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Dendritic Cells, Inflammation and Breast Cancer

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Abstract

Solid tumors are well known for their genomic heterogeneity. While some aspects of this derive from so-called driver mutations, it is now clear that tumor cells possess a seemingly limitless capacity to evade cell death pathway activation, maintain essential survival programming, and initiate resistance networks that block efficacy of cytotoxic and targeted therapy. Given this amazing survival capability, how then to design approaches for effective eradication of malignant cells? Also present within all solid tumors is a diverse assemblage of genomically-stable immune cell types. While some of these possess documented activities that foster tumor progression, others possess inherent activities, that when favored, lead to rapid tumor cell elimination. This review focuses on aspects of dendritic cell (DC) biology in solid tumors, especially breast cancers, which point to DCs as a tractable tool to exploit for immune-based therapies.

Keywords

cancer; inflammation; immunogenic cell death; dendritic cells; Th2

INTRODUCTION

Novel therapeutic approaches are urgently needed for patients with breast cancer. Immunotherapies are amongst the most promising of these, including immune checkpoint-blockade where select T cell regulatory mechanisms are blocked, reported to alter the natural history of some refractory cancers [1, 2]. For example, improved survival has been documented for patients with metastatic melanoma treated with a blocking antibody targeting CTLA-4 [3], a T cell-intrinsic regulatory molecule [4]. Furthermore, objective responses in pretreated metastatic non-small cell lung cancer patients has been observed with PD-1-targeted therapy [5] (a T cell-extrinsic regulatory molecule that delivers an inhibitory signal via binding of ligand, PDL-1, expressed on some cancers [6]). In addition, delivery of immune activating signals can enhance anti-tumor responses to standard therapy, as has been recently illustrated with CD40-targeted therapy in pancreatic cancer [7]. These promising clinical findings indicate the power and therapeutic potential of leveraging aspects of immune-mediated mechanisms as anti-cancer therapy. Herein, we discuss recent insights and advances in the understanding aspects of tumor-promoting inflammation in breast cancer, focusing on the role of dendritic cells (DCs).

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CHALLENGES IN BREAST CANCER THERAPY

Despite definitive reductions in breast cancer–related mortality, median survival of the ~ 25% of patients who develop metastatic disease remains poor at ~ 2–3 years [8]. While administration of preoperative therapies has not improved overall survival above that achieved with adjuvant therapy, the rate of pathologic complete response (pCR) after preoperative chemotherapy has been demonstrated to be a predictor of improved outcomes for estrogen receptor (ER)-negative breast cancer [9, 10]. Indeed, patients with triple-negative breast cancer (TNBC) that lacks ER, progesterone receptors (PR) and HER2, achieve higher pCR rates with preoperative chemotherapy as compared to ER-positive cancers, moreover, a pCR in TNBC predicts for highly favorable disease-free survival rates [11]. Conversely, patients with TNBC who do not achieve a pCR and have residual disease after preoperative chemotherapy, have a markedly increased risk of rapid recurrence, and death [11]. These patients represent a great unmet medical need as there is no known effective therapy that improves outcome. Many translational and clinical trials with new therapies for TNBC have been launched exploiting molecular insights to target tumor cell-intrinsic pathways regulating proliferation, survival, and chemoresistance (reviewed in [12]). With that TNNBC represents a heterogeneous assemblage of subtypes [13, 14], there is optimism for clinical trials evaluating sub-type-selective targeted-therapies for this patient group. That said, the inherent genomic instability and intratumoral heterogeneity of TNBC may instead limit efficacy, or enhance host toxicity that limits combinatorial strategies. Conversely, leveraging the diversity inherent to the immune response in these tumors for therapeutic gain has potential to overcome tumor cell genomic plasticity and clonal evolution.

While many immune effectors pathways are co-opted by tumors to foster neoplastic progression [15] other immune effector pathways can be harnessed to eliminate (breast) cancer cells. Perhaps the most compelling of these in humans is that observed by paraneoplastic diseases, some of which are neurological disorders that are a consequence of anti-tumor immune responses [16, 17]. Onconeural antigens (like cdr2), normally expressed on neurons, can also be expressed on breast cancer cells [16, 17]; some patients develop a strong antigen-specific $CD8^+$ T cell-mediated response against their breast cancer resulting in autoimmune cerebellar degeneration and severe neurological dysfunction [16, 17]. The presence of naturally occurring immunity against a broad range of tumor-associated antigens including HER-2/neu, MUC1, cyclin B1 and survivin has now been documented in patients with breast cancer [18]. Indeed, some early clinical studies are attempting to augment this intrinsic immunity in patients at high risk for disease recurrence [19–21]. However, the native immune response to the cancer co-exists with the cancer, and is therefore not protective, either because of tumor escape, for example, through clonal evolution, or because it might have been generated in an inappropriate immunosuppressive microenvironment.

There is accumulating evidence that chronic inflammatory pathways play a key role in the initiation and progression of cancer [15, 22]. There are (at least) two types of chronic inflammation having opposing effects on tumors: (a) chronic inflammation that promotes cancer cell survival and metastasis [23–25], and (b) acute inflammation that can trigger cancer cell destruction as illustrated by regression of bladder cancer after treatment with microbial preparations [26]. Although chronic inflammation is often linked with the presence of type 2 polarized responses involving alternatively-activated macrophages (variably referred to as type 2, M2, Th2-type), acute inflammation associated with cancer destruction is instead linked with classically-activated macrophages (variably referred to as type 1, M1, Th1-type) [27]. Type 1 macrophages are induced by type 1 cytokine like interferon (IFN)-γ, whereas type 2 macrophages are induced by type 2 cytokines including interleukins (IL)-4 and IL-13 [27]. Clinically, there is evidence that chronic inflammation

may increase the risk of breast cancer recurrence [28]; in a multi-center study of 734 women treated for early stage breast cancer, high levels of circulating acute phase proteins (APPs) approximately 3 years after treatment were associated with a two-fold elevated risk of disease recurrence and mortality [28]. Herein, we will discuss the mechanisms by which cancers can hijack dendritic cells (DCs) to promote chronic inflammation and accelerate tumor development, and how understanding this circuitry can offer new targets for cancer therapy.

DENDRITIC CELLS

Immunity results from a complex interplay between the innate arm of the immune system (which is antigen-nonspecific), and the adaptive arm of the immune system (which is antigen-specific). Cells of the innate arm utilize non-clonal recognition receptors, including lectins, Toll-like receptors (TLRs), NOD-like receptors (NLRs) and helicases for activation. B and T lymphocytes of the adaptive arm instead utilize clonal receptors that recognize antigens, or their derived peptides, in a highly specific manner. The nature of the immune response is regulated by DCs, a rare cell type in most tissues where under homeostatic conditions are key cellular sensors of microbes. DCs are linked to their environment through a wealth of molecular sensors enabling them to sense danger, and to transmit resulting information to lymphocytes. Thus, DCs provide an essential link between innate and adaptive immunity and thus represent an attractive vector for immunotherapy [29, 30].

DCs, discovered by Ralph Steinman in 1973, are bone marrow-derived cells that seed all tissues (reviewed in [31]), where they sample their environment and transmit information to adaptive immune cells [30, 31]. In peripheral tissues, DCs capture antigens (Ags) through several complementary mechanisms. DCs launch immune responses by presenting captured Ag in the form of peptide- major histocompatibility complex (MHC) complexes to naïve, i.e., antigen-inexperienced, T cells in lymphoid tissues. Upon interaction with DCs, naïve $CD4^+$ and $CD8^+$T cells differentiate into antigen-specific memory T cells with distinct functions. $CD4^+$ T cells for example, can become T helper (Th)-1, Th2, or Th17 cells, T follicular helper cells (Tfh) that help B cells differentiate into antibody secreting cells, or regulatory T cells (Tregs) that modulate functions of other lymphocytes. Naïve $CD8^+$ T cells can give rise to cytotoxic effector lymphocytes (CTLs).

In the steady state, non-activated (immature) DCs present self-antigens to T cells, thereby inducing tolerance either through T cell deletion or differentiation of regulatory/suppressor T cells [32]. These immature DCs have special characteristics including: 1) ability to efficiently capture Ags, 2) accumulation of MHC class II molecules in the late endosome-lysosomal compartment, 3) low level expression of costimulatory molecules, 4) a unique set of chemokine receptors allowing their migration to lymphoid tissues (e.g. CCR7), and 5) limited capacity to secrete cytokines [33]. In contrast, mature Ag-loaded DCs can launch differentiation of Ag-specific T cells into effector cells with unique functions and cytokine profiles. DC maturation is associated with: 1) down-regulation of Ag-capture activity, 2) increased expression of surface MHC class II molecules and costimulatory molecules, 3) ability to secrete cytokines [33], and 4) acquisition of CCR7 expression thus enabling migration of DCs into draining lymph nodes [33]. However, DC maturation does not result in a unique phenotype. Rather, in response to various signals provided by different microbes, either directly or through the surrounding cells, DCs acquire distinct phenotypes that eventually contribute to diverse immune responses. In addition to cytokines or direct microbial signals, ligation of CD40 represents an essential signal for differentiation of fully mature DCs able to launch adaptive T cell immunity [34]. The plasticity of DCs in response to extrinsic signals, and the existence of distinct DC subsets with specific functions, contributes to the mounting of highly diverse immune responses. DCs that sit in tissues under steady state are dependent upon FLT3 (fms-related tyrosine kinase receptor 3) and

macrophage-colony stimulating factor receptor (MCSF-R) [35]. However, inflammatory processes such as those initiated by microbial invasion, or developing cancers, substantially alter DC compartments. While the origin of DCs recruited to sites of inflammation is still unclear, it is clear that monocytes give rise to inflammatory DCs in vivo [36].

Human blood DC subsets can be distinguished by differential expression of three surface molecules: CD303 (BDCA-2), CD1c (BDCA-1), and CD141 (BDCA3) [37]. $CD303^+$ plasmacytoid DCs (pDCs) represent a front-line of anti-viral immunity through their ability to secrete large quantities of type I IFN in response to viral encounter [38]. Their pre-synthesized stores of MHC class I permit a rapid initial $CD8^+$ T cell response to viral infection [39]. pDC-derived type I IFN may promote the immunogenic maturation of other DC populations [40] therefore helping to activate novel T cell clones. In their resting state, pDCs are considered to play an important role in tolerance, including oral tolerance [40]. This functional plasticity could be exploited in cancer as we will discuss below.

Human $CD141^+CD1c^-$ DCs uniquely express Toll-like Receptor 3 (TLR3), produce IL-12, and efficiently cross-prime $CD8^+$ T cells when activated by the TLR3 ligand, poly I:C [41–47]; however, other human DC subsets such as Langerhans cells (LCs) [48, 49] and $CD1c^+$ DCs also cross-present antigens to $CD8^+$ T cells [43, 45, 46]. Recent data from humanized mice, human blood, and lung tissue reveal that both $CD1c^+$ and $CD141^+$ DC subsets can acquire viral antigens and thereby drive antiviral effector $CD8^+$ T cell responses [50]. In contrast, $CD1c^+$ DCs are uniquely able to drive differentiation of $CD103^+CD8^+$ mucosal T cells [50]. This is important because CD103 expression by CTLs mediates adherence to E-cadherin resulting in tumor cell rejection [51]. Indeed, mucosal homing and retention of $CD8^+$ T cells is important for mucosal cancer vaccine efficacy [52]. These results highlight the critical role the route of immunization plays in trafficking of effector T cells [53, 54], and the critical role tissue DCs play in imprinting the trafficking patterns of elicited T cells [55].

The human skin hosts epidermal LCs and dermal interstitial DCs (dermal DCs). The dermal DCs can be further subdivided into $CD1a^+$ DCs and $CD14^+$ DCs. Earlier studies of human cutaneous DCs demonstrated their phenotypic and functional heterogeneity with regards to cellular immunity and priming of highly efficient CTLs [56]. Our studies concluded that human $CD14^+$ DCs can directly help activated B cells, as well as induce naïve T cells, differentiate into cells with properties of T follicular helper cells (Tfh) [48], thus, they may be specialized for development of humoral responses [48]. On the contrary, LCs are more efficient in cross-presenting peptides from protein Ags to $CD8^+$ T cells, and in priming $CD8^+$ T cells in becoming potent CTLs [48]. With this evolving understanding of the biologic function of DCs, the challenge becomes deciphering which DCs control T cell differentiation and trafficking in vivo in human breast cancer.

$CD4^+$ T CELLS IN BREAST CANCER-ASSOCIATED INFLAMMATION

An expanding list of Th subsets, specialized for promoting particular types of inflammation, function through secretion of a restricted set of cytokines leading to unique classes of immune response (reviewed in [57]). Thus, in response to intracellular microbes, such as viruses and certain bacteria, $CD4^+$ T cells differentiate into Th1 cells, secrete IFN-γ, and possess a specific range of functions. In contrast, extracellular pathogens such as helminths induce development of Th2 cells, whose cytokines [IL-4, IL-5, IL-10 and IL-13] direct immunoglobulin E- and eosinophil-mediated destruction of pathogens [57]. Since discovery of Th1 and Th2, a large spectrum of $CD4^+$ T cell phenotypes have been described based on their cytokine secretion profiles and function (reviewed in [57]). The main subsets of $CD4^+$ T cells also express unique transcription factors: Th1 cells can be identified by expression of T-bet, Th2 cells express GATA-3, Th17 cells express RORγT, whereas Tregs express Fox-

P3 [57]. All of these $CD4^+$ T cell types contribute to tumorigenesis in various ways. For example, Tregs can inhibit effector functions of $CD8^+$ T cells thereby preventing tumor rejection [58]. Although in general favoring tumor rejection, Th1 cells can contribute to tumor escape via secretion of IFN-γ which in turn triggers expression of programmed cell death ligand (PDL)-1 in tissues providing an off-signal to effector T cells [6]. Furthermore, selective evolutionary pressure exerted by IFN-γ can lead to tumor editing and selection of resistant clones, thereby also facilitating tumor development [59].

Such plasticity of cells and outcomes is even further exemplified by the more recently identified Th17 cells [60] that exert pro- and anti-tumor activity depending on the tissue environment in which they find themselves. Th17 cells are detected at strikingly high frequency in tumors, but not blood, of patients with diverse cancer types, including ovarian and pancreatic cancer (reviewed in [61]). The major pro-tumor effects of Th17 cells are manifest by their capacity to promote angiogenesis, and recruit other immune cells, in particular neutrophils, which in turn can secrete elastase, another pro-tumor factor [61]. Interestingly, IL-17, derived from Th17 cells, can synergize with IFN-γ to induce secretion of Th1 type chemokines, CXCL9 and CXCL10, by tumor cells, which in turn attract effector T cells to tumor sites [61]. IFN-γ^+IL17$^+$T cells have been reported in human tumors and in patients with autoimmune disease [61]. Whereas they are pathogenic in autoimmune disease, the synergistic effects of IL-17 and IFN-γ could be exploited for cancer therapy. Another recently characterized pathway for pro-tumor inflammation that can be a target for therapy is Th2 inflammation, discussed in greater detail hereunder.

Th2 INFLAMMATION IN PATHOGENESIS OF EPITHELIAL TUMORS

Breast and pancreatic cancers contain significant presence of inflammatory Th2 (iTh2) cells [62] (Figure 1). These iTh2 cells are differentiated from the classical Th2 cells by their co-expression of tumor necrosis factor (TNF)-α and lack of IL-10 secretion [63]. They are driven by OX40L (CD134)-expressing DCs in response to cancer-derived thymic stromal lymphopoietin (TSLP) [64]. These iTh2 cells accelerate breast tumor development in humanized mouse models through production of IL-13 [62]. Blocking of OX40L and/or TSLP in vivo results in inhibition of IL-13 secretion, and consequently leads to inhibition of breast cancer development [64]. In genetically-engineered mouse models of mammary cancer, Th2 cells accelerate development of pulmonary metastasis via IL-4R signaling [65]. IL-4 and IL-13 can contribute to tumorigenesis in several ways. For example, IL-13 produced by NKT cells induces myeloid cells to secrete transforming growth factor (TGF)-β;, which ultimately inhibits CTL function (reviewed in [66]). Spontaneous autochthonous breast carcinomas arising in Her-2/neu transgenic mice arise with shorter latency when mice are depleted of T cells, thus providing evidence for T cell–mediated immunosurveillance slowing tumor growth [66]. This immunosurveillance can be further enhanced by blockade of IL-13, which slows appearance of autologous tumors as compared to controls [66]. IL-4 and IL-13 can also generate type 2 macrophages [67] that promote tumor development via several mechanisms [68] including secretion of growth factors such as epidermal growth factor (EGF), enzymes involved in tissue remodeling such as cathepsins, as well as through direct inhibitory effects on $CD8^+$ T cell function (reviewed in [69]).

Autocrine IL-13 is important in the pathophysiology of Hodgkin's disease (reviewed in [70]). IL-13 and IL-13R are frequently expressed by Hodgkin's and Reed-Sternberg cells, where IL-13 stimulates growth. Similar to Hodgkin's cells, breast cancer cells express pSTAT6 [62], indicating that IL-13 may regulate cancer cell physiology. Phosphorylation of STAT6 can lead to up-regulation of anti-apoptotic pathways in cancer cells [71] leading to chemotherapy resistance, or to immune-mediated cytotoxicity driven by granzymes and resulting in tumor growth rather than rejection. Clinically, the Th2 signature in breast cancer [14, 72, 73] and the expression of the Th2 master regulator GATA-3 in pancreatic cancer

[74] are associated with poor outcomes. Furthermore, the pathogenic TSLP/IL-13 pathway has also been detected in the context of *Helicobacter pylori* infection which leads to chronic gastritis, the causative factor in gastric cancer [75]. There, *H. pylori* triggers human gastric epithelial cells to produce TSLP [75]. DCs exposed to supernatants of *H. pylori*-infected epithelial cells trigger naïve $CD4^+$ T cells to produce high levels of the Th2 cytokines IL-4 and IL-13, and of inflammatory cytokines TNF-α and IFN-γ [75]. Thus, disrupting this inflammatory, pro-tumor TSLP-OX40L-IL13/4 axis could be considered as a novel investigational therapeutic approach for several cancers. The molecular and cellular factors contributing to global IL-4/IL-13 production in epithelial cancers likely extend beyond TSLP, and are topics of intense study.

MODULATING DCs IN THE TUMOR ENVIRONMENT

DCs are found in most tumors in humans and mice. Tumors can prevent Ag presentation and establishment of tumor-specific immunity through a variety of mechanisms. Tumor-derived factors can alter DC maturation so as to yield cells that indirectly help tumor growth ("pro-tumor" inflammation) as discussed above. Furthermore, by converting immature DCs into macrophages, i.e., through IL-6 and M-CSF, breast cancers can prevent priming of tumor-specific T cells [76, 77]. Alternatively, the tumor glycoproteins carcinoembryonic antigen (CEA) and MUC-1 (mucin-1) that are endocytosed by DCs may stay confined in early endosomes, therefore preventing efficient processing and presentation to T cells [78].

pDCs that infiltrate breast carcinomas produce little type I interferon upon TLR ligation [79]. These pDCs induce naïve $CD4^+$T cells to differentiate into IL-10-producing T cells having suppressive functions. Such inhibition of type I interferon secretion might also impact generation of effector T cells as DCs require type I interferon signals to cross-present tumor Ags [80, 81]. Whether this mechanism explains why pDC are associated with poor prognosis in early breast cancer [82] remains to be determined. Consistently however, pDC depletion delayed tumor growth in vivo, and intratumoral administration of TLR7L led to pDC activation, and displayed potent curative effects [83].

Recent studies point to an unexpected role for DCs in response to cancer therapy via so-called "immunogenic cancer cell death" [84]. Certain cytotoxic agents such as anthracyclines or oxaliplatin can induce immunogenic cancer cell death, characterized by secretion of HMGB1 (high mobility group protein B1) from dying cells that engages TLR4 on DCs [84]. This signal facilitates cancer Ag processing and presentation by DCs to T cells [84] that in turn plays an important role in boosting anti-cancer immunity via endogenous vaccination. Indeed, absence of HMGB1 expression by dying tumor cells compromises DC-dependent T cell priming by tumor-associated Ags [85]. Furthermore, early stage breast cancer patients who carry a TLR4 loss-of-function allele have a higher risk of recurrence following radiotherapy and chemotherapy than those who carry the wild type TLR4 allele [86]. Exploiting this unique molecular mechanism of Ag delivery and DC activation could be another way to harness DCs for breast cancer immunotherapy.

Conclusions

Interrogating the functions of DCs in tumor parenchyma is a fertile area for investigation. Ultimately, re-programming patients' "pro-tumor" DCs into "anti-tumor" DCs may be part of effective cancer immunotherapy.

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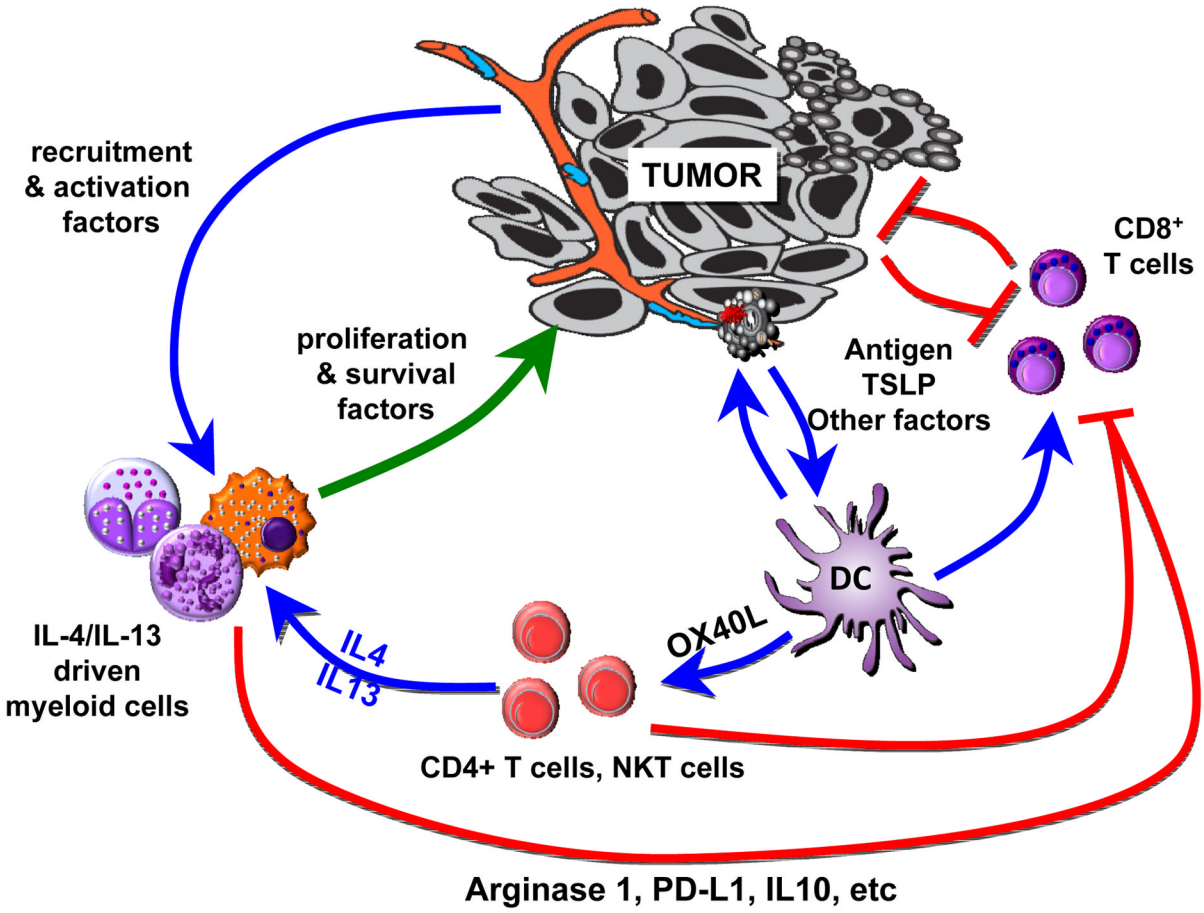

Figure 1. Pathogenic type 2 cytokine loop in breast cancer

DCs in breast cancer are exposed to cancer-derived factors—for example, TSLP—that skew their maturation toward expression of OX40L and capacity to activate $CD4^+$ T cells to secrete IL-13 and IL-4, type 2 cytokines. In this environment, responding lymphocytes secreting IL-4 and IL-13 promote tumor development either directly or indirectly via myeloid cells including macrophages. Direct effects include triggering anti-apoptotic pathways and steroid metabolism in epithelial cancer cells, as well as promoting stromal fibroblast proliferation and differentiation. Indirect effects include triggering secretion of growth (EGF) and pro-angiogenic (VEGF) factors by tumor-infiltrating macrophages as well as PDL-1 expression and IL-10 secretion that blunt $CD8^+$ T cell effector function. Cancers cells are likely to also directly activate innate lymphocytes secreting IL-13. The molecular and cellular factors contributing to the global IL-13 production in epithelial cancers likely extend beyond TSLP, and are topics of intense study. Another active area is the question of TSLP regulation, whether all breast cancer express it, and at which stages, as well as its role in metastatic niche formation.