancer, its success has been limited to a subset of patients with triple-negative disease, whose durability of observed responses remain unclear. The lack of detailed understanding of breast tumor immune evasion mechanisms and the treatment of patients with highly heterogeneous metastatic disease contribute to these disappointing results. Here we discuss the current knowledge about immune-related changes during breast tumor progression, with special emphasis on the *in situ*-to-invasive breast carcinoma transition that may represent a key step of immunoediting in breast cancer. Comprehensive characterization of early-stage disease and better understanding of immunologic drivers of disease progression will likely expand the tools available for immunotherapy and improve patient stratification.

Introduction

Immunoediting is a dynamic process by which the immune system shapes the evolution of tumors. It is marked by three phases: elimination, equilibrium, and escape (ref. 1; **Fig. 1A**). Most malignant cells are eliminated by immunosurveillance before clinical presentation (**Fig. 1B**). In this elimination phase, antitumor immunity is stimulated through innate and adaptive immune responses. During the equilibrium phase pro- and antitumor immunity fail to fully eradicate tumors, but keep them under control. In the escape phase, cancer cells completely evade immune control as demonstrated in experimental models and in patients with cancer (1). Mechanisms of immune escape include decreased immune detection, downregulation of costimulatory molecules, and/or overexpression of coinhibitory molecules, resulting in reduced $CD8^+$ T-cell activity (**Fig. 1C**). Immune escape is a requirement for breast tumor progression and a critical step in the transition from preinvasive to potentially lethal invasive disease. In this review, we discuss immune-related changes during breast cancer progression with special emphasis on the preinvasive-to-invasive transition.

Breast Tumor Progression

Histopathologic progression and classification

Ductal carcinoma, the most common histologic subtype of breast cancer, begins as abnormal epithelial proliferation in milk ducts of mammary glands, then progresses to ductal carcinoma *in situ* (DCIS), followed by invasive ductal carcinoma (IDC), and finally metastatic disease (**Fig. 1A**; ref. 2). DCIS is characterized by proliferation of cancer cells inside mammary ducts, which are surrounded by an intact layer of myoepithelial cells and basement membrane separating the epithelium from stroma. In contrast, IDC lacks myoepithelium and tumor epithelial cells invade the stroma. The major clinical and molecular breast cancer subtypes, defined by the presence of estrogen (ER) and progesterone (PR) receptors, HER2, and luminal or basal differentiation status, are present in preinvasive and invasive disease (3). Thus, tumors are classified as luminal (ER^+ and/or PR^+), $HER2^+$, or triple-negative (lacking ER/PR/HER2). However, pure DCIS (no evidence of invasion) is not routinely tested for these classifying markers aside from ER, as most patients with DCIS do not receive systemic adjuvant therapy. On the basis of a comprehensive meta-analysis of all prior publications, African-American race, premenopausal status, detection by palpation, high histologic grade, involved margins, and high p16 expression are all significantly associated with risk of invasive recurrence (4). The Oncotype DCIS Score is a commercial gene signature test predicting the probability of recurrence in women >50 years of age, reducing the need of radiotherapy for low-risk patients (5). However, this score is not routinely used in the clinic to inform treatment decisions in patients with DCIS.

Molecular changes in tumor epithelial cells

Despite significant genetic and gene expression changes during tumor progression, mutations or gene signatures that consistently differentiate DCIS from IDC are unknown (6). Attempts to improve classification by stratification according to intrinsic subtypes and comparing DCIS and IDC within the same subtype did not yield consistent *in situ* and invasive epithelial gene signatures (7). Similar to IDC, the top mutated genes in high-grade DCIS include *PIK3CA*, *TP53*, *GATA3*, and *KMT2C*, with *TP53* inactivation being a common event at the pathway level (8). High-grade DCIS also has frequent copy number aberrations including gain of chromosomes 1q, 8q, 11q13, 17q12, and 20q13 (9). *PIK3CA* mutations, more common in ER^+ luminal cases, are sometimes discordant between IDC and adjacent DCIS (10).

Comparing genomic copy number profiles of IDC and adjacent synchronous DCIS at single-cell resolution complemented with exome sequencing confirmed known copy number alterations in breast cancer and revealed many shared clones between *in situ* and invasive regions of the same tumor, suggesting a multiclonal invasion model (11). However, analysis of pure DCIS and subsequent IDC recurrences is required to validate this model.

Gene expression changes in the stroma

Because of inability to define consistent epithelial genetic changes between DCIS and IDC and the role of microenvironment in tumor progression, researchers have profiled various stromal cells to find potential drivers of invasiveness (12). Contrary to the heterogeneity of

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [2]Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts. [3]Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Corresponding Author: Kornelia Polyak, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-2106; Fax: 617-582-8490; E-mail: kornelia_polyak@dfci.harvard.edu

Cancer Immunol Res 2020;8:422–7

doi: 10.1158/2326-6066.CIR-19-0786

epithelial changes, stromal cell epigenetic and gene expression profiles show significant and consistent differences between normal breast tissue, DCIS, and IDC (13, 14). For instance, DCIS-associated myoepithelial cells are distinct from normal myoepithelia, with alterations in numerous genes encoding secreted proteins and extracellular matrix components (14). The myoepithelium contracts ducts during lactation for milk expulsion, controls mammary gland function via regulation of epithelial cell polarity, branching, and differentiation (15), and is a natural tumor suppressor by restricting angiogenesis and invasion (16, 17). However, myoepithelial cells lose this function during tumor progression and are absent in IDC. Molecular changes in DCIS-associated myoepithelium reflect perturbed differentiation and upregulation of genes related to angiogenesis and invasion (14, 18). Several genes altered in DCIS-associated myoepithelium have immune-related functions, implying a potential role for myoepithelial cells in immune regulation (14).

Immune-Related Changes during Breast Cancer Progression

Myeloid cells and lymphocytes

Leukocytes, which mount antitumor immune responses, present a barrier and selective pressure in tumor progression (19). Innate immune responses do not rely on antigens for activation, represent the first-line of defense against pathogens and cancer, and are responsible for activating adaptive immunity. In normal breast, $CD45^+$ leukocytes are relatively rare, but detectable in both stroma and within mammary ducts (20). In DCIS, leukocytes are abundant in the stroma surrounding the ducts (especially in high-grade and $HER2^+$ lesions), but intraepithelial leukocytes are rarely detectable (21). Leukocytes also localize to sites of myoepithelial cell layer disruption/microinvasion (21). This limited interaction between leukocytes and cancer cells in DCIS may underlie a mechanism by which tumors evade immune surveillance. Therefore, in DCIS, tumors could still exist in the equilibrium phase, with immune escape likely occurring during or just prior to invasive transition (**Fig. 1A**).

Both, myeloid and lymphoid lineage immune cells are recruited at all stages of breast tumor progression. However, leukocyte composition and relative abundances of innate and adaptive immune cells change according to histologic stage, underscoring the importance of both arms of immunity in tumor progression (21). For example, the relative fraction of neutrophils increases as cancers progress from normal to DCIS to IDC (21). Similarly, the relative proportion of macrophages (Mφ) increases in $HER2^+$ and triple-negative IDC compared with DCIS, while the fraction of dendritic cells (DC) decreases as tumors progress (21). Besides changes in relative abundance, the role of neutrophils and DCs in the DCIS-to-IDC transition is largely unknown, although they have been extensively studied in invasive disease.

DCs can have pro- or antitumor effects. They are functionally defective in patients with breast cancer potentially because of perturbed metabolism (22). However, HER2-targeting DC vaccines have been tested in patients with $HER2^+$ DCIS to prevent invasive progression with some promising results (23). Neutrophil infiltration associates with breast tumor grade and the triple-negative subtype (TNBC; ref. 24). TNBC can be classified into subtypes enriched for either Mφ or neutrophils, with a Mφ-to-neutrophil conversion mediating immune checkpoint blockade resistance (25). Neutrophils modulate local and systemic immune environments and promote breast cancer metastasis (26).

Mφ can also have pro- or antitumor effects. Tumor-associated Mφ (TAM) infiltration associates with poor survival in breast cancer (27). TAMs and their progenitors, monocytes, have distinct antitumor expression profiles in IDC when compared with their homeostatic tissue counterparts, correlating with subtype and tumor aggressiveness (28). In $HER2^+$ breast tumor models, Mφ recruited by tumor-derived CCL2 promote progression and early dissemination even from preinvasive lesions (29), which can be prevented by anti-CSF1R or CCR2 inhibition. In DCIS, $CD68^+$ Mφ are detected within ducts near cancer cells with lower E-cadherin levels implying that they may play a similar progression-promoting role (29).

High-grade DCIS has significantly more tumor-infiltrating lymphocytes (TIL) than low-grade DCIS (21, 30–33), particularly $CD68^+$ Mφ, $CD4^+$ T cells, $CD20^+$ B cells, and HLA-DR^+ and $FoxP3^+$ cells (30). High TIL content associates with high-grade, comedo necrosis, apocrine features, high $CD8^+$ T cells, and $HER2^+$/triple-negative subtypes (21). DCIS with microinvasion or adjacent to IDC have higher TIL density compared with pure DCIS, with $CD8^+$, $CD4^+$, and $CD38^+$ cells being more common in adjacent DCIS lesions (21, 34).

The spatial distribution of TILs is also highly heterogeneous in DCIS and IDC. In DCIS, some ducts are surrounded by TILs while other regions are devoid of leukocytes; however, the biological mechanism underlying this heterogeneity and its potential clinical relevance are unknown. In IDC, TILs are found in discrete spatial arrangements. For instance, in TNBC there are four distinct topologic patterns correlating with gene signatures and clinical outcomes: inflamed (TILs are fully intermingled with malignant cells), stroma restricted (TILs are within the tumor, but only detected in stroma), margin restricted (TILs surround the tumor, but do not enter), and immune desert (few to complete lack of TILs; refs. 35–37).

Distant metastatic tumors appear to have lower TIL densities compared with matched primary tumor sites, with brain metastases having the lowest T-cell infiltration among all metastatic sites (38–40). However, these studies have several limitations, including small cohorts (<10 cases/subtype), varying metastatic sites (e.g., lung, brain, and liver), and the use of post-systemic therapy recurrent patients. Regardless, these findings suggest a decline in antitumor immunity with metastatic progression making immunotherapy less effective. One reason for this decline could be an increase in intratumor heterogeneity because subclonal neoantigens generate less-effective antitumor immune responses (41). Indeed, immunotherapy in adjuvant and neoadjuvant settings has induced robust immune responses. Even in metastatic disease, earlier treatment was more effective (42). One limitation of applying immunotherapies to earlier settings in patients who may have complete responses with standard of care is the high frequency of serious and lasting side effects. Thus, improving patient selection is critical for broader use of immunotherapies in earlier stage disease.

Increased immunosuppression leads to immune escape

Paradoxically, whereas leukocyte infiltration increases from normal to DCIS and IDC progression, there is a marked decrease in the frequency of activated immune cells and a progressively suppressive immune microenvironment. The relative fraction of cytotoxic $CD8^+$ T cells is also variable based on tumor subtype, with triple-negative and $HER2^+$ pure DCIS having a higher proportion compared with DCIS adjacent to IDC (21). This decline was also observed in patients diagnosed with pure DCIS who underwent lumpectomy, but years later recurred locally with IDC (21). Gene set enrichment analysis of $CD3^+$ T cells from DCIS compared with IDC also demonstrates a

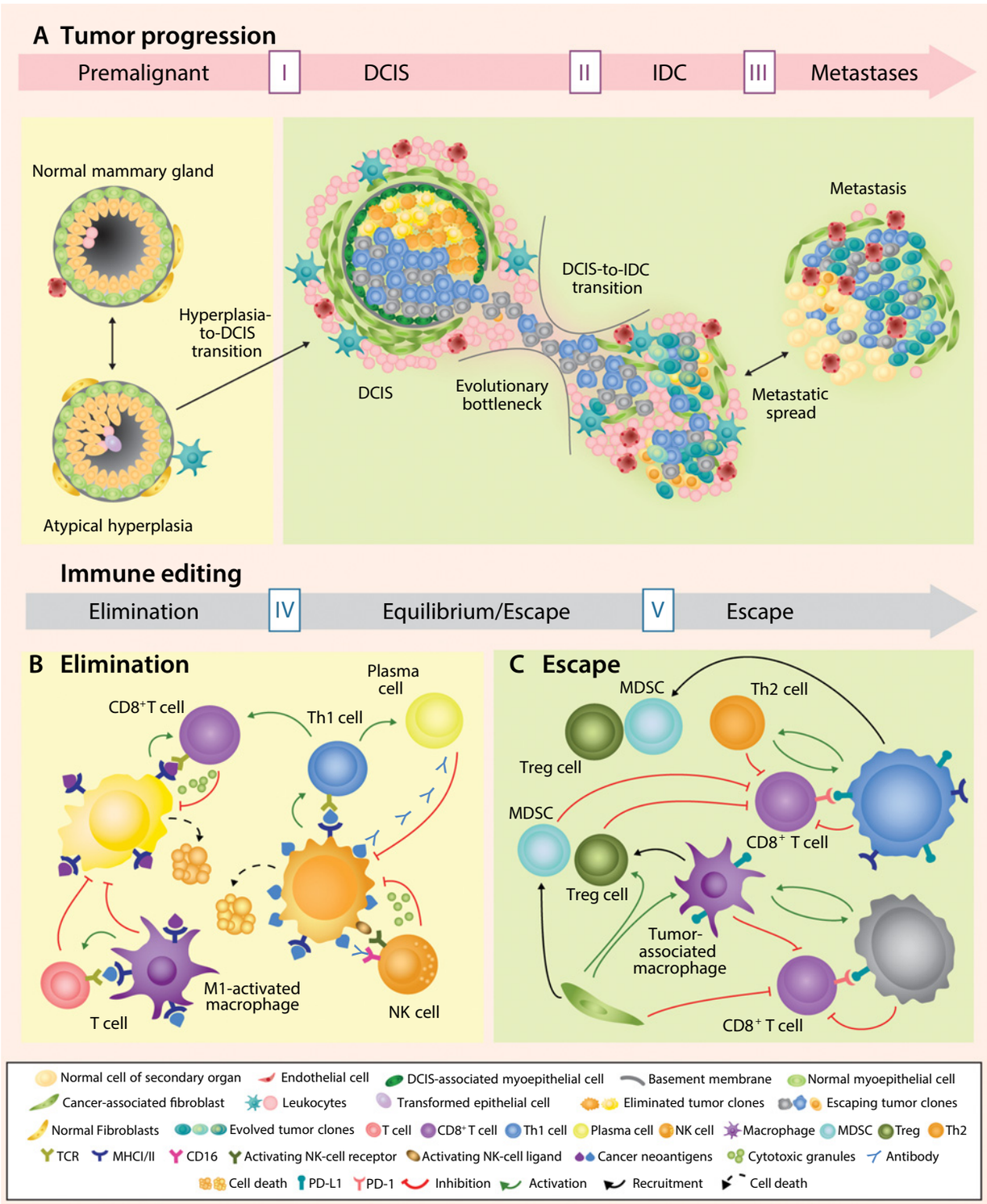

Figure 1.

Breast tumor progression in the context of immunoediting. **A,** Breast tumor progression (top) is accompanied by numerous stromal modifications, particularly immunoediting (bottom). Normal milk ducts, lined by luminal epithelial cells, myoepithelial cells, and basement membrane, progress to atypical hyperplasia through aberrant epithelial proliferation initiated by genetic and/or epigenetic alterations. Further acquisition of hereditary changes in combination with selection (**I**) promotes progression to DCIS, which is accompanied by an exclusion of leukocytes from the ducts (**IV**), recruitment and activation of surrounding immune infiltrates such as cytotoxic $CD8^+$ T cells (**IV**), and extensive stromal reprogramming (**I**). (*Continued on the following page.*)

switch from cytotoxic T-cell to immunosuppressive regulatory T-cell (Treg) signatures (21).

Multiple mechanisms contribute to the progressively suppressive immune environment during breast tumor evolution. The 9p24 amplicon containing *CD274* (encoding for PD-L1) is present in approximately 20% of primary TNBC, increasing in residual tumors after neoadjuvant chemotherapy (43). In triple-negative pure DCIS and IDC comparison, *CD274* amplification was only detected in IDC and associated with higher tumor cell expression of PD-L1 (21). Similarly, the 17q12 amplicon in close proximity to *ERBB2* (encoding HER2) contains a cluster of chemokine (CC) genes with diverse functions. In $HER2^+$ pure DCIS and IDC, amplification of *ERBB2* associates with coamplification of this CC, which inversely correlates with the frequency of intratumoral $GZMB^+CD8^+$ T cells (21).

HER2 itself can trigger an antitumor immune response in *ERBB2*-amplified tumors. Progressive loss of anti-HER2 Th1 function is found when comparing healthy individuals with patients diagnosed with $HER2^+$ DCIS and $HER2^+$ IDC, and this associates with a functional shift in IFNγ:IL10 producing phenotypes, potentially reflecting a mechanism of immune evasion in HER2-driven breast tumors (44). A vaccine against HER2 tested as an invasive breast cancer prevention strategy in patients with $HER2^+$ DCIS yielded promising results (45). However, HER2-targeted immune responses could favor the outgrowth of $HER2^-$ breast tumors with less favorable prognoses.

Changes in TIL composition, such as increased accumulation of Treg cells during tumor progression, contribute to immune suppression. Synchronous DCIS and IDC cases have increased infiltration of Treg cells in DCIS compared with normal breast and a further increase in IDC compared with DCIS (46). Higher Treg infiltration associates with high grade but not tumor subtype, size of the invasive tumor, lymph node status, or disease stage (46). Expression of CTLA-4 also significantly increases in T cells from IDC compared with DCIS regardless of subtype (21), potentially contributing to immune exhaustion.

In breast cancer, aberrant maturation and differentiation of DCs (47, 48), downregulation of neoantigen peptide loading genes including MHC class I (49, 50), and upregulation of HLA-G (21, 50), which is highly expressed in placenta and results in a tolerogenic phenotype permissive for embryo development, associate with malignant progression. In other cancer types, such as lung cancer and melanoma, downregulation of neoantigens results in decreased immune recognition (51). Following anti-PD-L1 or anti-CTLA-4 treatment of lung cancer, seven to 18 putative mutation-associated neoantigens are lost in therapy-resistant clones, potentially mediating tumor recurrence (51). Loss of heterozygosity in human leukocyte antigens genes or depletion of expressed neoantigens via promoter methylation are reported in early-stage, immune-infiltrated lung cancer (52). Interestingly, intratumoral genetic heterogeneity induced by cytotoxic chemotherapy, which leads to increased subclonal neoantigens, correlates with worse outcomes in early-stage lung cancer and melanoma (53). As intratumoral subclonal neoantigen heterogeneity increases, immune responses and immune infiltration decrease, possibly due to dilution/overwhelming of the immune system with neoantigens that might be only subclonal or not reactive (41). Evolutionary studies like these have not been conducted in breast cancer in part due to difficulties with acquisition of fresh tissue from early-stage tumors and the limited success of immunotherapy.

Immunotherapy in Breast Cancer

The first FDA approval for a breast cancer immunotherapy was in April 2019 for atezolizumab (anti–PD-L1) in combination with nab-paclitaxel for triple-negative metastatic disease. This led to a sustained enthusiasm for immunotherapy, with around 300 trials exploring immunotherapies in breast cancer, the vast majority being phase I or I/II trials for immune checkpoint blockade (45). Vaccination against HER2 is being tested in the clinical setting and elicits tumor-specific T-cell responses (45). In the adjuvant setting, vaccination against HER-2 resulted in no tumor recurrences after a 34-month period (45). In addition, current clinical trials test vaccines in combination with immune checkpoint blockade (NCT03362060, NCT03199040, and NCT02826434), adoptive natural killer cell therapy (NCT02844335 and NCT02843126), and chimeric antigen receptor T cells targeting overexpressed proteins in breast cancers including HER2 (NCT02713984, NCT01837602, NCT02792114, and NCT02587689).

A concerted action targeting both types of immunity will lead to stronger immune responses in a wider set of patients (27). For example, targeting TAMs may lift immunosuppression on effector cells and reestablish Mφ antitumor effects (27). Several clinical trials aim at depleting Mφ by targeting CSF1R (NCT01346358, NCT02265536, and NCT03153410) or through specially formulated inorganic bisphosphonates, such as zoledronate (NCT02347163) or clodronate (NCT00127205). Decreasing TAM recruiting by CCL2-neutralizing antibody treatment and Mφ reprogramming have also been evaluated in preclinical models. For example, inhibition of class IIA HDACs in luminal B breast cancer increased the efficacy and durability of immune checkpoint inhibitor and chemotherapy (54), while neutralization of the macrophage receptor with collagenous structure (MARCO) on a subset of inflammatory TAMs inhibited tumor metastasis (55). $MARCO^+$ TAMs are also present in human breast cancers, particularly in basal/triple-negative tumors. Thus, repolarizing TAMs through MARCO may be therapeutically beneficial for

(*Continued.*) The transition from DCIS to IDC represents an evolutionary bottleneck, which associates with lack of myoepithelium and basement membrane (**II**) as well as further stromal alterations (**II**); see **B** and **C** for more detail. Only clones capable of immune evasion and survival in the stromal microenvironment persist. Immune escape might occur through various mechanisms before or during the DCIS-to-IDC transition (**V**), including downregulation of cancer neoantigens or MHCI/II genes, aberrant polarization of immune cells, recruitment of immune-suppressive cells, and reduced cytotoxic immune responses. Distant metastasis, arising from single or multiple dissemination events (**III**), is characterized by an even more suppressive immune environment than IDC. **B,** Innate and adaptive immune responses can eliminate malignant cells. Antigen-presenting cells, including macrophages, prime lymphocytes such as $CD8^+$ and $CD4^+$ Th cells and induce their clonal expansion. M1-activated macrophages also engulf and destroy cancer cells. Activated $CD8^+$ T cells bind cognate neoantigens presented on MHC I/II via T-cell receptors (TCR) and secrete cytotoxic granules. Th1 cells aid in $CD8^+$ T-cell activation and differentiation of B cells into neoantigen-targeted antibody-producing plasma cells. Natural killer (NK)–cell cytotoxicity is activated by the NK-cell receptor in an antigen-independent manner but can also be triggered by antibody binding via CD16. **C,** Immune-escaper clones interact with stromal cells such as cancer-associated fibroblasts (CAF) to secrete/induce secretion of immunomodulatory cytokines that polarize immune responses. This results in a shift from immune-reactive to immune-suppressive microenvironments, marked by recruitment and polarization of TAMs, Th2 polarization, and recruitment of Tregs and myeloid-derived suppressor cells (MDSC). TAMs, MDSCs, Th2, and Treg cells inhibit the cytotoxic immune response, while CAFs also promote exclusion of $CD8^+$ T cells from tumors. Tumor cells downregulate neoantigens and the antigen-presentation machinery and upregulate immune checkpoint proteins including PD-L1, which promotes exhaustion of $CD8^+$ T cells through the PD-1 receptor. This immune-suppressive environment ultimately promotes tumor progression and metastasis.

patients with TNBC. Toll-like receptor (TLR) activation by bacterial particles polarizes plasmacytoid DCs and Mφ toward a proinflammatory phenotype, leading to antitumor effects in breast cancer (56). The TLR7 agonist, imiquimod, increases lymphoid immune infiltration and tumor regression of skin metastases from breast cancers in patients (57).

Summary and Future Directions

With immune escape marking the DCIS-to-IDC transition, we hypothesize that it is a requirement for invasive progression and tumor dissemination, because only cancer cells evading immune surveillance can contribute to tumor formation (**Fig. 1**). Therefore, the *in situ*-to-invasive carcinoma transition represents an evolutionary bottleneck, which may be determined by the host's immune status. Thus, assessing systemic and local immune environments in patients with DCIS could serve as a risk predictor of invasive progression. Comprehensive characterization of pure DCIS and their local invasive recurrences at the single-cell level while preserving topology could reveal mechanisms underlying immune escape, which can facilitate the design of more effective immunotherapies for the treatment of both early and advanced stage disease.

One limitation of implementing immunotherapies in breast cancer is the scarcity of preclinical models that reproduce the natural progression of human breast cancer. Engineered and spontaneous mouse mammary tumors neither recapitulate the histopathologic progression nor the immune microenvironment of human breast tumors. Carcinogen-induced mammary tumors in Sprague Dawley and Wistar-Furth rats show remarkable similarities to human disease with regards to hormone dependence and histopathologic stages of progression (58), but their immune environments remain to be characterized. However, based on data highlighting the importance of the microbiome in antitumor immunity and success of immunotherapy (59), no preclinical model faithfully reproduces the complexity of the human body, limiting the predictive power of such models. Therefore, improved molecular and cellular understanding of how tumors evade immune surveillance in patients with breast cancer coupled with rationally designed clinical trials with strong correlative studies are necessary to make progress.

Disclosure of Potential Conflicts of Interest

K. Polyak is a scientific advisory board member for Farcast Biosciences and is a consultant/advisory board member for Acrivon Therapeutics. No potential conflicts of interest were disclosed by the other authors.

Acknowledgments

The authors would like to thank Drs. Judith Agudo and Jennifer Guerriero, and members of the Polyak laboratory for critical reading of this article and for helpful discussions. This work was supported by the NCI (R35CA197623; to K. Polyak), the Breast Cancer Research Foundation (to K. Polyak), and the Claudia Adams Barr Program (to C.R. Gil Del Alcazar).

References

1. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
2. Bombonati A, Sgroi DC. The molecular pathology of breast cancer progression. J Pathol 2011;223:307–17.
3. Sanati S. Morphologic and molecular features of breast ductal carcinoma in situ. Am J Pathol 2019;189:946–55.
4. Visser LL, Groen EJ, van Leeuwen FE, Lips EH, Schmidt MK, Wesseling J. Predictors of an invasive breast cancer recurrence after DCIS: a systematic review and meta-analyses. Cancer Epidemiol Biomarkers Prev 2019;28:835–45.
5. Nofech-Mozes S, Hanna W, Rakovitch E. Molecular evaluation of breast ductal carcinoma in situ with oncotype DX DCIS. Am J Pathol 2019;189:975–80.
6. Polyak K. Molecular markers for the diagnosis and management of ductal carcinoma in situ. J Natl Cancer Inst Monogr 2010;2010:210–3.
7. Lesurf R, Aure MR, Mork HH, Vitelli V; Oslo Breast Cancer Research Consortium (OSBREAC), Lundgren S, et al. Molecular features of subtype-specific progression from ductal carcinoma in situ to invasive breast cancer. Cell Rep 2016;16:1166–79.
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;75:3980–90.
9. Casasent AK, Edgerton M, Navin NE. Genome evolution in ductal carcinoma in situ: invasion of the clones. J Pathol 2017;241:208–18.
10. Sakr RA, Weigelt B, Chandarlapaty S, Andrade VP, Guerini-Rocco E, Giri D, et al. PI3K pathway activation in high-grade ductal carcinoma in situ–implications for progression to invasive breast carcinoma. Clin Cancer Res 2014;20:2326–37.
11. 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;172:205–17.
12. Nelson AC, Machado HL, Schwertfeger KL. Breaking through to the other side: microenvironment contributions to DCIS initiation and progression. J Mammary Gland Biol Neoplasia 2018;23:207–21.
13. Hu M, Yao J, Cai L, Bachman KE, van den Brule F, Velculescu V, et al. Distinct epigenetic changes in the stromal cells of breast cancers. Nat Genet 2005;37:899–905.
14. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 2004;6:17–32.
15. Forster N, Saladi SV, van Bragt M, Sfondouris ME, Jones FE, Li Z, et al. Basal cell signaling by p63 controls luminal progenitor function and lactation via NRG1. Dev Cell 2014;28:147–60.
16. Polyak K, Hu M. Do myoepithelial cells hold the key for breast tumor progression? J Mammary Gland Biol Neoplasia 2005;10:231–47.
17. Hilson JB, Schnitt SJ, Collins LC. Phenotypic alterations in ductal carcinoma in situ-associated myoepithelial cells: biologic and diagnostic implications. Am J Surg Pathol 2009;33:227–32.
18. Ding L, Su Y, Fassl A, Hinohara K, Qiu X, Harper NW, et al. Perturbed myoepithelial cell differentiation in BRCA mutation carriers and in ductal carcinoma in situ. Nat Commun 2019;10:4182.
19. Kroemer G, Senovilla L, Galluzzi L, Andre F, Zitvogel L. Natural and therapy-induced immunosurveillance in breast cancer. Nat Med 2015;21:1128–38.
20. Tower H, Ruppert M, Britt K. The immune microenvironment of breast cancer progression. Cancers 2019;11. pii: E1375.
21. 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;7:1098–115.
22. Gervais A, Leveque J, Bouet-Toussaint F, Burtin F, Lesimple T, Sulpice L, et al. Dendritic cells are defective in breast cancer patients: a potential role for polyamine in this immunodeficiency. Breast Cancer Res 2005;7:R326–35.
23. 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;23:2961–71.
24. Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. Asian Pac J Cancer Prev 2017;18:2689–93.
25. Kim IS, Gao Y, Welte T, Wang H, Liu J, Janghorban M, et al. Immuno-subtyping of breast cancer reveals distinct myeloid cell profiles and immunotherapy resistance mechanisms. Nat Cell Biol 2019;21:1113–26.
26. Janiszewska M, Tabassum DP, Castano Z, Cristea S, Yamamoto KN, Kingston NL, et al. Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments. Nat Cell Biol 2019;21:879–88.
27. Cassetta L, Pollard JW. Targeting macrophages: therapeutic approaches in cancer. Nat Rev Drug Discov 2018;17:887–904.

28. Cassetta L, Fragkogianni S, Sims AH, Swierczak A, Forrester LM, Zhang H, et al. Human tumor-associated macrophage and monocyte transcriptional landscapes reveal cancer-specific reprogramming, biomarkers, and therapeutic targets. Cancer Cell 2019;35:588–602.
29. 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;9:21.
30. 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;161:17–28.
31. 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;29:249–58.
32. Morita M, Yamaguchi R, Tanaka M, Tse GM, Yamaguchi M, Kanomata N, et al. CD8(+) tumor-infiltrating lymphocytes contribute to spontaneous "healing" in HER2-positive ductal carcinoma in situ. Cancer Med 2016;5:1607–18.
33. Kim A, Heo SH, Kim YA, Gong G, Jin Lee H. An examination of the local cellular immune response to examples of both ductal carcinoma in situ (DCIS) of the breast and DCIS with microinvasion, with emphasis on tertiary lymphoid structures and tumor infiltrating lymphoctytes. Am J Clin Pathol 2016;146: 137–44.
34. Beguinot M, Dauplat MM, Kwiatkowski F, Lebouedec G, Tixier L, Pomel C, et al. Analysis of tumour-infiltrating lymphocytes reveals two new biologically different subgroups of breast ductal carcinoma in situ. BMC Cancer 2018;18:129.
35. Gruosso T, Gigoux M, Manem VSK, Bertos N, Zuo D, Perlitch I, et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. J Clin Invest 2019;129:1785–800.
36. Li X, Gruosso T, Zuo D, Omeroglu A, Meterissian S, Guiot MC, et al. Infiltration of CD8(+) T cells into tumor cell clusters in triple-negative breast cancer. Proc Natl Acad Sci U S A 2019;116:3678–87.
37. Keren L, Bosse M, Marquez D, Angoshtari R, Jain S, Varma S, et al. A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. Cell 2018;174:1373–87.
38. Stover DG, Gil Del Alcazar CR, Brock J, Guo H, Overmoyer B, Balko J, et al. Phase II study of ruxolitinib, a selective JAK1/2 inhibitor, in patients with metastatic triple-negative breast cancer. NPJ Breast Cancer 2018;4:10.
39. Dieci MV, Tsvetkova V, Orvieto E, Piacentini F, Ficarra G, Griguolo G, et al. Immune characterization of breast cancer metastases: prognostic implications. Breast Cancer Res 2018;20:62.
40. Cimino-Mathews A, Ye X, Meeker A, Argani P, Emens LA. Metastatic triple-negative breast cancers at first relapse have fewer tumor-infiltrating lymphocytes than their matched primary breast tumors: a pilot study. Hum Pathol 2013;44: 2055–63.
41. Wolf Y, Bartok O, Patkar S, Eli GB, Cohen S, Litchfield K, et al. UVB-induced tumor heterogeneity diminishes immune response in melanoma. Cell 2019;179: 219–35.
42. Kwa MJ, Adams S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): where to go from here. Cancer 2018;124:2086–103.
43. Balko JM, Giltnane JM, Wang K, Schwarz LJ, Young CD, Cook RS, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov 2014;4:232–45.
44. Datta J, Rosemblit C, Berk E, Showalter L, Namjoshi P, Mick R, et al. Progressive loss of anti-HER2 CD4(+) T-helper type 1 response in breast tumorigenesis and the potential for immune restoration. Oncoimmunology 2015;4:e1022301.
45. Adams S, Gatti-Mays ME, Kalinsky K, Korde LA, Sharon E, Amiri-Kordestani L, et al. Current landscape of immunotherapy in breast cancer: a review. JAMA Oncol 2019 Apr 11 [Epub ahead of print].
46. Lal A, Chan L, Devries S, Chin K, Scott GK, Benz CC, et al. FOXP3-positive regulatory T lymphocytes and epithelial FOXP3 expression in synchronous normal, ductal carcinoma in situ, and invasive cancer of the breast. Breast Cancer Res Treat 2013;139:381–90.
47. Bell D, Chomarat P, Broyles D, Netto G, Harb GM, Lebecque S, et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. J Exp Med 1999;190: 1417–26.
48. Michea P, Noel F, Zakine E, Czerwinska U, Sirven P, Abouzid O, et al. Adjustment of dendritic cells to the breast-cancer microenvironment is subset specific. Nat Immunol 2018;19:885–97.
49. Liu Y, Komohara Y, Domenick N, Ohno M, Ikeura M, Hamilton RL, et al. Expression of antigen processing and presenting molecules in brain metastasis of breast cancer. Cancer Immunol Immunother 2012;61:789–801.
50. de Kruijf EM, Sajet A, van Nes JG, Natanov R, Putter H, Smit VT, et al. HLA-E and HLA-G expression in classical HLA class I-negative tumors is of prognostic value for clinical outcome of early breast cancer patients. J Immunol 2010;185: 7452–9.
51. Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, White J, et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. Cancer Discov 2017;7:264–76.
52. Rosenthal R, Cadieux EL, Salgado R, Bakir MA, Moore DA, Hiley CT, et al. Neoantigen-directed immune escape in lung cancer evolution. Nature 2019;567: 479–85.
53. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9.
54. Guerriero JL, Sotayo A, Ponichtera HE, Castrillon JA, Pourzia AL, Schad S, et al. Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. Nature 2017;543:428–32.
55. Georgoudaki AM, Prokopec KE, Boura VF, Hellqvist E, Sohn S, Ostling J, et al. Reprogramming tumor-associated macrophages by antibody targeting inhibits cancer progression and metastasis. Cell Rep 2016;15:2000–11.
56. Le Mercier I, Poujol D, Sanlaville A, Sisirak V, Gobert M, Durand I, et al. Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment. Cancer Res 2013;73:4629–40.
57. Adams S, Kozhaya L, Martiniuk F, Meng TC, Chiriboga L, Liebes L, et al. Topical TLR7 agonist imiquimod can induce immune-mediated rejection of skin metastases in patients with breast cancer. Clin Cancer Res 2012;18:6748–57.
58. Crist KA, Chaudhuri B, Shivaram S, Chaudhuri PK. Ductal carcinoma in situ in rat mammary gland. J Surg Res 1992;52:205–8.
59. Kroemer G, Zitvogel L. Cancer immunotherapy in 2017: the breakthrough of the microbiota. Nat Rev Immunol 2018;18:87–8.